Selenium speciation and bioavailability in Dutch agricultural soils: The role of soil organic matter

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This thesis was conducted under the auspices of the Graduate School of Socio-Economic and Natural Sciences of the Environmental (SENSE)

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Thesis
submitted in fulfilment of the requirements for the degree of doctor
at Wageningen University
by the authority of the Rector Magnificus
Prof. Dr A.P.J. Mol
in the presence of the
Thesis Committee appointed by the Academic Board
to be defended in public
on Tuesday 6 December 2016
at 1.30 p.m. in the Aula.

Supriatin Supriatin
Selenium speciation and bioavailability in Dutch agricultural soils: The role of soil organic matter
265 pages.

PhD thesis, Wageningen University, Wageningen, NL (2016)
With references, with summary in English

ISBN: 9789462579309
DOI: http://dx.doi.org/10.18174/390125

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Chapter 1

General Introduction

1.1 The importance of Se for human and animal heath

Selenium (Se) is an essential micronutrient for human and animal (livestock) health. The importance of Se for human health was reported after early 1970s when the Keshan disease and Keshin-Beck disease were observed in humans in 16 provinces in China with low Se content in the top soils, i.e. < 0.125 mg kg^{-1} (Tan (1989) *in* Tan *et al.* (2002)). Keshan disease is an endemic cardiomyopathy, a type of endemic heart disease (Xiang, 1993; Tan *et al.*, 2002) and Keshin-Beck disease is an endemic osteoarthropathy, an endemic of pathological changes in joints all over body including thickened and deformed joints, and difficulty to bend or stretch (Tan *et al.*, 2002). In the human body, Se, which is mainly found as selenoproteins, plays a role as a component of the antioxidant enzyme glutathione peroxidase and a catalyst for the production of active thyroid hormone (Rotruck *et al.*, 1973; Rayman, 2000, 2002). Previous studies suggest that Se has benefits to human health, for instance, it can enhance the immune system, thus can further counteract certain viral infections; it is required for sperm maturity and motility; it can improve mood states; and reduce cardiovascular disease and cancer risks (Rayman, 2002; Shin *et al.*, 2007; Kato *et al.*, 2010; Hatfield *et al.*, 2014). The importance of Se for animal (livestock) health has been identified about two decades earlier than that for human health when Se supplementation to livestock resulted in prevention of exudative diathesis in chicken, white muscle disease in calves and lambs, unthrifty condition in weaned lambs, and hepatosis dietetica in pigs (Patterson *et al.*, 1957; Muth *et al.*, 1958; Drake *et al.*, 1960; Oldfield, 2002). In addition to enhancing the immune system, Se supplementation can increase growth rate and fertility of livestock as well (Oldfield *et al.*, 1960; Wilson, 1964; Hidiroglou, 1979; Harrison *et al.*, 1984).

Previous reviews showed that Se daily intake by humans worldwide including 30 countries in Europe (including Russia), 5 countries in Asia (including China with low-Se area), New Zealand (low-Se area) and 1 country in Africa (Libya) is in the range of 2 to 70 µg Se day^{-1}, in which the intake is mostly < 40 µg day^{-1} (Rayman, 2004; Surai *et al.*, 2008). Specifically, the Se intake in the Netherlands is in the range of 39 to 54 µg Se day^{-1} (Van Dokkum, 1995). On the other hand, in some other countries, such as Australia, Canada, Japan, New Zealand (high-Se area), USA, and Venezuela, the Se daily intake is higher, i.e. 55 to 350 µg day^{-1} (Rayman, 2004), and an exceptionally high intake of up to 4990 µg day^{-1} was found in some seleniferous areas in China (Rayman, 2004). These findings indicate that in general the Se intake by humans worldwide is lower than the Dietary Reference Intake of Se proposed by The Panel on Dietary Antioxidants and Related Compounds, USA, i.e. 55 µg Se day^{-1} for both adult men and women, which is a level associated with the highest activity of antioxidant enzymes (Monsen, 2000). The study of Wanger (1998) suggested that the minimum Se daily intake required for humans is 40 µg Se day^{-1}, and the maximum safe intake on a long term basis is 400 µg Se day^{-1}. A very high intake of Se (i.e. > 600 µg Se day^{-1}) can cause Se toxicity (i.e. selenosis) in humans (Wanger, 1998).

The low Se intake by humans, especially in European countries, is mainly due to a low available Se content in soils that can be taken up by plants (as food crops and fodder crops). Grains are a major source of human Se intake, although the sources of Se intake for humans can be also from meat, eggs, dairy products such as milk and cheese, and seafood, such as fish and oyster. The low Se content in fodder crops made it necessary to supply Se to livestock, which has been in practice for several decades in European countries, such as Finland, Norway, Denmark, Sweden and Britain, in order to have a sufficient amount of Se intake by livestock (Rayman, 1997; Oldfield, 2002).

1.2 Selenium content and speciation in soils

1.2.1 Selenium content in soils

Total Se content in soils worldwide is in the range of 0.03 to 2.0 mg kg^{-1}, with an average of 0.40 mg kg^{-1} (n = 1623) (Ure and Berrow (1982) *in* Yamada *et al.* (2009)). However Se content in some seleniferous soils can be quite high, i.e. up to

Table 1. Total Se content in (agricultural) soils in several countries in Europe.

Country	Number of samples	Se content (mg kg^{-1})		Reference
		Range	Average	
Belgium	539	0.14 – 0.70	0.25[a] – 0.46[a]	De Temmerman *et al.* (2014)
Sweden	297	0.065 – 1.70	0.28	Johnsson (1992)
Germany	499	0.016 – 0.65	0.12 (AB[b]) 0.16 (GL[c])	Hartfiel and Bahners (1988)
Spain (Mediterranean)	32	0.06 – 1.51	0.48	Roca-Perez *et al.* (2010)
Spain (semi-arid Mediterranean)	24	0.20 – 4.38	0.83	Rodriguez *et al.* (2005)
13 European countries	128	0.06 – 0.82	0.32	Wang and Sippola (1990)
Finland	93	0.05 – 0.633	0.21	Yläranta (1983) *in* Hartfiel and Bahners (1988)
Denmark		0.14 – 0.52		Hamdy and Gissel-Nielsen (1976) *in* Gupta and Gupta (2000)
England and Wales	229	0.02 – 2.0	0.50	Archer and Hodgson (1987)
Netherlands	341	0.05 – 2.0		Mol *et al.*(2012)

[a] Based on soil types.
[b] Arable land.
[c] Grassland.

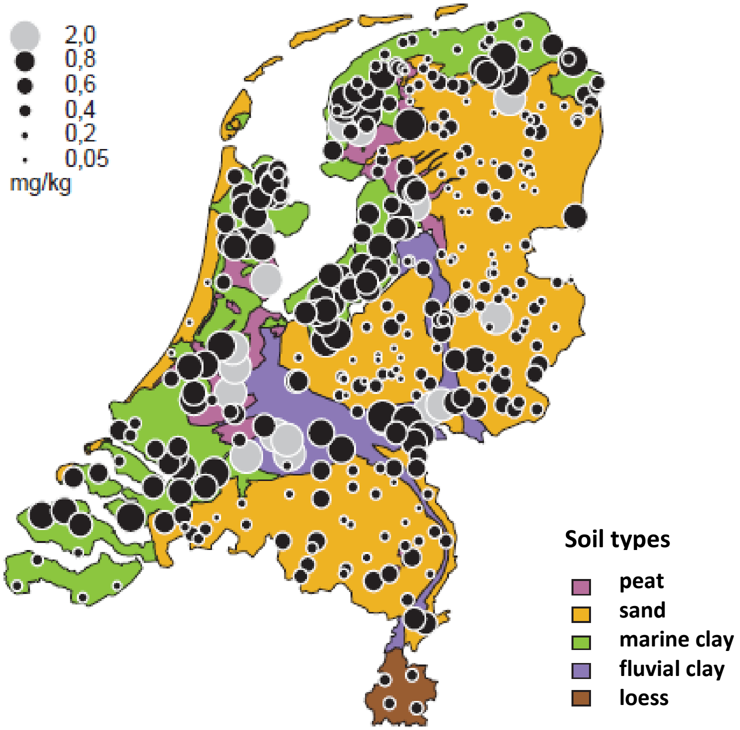

Fig. 1. Map of Se content in soils in the Netherlands. Source: Mol *et al.* (2012).

3.81 mg kg^{-1} in soils developed from carbonaceous shale in Enshi, Hubei Province, China (Tan *et al.*, 2002) and up to 4.91 mg kg^{-1} in soils developed from Se-rich sediments in Punjab, Northwestern India (Dhillon and Dhillon, 2014). In general, the total Se content of soils in Europe is within the normal range worldwide, in which the Se content is mostly < 1 mg kg^{-1} (Table 1), including the total Se content in various soil types in the Netherlands (Fig. 1). Previous studies proposed different ranges of total Se content in soils to classify its potential effects on plant, animal and human health into deficient and toxic levels. Based on the relationship between total soil Se and Se deficiency diseases (Keshan-disease and Keshin-Beck disease) or Se toxicity (selenosis) in humans in China, Tan *et al.* (2002) suggested that a total soil Se of < 0.125 mg kg^{-1} can be considered as deficient, whereas a total soil Se of > 3.00 mg kg^{-1} is considered as toxic. On the other hand, Gupta and Winter

(1975) suggested that a total soil Se of < 0.60 mg kg^{-1} is likely to produce crops with insufficient amount of Se for livestock intake. Moreover, Allaway (1969) *in* Girling (1984) indicated that a total soil Se of < 0.04 mg kg^{-1} can be considered as low Se soils, which can cause Se deficiency in animals; soils with a total Se of 0.5 to 5.0 mg kg^{-1} can be considered as moderate Se soils; and soils with a total Se of 1 to 100 mg kg^{-1} can be considered as high Se soils.

Total Se (and other micronutrients) content in soils is mainly determined by the geological parent materials of the soils and pedogenic processes (White and Zasoski, 1999), resulting in Se rich soils or Se deficient soils, which further influence Se content in the food chain (plants, animals, humans). In general, Se contents in sedimentary rocks, especially shales, coals and organic rich deposits, are higher than those in igneous rocks, with an exception that particular sedimentary rocks, such as sandstone and limestone, contain relatively low Se (Fordyce, 2013). Selenium in shales, coal and organic rich deposits is mainly present as organo-selenium compounds, adsorbed element and organo-selenium complexes (Jacobs (1989) *in* Fordyce (2013)) and (Kulp and Pratt, 2004). Moreover, clay soils in general contain higher level of Se than sandy soils due to the presence of Se-enriched minerals, such as biotite, and, of aluminium and iron-(hydr)oxides that can contain adsorbed Se (Keskinen *et al.*, 2009; Ros *et al.*, 2014). In relation to pedogenic processes, Tan *et al.* (2002) showed an example of Se content variability in soils from different climate zones in China. The soils developed in tropic and sub-tropic zones such as laterite, yellow soil and red soil, contain higher Se (> 0.30 mg kg^{-1}) than the soils developed in temperate (dry) steppe and desert zones, such as chernozem, chestnut soil, calcic brown soil, desert soil, and solonchak (0.14 to 0.30 mg kg^{-1}). Moreover, the soils developed in temperate humid and sub-humid zones, such as brown earth, dark brown, and drab brown, loessial soil, purple soil and red drab contain very low Se (< 0.14 mg kg^{-1}). The higher Se content in the soils in the tropic and sub-tropic zones is related to their parent materials, such as red limestone and lateritic red earth; in the temperate (dry) steppe and desert zones Se has been accumulated through hydrological and aerial transport of fine particles and evaporation of water, which form alkaline and oxidized soils that contain mainly selenate; and in the temperate humid and sub-humid zones, the low Se content in the soils is related to low Se content in the parent materials of the soils (Wang and Gao, 2001). The endemic Keshan disease and Keshin-Beck disease include areas with dark brown, drab brown, brown earth, yellow brown, red drab, loessial soil, purple soil and black soil, whereas the disease free areas include laterite, yellow soil, red soil, desert soil, chernozem, chestnut soil, and calcic brown soil (Tan *et al.*, 2002).

In addition to the geological parent materials and pedogenic processes, the total Se content in soils is determined by soil organic matter content due to the propensity of Se to associate with organic materials (Fordyce, 2013). This association is possibly due to either selenium-metal-organic matter complexation, such as Se-Fe(III)-organic matter complexes, or due to biotic formation via microbial reduction of Se and further incorporation into soil organic matter structure (Gustafsson and Johnsson, 1994; Fernández-Martínez and Charlet, 2009). Most studies indicated that the association relies more on biotic formation than on abiotic complexation (Fernández-Martínez and Charlet, 2009).

1.2.2 Selenium speciation in soils

In soil, Se is present in inorganic Se and organic Se species. The inorganic Se species in soils include selenate (SeO_4^{2-}) and selenite (SeO_3^{2-}) that are present in oxidized alkaline to acidic soils, respectively, and elemental Se (Se^0) and selenides (Se^{2-}) that are present in reduced soils (Fig. 2). The reduction of Se into elemental Se and selenides is possible via soil microbial activity during anaerobic oxidation of organic substrates including acetate, lactate and aromatics, in which the soil microorganisms can use Se (selenate or selenite) as terminal electron acceptors (Stolz and Oremland, 1999). In addition to these inorganic Se species, different organic Se species are present in soils, but factors that influence organic Se speciation have not been identified. Soluble organic Se, such as selenomethionine ($C_5H_{11}NO_2Se$), methane seleninic acids (CH_3SeOOH) and trimethyl selenium ion

($(CH_3)_3Se^+$) (Abrams and Burau, 1989; Yamada and Hattori, 1989; Abrams *et al.*, 1990a; Tolu *et al.*, 2011; Stroud *et al.*, 2012; Tolu *et al.*, 2014), and solid organic Se associated with humic and fulvic acid fractions of soil organic matter (Kang *et al.*, 1991b; Qin *et al.*, 2012) that is possibly present as seleno-amino acids incorporated into protein or peptides (Kang *et al.*, 1991b), have been detected in soils. Volatile organic Se compounds, such as dimethylselenide ($(CH_3)_2Se$) and dimethyldiselenide ($(CH_3)_2Se_2$), are also formed in soils via soil microbial processes (Abu-Erreish *et al.*, 1968; Doran and Alexander, 1977a).

The amount and predominance of Se species present in soils is influenced by the chemical and mineralogical composition of the soils (organic matter, metal-(hydr)oxides, clay content), pH, redox condition and soil microbial activity (Dhillon and Dhillon, 1999; Wang and Chen, 2003; Coppin *et al.*, 2006; Lyons, 2010), which control the transformation mechanisms of Se in soils, i.e. oxidation vs. reduction, mineralization vs. immobilization, volatilization (Ros *et al.*, 2014) and adsorption vs. desorption. Previous studies have shown that the low Se soils (total Se < 1.0 mg kg^{-1}) with high organic matter content tend to be dominated by organic Se (Yamada *et al.*, 2009), whereas the low Se soils with high Fe- and Al-(hydr)oxide content but low in organic matter content (i.e. < 5%) tend to be dominated by inorganic Se species (adsorbed on the metal-(hydr)oxides) (Wang and Chen, 2003). The role of pH and redox status in Se speciation in soils has been described above and presented in Figure 2. Soil microbial activity can reduce Se in soils into insoluble Se (Se^0 and H_2Se) (Doran and Alexander, 1977b; Herbel *et al.*, 2003; Fernández-Martínez and Charlet, 2009) and can convert Se into volatile Se compounds (Abu-Erreish *et al.*, 1968; Doran and Alexander, 1977a).

Methodology to determine Se speciation in soils has been explored intensively either using various single step soil extractions (Yamada and Hattori, 1989; Abrams *et al.*, 1990a; Kang *et al.*, 1991b; Gustafsson and Johnsson, 1992; Johnsson, 1992; Gustafsson and Johnsson, 1994; Alam *et al.*, 2000; Keskinen *et al.*, 2009; Tolu *et al.*, 2011; Qin *et al.*, 2012; Stroud *et al.*, 2012) or sequential soil extractions (Chao and Sanzolone, 1989; Tokunaga *et al.*, 1991; MacLeod *et al.*, 1998; Mao and Xing, 1999; Wang and Chen, 2003; Hagarová *et al.*, 2005). In general, these soil extractions represent different Se pools or Se fractions present in soils that are removed by the corresponding extractants, such as soluble Se, exchangeable Se, Se adsorbed to metal-(hydr)oxides or carbonates, and Se bound to organic matter or humic compounds, instead of specific Se species (Tokunaga *et al.*, 1991). In comparison with single step soil extractions, the sequential soil extractions seem inappropriate for low Se soils and increase the risk of Se contamination, loss and Se species redistribution (Tolu *et al.*, 2011). In addition to various chemical soil extractions, further analyses using certain separation techniques, such as High Performance Liquid Chromatography (HPLC), Donnan Membrane Technique (DMT), XAD-8 resin, and high voltage paper electrophoresis, have been performed to identify specific inorganic Se species and/or organic Se species in different soil extractions (Van Dorst and Peterson, 1984; Abrams and Burau, 1989; Ochsenkühn-Petropoulou *et al.*, 2003; Weng *et al.*, 2011; Stroud *et al.*, 2012). However, identification of organic Se species using these separation techniques is still challenging due to the fact that the majority of organic Se species in the soil extracts is unknown.

1.3 Selenium content and metabolism in plants

The amount of Se in plants is determined by biological factors including plant species and metabolism of Se in plants; soil factors including geological parent materials of soil, soil type and chemical species of Se present in soil; and external factors including fertilizer applications (Gissel-Nielsen *et al.*, 1984). In this section, only the biological factors influencing Se content in plants are discussed, whereas the soil factors and external factor of fertilizations are described in details in Section 1.2 and 1.4.

Table 2. Selenium content in food crops and fodder crops from several countries in Europe.

Country	Crop	Number of fields	Se content (μg kg^{-1})		Reference
			Range	Average	
UK (1982; 1992; 1998)	Wheat grain	180; 187; 85		25; 33; 25	Adams *et al.* (2002)
UK (2006 – 2007)	Wheat grain	10	15.5 – 44		Stroud *et al.* (2010a)
Denmark (1972 – 1973)	5 cereal plants	25 – 178	2 – 110	16 – 21	Gissel-Nielsen (1975)
	Grass, clover, alfalfa	15 – 98		32 – 44	
Greece (2004 – 2005)	Fruits		1.4 – 6.3	3.4	Pappa *et al.* (2006)
	Vegetables		2.3 – 13.7	6.5	
	Nuts		7.0 – 32.7	19.6	
Belgium (2001 – 2012)	Wheat grain	182		55	De Temmerman *et al.* (2014)
	Root- and tuber vegetables	18 – 121		28 – 43	
	Leafy vegetables	2 – 7		62 – 169	
	Vegetable fruits	3 – 4		12 – 53	
	Allium species	6 - 25		41 – 83	
	Brassica species	2 – 6		29 – 247	
Germany	Grass	304	11 – 123	45	Hartfiel and Bahners (1988)
13 European countries	Winter wheat plant	128	4 – 127	42	Wang and Sippola (1990)
Czech Republic	29 plant families of uncultivated meadows	2	Undetectable – 158	17 and 25	Száková *et al.* (2015)

Selenium content in plants varies among plant species. Based on their ability to take up and accumulate Se, plants are divided into three groups: (1) primary Se accumulator plants (*Astragalus, Morinda, Neptunia, Oonopsis, Xylorhiza*); (2) secondary Se accumulator plants (*Aster, Astragalus, Atriplex, Castilleja, Comandra, Grayia, Grindelia, Gutierrezia, Machaeranthera, Brassica*); and (3) non-accumulator plants (grasses or forage crops, cereals and oil-meal crops) (Surai *et al.*, 2008). When grown on seleniferous soils, primary Se accumulator plants can absorb > 1000 mg kg^{-1} Se, secondary Se accumulator plants can accumulate 50 to 100 mg kg^{-1} Se, and non-accumulator plants accumulate < 50 mg kg^{-1} Se (Fordyce, 2013). In general, Se content in plants ranges from 0.005 mg kg^{-1} in plants grown on Se deficient soils up to 5500 mg kg^{-1} in primary Se accumulator plants, however, the majority of plants contain < 10 mg Se kg^{-1} (Fordyce, 2013). Specifically, Se content in food crops and fodder crops from several countries in Europe ranges from undetectable up to 158 µg kg^{-1}, with an average mostly < 60 µg kg^{-1} (Table 2). In general, the Se contents in the food crops and fodder crops in these European countries are below the nutritional minimum amount of Se for humans and animals, i.e. 50 to 100 µg Se kg^{-1} dry weight of food or fodder (Gardiner and Gorman, 1963; Gissel-Nielsen *et al.*, 1984).

Metabolism and translocation of individual Se specie taken up from the soil are different in plants, which also influence the Se content in edible parts of the crops. Selenate is very mobile within plant tissue and easily transported from roots into shoots in the form of selenate, and accumulated as selenate in the accumulator plants (Zayed *et al.*, 1998) or converted and accumulated as selenomethionine in non-accumulator plants (Beilstein *et al.*, 1991; Stadlober *et al.*, 2001). Selenite and selenomethionine are converted directly into organic Se (i.e. selenomethionine-like compounds) inside the roots, and then transported (limitedly, especially for selenite) into shoots (Zayed *et al.*, 1998; Li *et al.*, 2008). In general, plants tend to accumulate Se mainly in shoots when supplied with selenate and mainly in roots when supplied with selenite or selenomethionine (Zayed *et al.*, 1998). Accumulator plants store Se in the form of non-protein bound Se, such as Semethylselenocysteine, γ-glutamyl-methylselenocysteine, selenocystathionine, selenate and selenite (Pickering *et al.*, 2003; Freeman *et al.*, 2006), whereas non-accumulator plants store Se in the form of protein bound-Se, such as selenomethionine (Beilstein *et al.*, 1991; Stadlober *et al.*, 2001). The accumulation of non-protein bound-Se allows the accumulator plants to become Se tolerant (Brown and Shrift, 1981). The accumulation of Se in the form of selenomethionine in the non-accumulator plants, such as cereals, increases the bioavailability of Se for human and animal intake because selenomethionine is easily absorbed by small intestine of humans and animals compared to inorganic selenite and selenate (Wanger, 1998; Stadlober *et al.*, 2001; Juniper *et al.*, 2006; Čobanová-Boldižárová *et al.*, 2008). Selenium in plants can also be converted into methylated compounds, such as dimethylselenide ($(CH_3)_2Se$), and is lost into atmosphere through volatilization (phyto-volatilization) (Hawkesford and Zhao, 2007). The study of Zayed *et al.* (1998) indicated that the rate of Se volatilization from plants follows the order of selenomethionine > selenite > selenate, in which the volatilization via roots is much higher than that via shoots.

1.4 Selenium bioavailability in soils and the influencing factors

Instead of being determined by the total soil Se per se, the amount of Se in plants, and consequently in animals and humans, depends on the bioavailability of Se in soils. For example, the low Se soils (Se content of 0.20 to 0.86 mg kg^{-1}, with an average of 0.38 mg kg^{-1}) in the semi-arid Mediterranean of Central Spain result in high Se content in plants, i.e. 0.06 to 14.3 mg kg^{-1}, in which the Se content is mostly > 1.0 mg kg^{-1} (Rodriguez *et al.*, 2005). These soils have apparently high bioavailability of selenate, which is readily available for plant uptake, due to the well aerated alkaline soils (pH 7.1 to 8.7) (Rodriguez *et al.*, 2005).

The directly bioavailable Se is present in soil solution as a fraction of total soluble Se, in the forms of soluble inorganic selenate or selenite and soluble organic Se, such as selenomethionine and selenocystine (Abrams *et al.*, 1990b; Williams and

Mayland, 1992; Zayed *et al.*, 1998; Hopper and Parker, 1999; Li *et al.*, 2008; Kikkert and Berkelaar, 2013). Both selenate and selenite can bind to soil mineral surfaces, and can be incorporated into soil organic matter upon reduction. However, selenate is more mobile in soils and readily available for plant uptake than selenite due to a weaker adsorption, especially on soil mineral surfaces (Fig. 2). The presence of seleno-amino acids in soils, such as selenomethionine and selenocystine, has been detected, however, other forms of soluble organic Se in soil solution that are directly available for plant uptake are not well understood. The studies of Zayed *et al.* (1998) and Kikkert and Berkelaar (2013) indicated that the bioavailability and plant uptake of Se species in culture solutions follow the order of selenate > selenomethionine > selenocystine > selenite.

The amount and speciation of the bioavailable Se in soil solution is influenced by soil pH, redox condition, clay content, metal oxide content and organic matter content, and the availability of competitive anions, i.e. sulphate, phosphate and organic acid anions (Mikkelsen *et al.*, 1989; Wijnja and Schulthess, 2000; Øgaard *et al.*, 2006; Fordyce, 2013). Soil pH and redox condition determine the major chemical species of Se present in soil solution (Fig. 2) as described in Section 1.2.2. In contrast to selenate (Se^{+6}) and selenite (Se^{+4}), elemental Se (Se^0) and selenides (Se^{2-}) are insoluble and unavailable for plant uptake (Fig. 2). Selenium bioavailability in soils increases in general with the increase of soil pH, due to a weaker adsorption of selenate and selenite to soil minerals, and a faster mineralization of organic Se. On the other hand, bioavailability of Se also increases with the increase of redox potential or oxidizing condition. An oxidizing condition can firstly prevent reduction of Se to form insoluble elemental Se or selenides, or incorporation of Se into organic matter, and secondly an oxidizing condition favours mineralization of soil organic matter, releasing bioavailable organic and inorganic Se. In relation to soil mineralogy and organic matter content, the bioavailability of Se is in general decreased with an increase of clay content, organic matter content (Bisbjerg and Gissel-Nielsen, 1969; Gissel-Nielsen, 1971; Yläranta, 1983; Johnsson, 1991) and Fe-(hydr)oxide content, due to adsorption or immobilization of Se by those soil minerals and organic matter (Gissel-Nielsen and Hamdy, 1977; Hamdy and Gissel-Nielsen, 1977; Balistrieri and Chao, 1990; Gustafsson and Johnsson, 1992). In any case, it can be expected that the amount of soluble selenomethionine, selenocystine and other possible bioavailable organic Se species is influenced by factors affecting soil organic matter mineralization, such as pH and soil moisture.

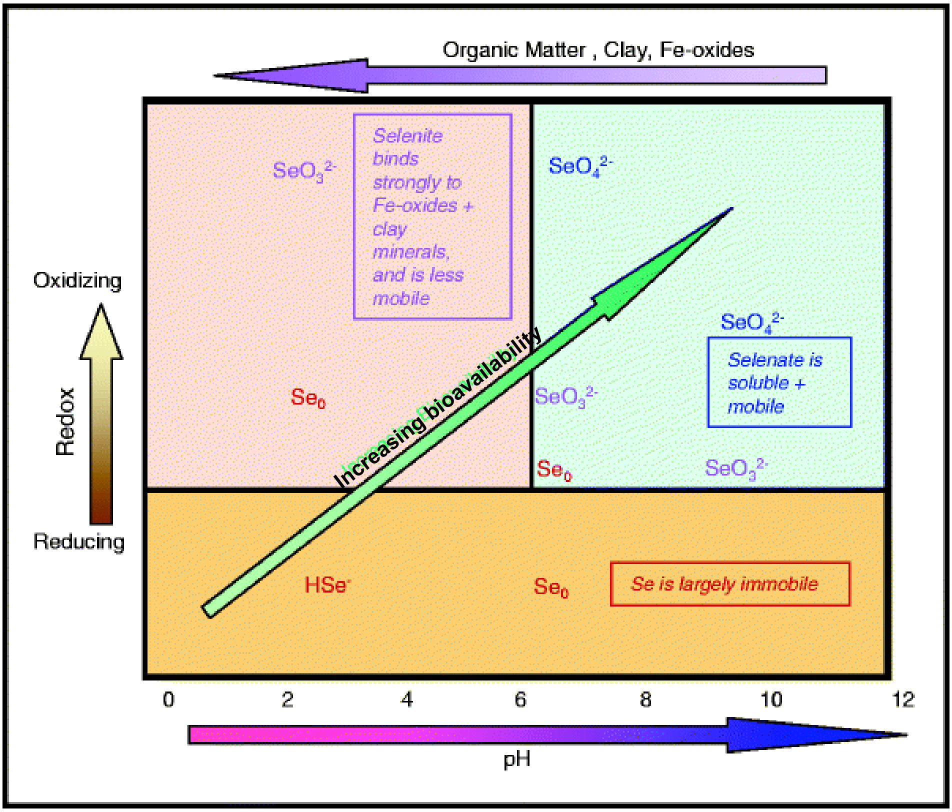

Fig. 2. Influence of soil pH, redox condition, clay, organic matter, and Fe-(hydr)oxides on Se speciation and bioavailability in soil. Source: Fordyce (2013).

In addition to soil properties, the concentration of oxyanions in soils, i.e. sulphate and phosphate, and organic anions, such as oxalate and citrate, influences bioavailability of Se via competition for adsorption on soil mineral surfaces and plant uptake. It has been found that addition of phosphate into soils increases Se plant uptake, due to an increase of competition between phosphate and Se (mainly selenite) for the adsorption on soil mineral surfaces, releasing the adsorbed Se into soil solution becoming available for plant uptake (Carter *et al.*, 1972; Liu *et al.*, 2004; Eich-Greatorex *et al.*, 2010). Sulphate competes stronger with selenate than with selenite for plant uptake, and the competition between sulphate and selenate is stronger than that between phosphate and selenite (Hopper and Parker, 1999; Cartes *et al.*, 2006). When the plant contains sufficient S, application of sulphate increases Se plant uptake, whereas a decrease of Se uptake happens when the plant is in S deficient condition (Stroud *et al.*, 2010b). The study of Adam *et al.* (2002) also indicated a decrease of Se content in wheat grain with an increase of soil sulphate (as gypsum) fertilization, and the S content in grain showed a negative effect on Se content. On the other hand, the presence of organic acids in soils increased Se uptake in wheat grain, especially at pH > 6.0 (Øgaard *et al.*, 2006), due to a decrease of Se (selenate and selenite) adsorption on soil mineral surfaces in the presence of organic acids, releasing the adsorbed Se into soil solution (Dynes and Huang, 1997; Wijnja and Schulthess, 2000; Øgaard *et al.*, 2006). The competitive effects of organic acids on Se adsorption on soil mineral surfaces depend on concentration and structure of the organic acids and the stability constant of oxide-organic acid complexes (Dynes and Huang, 1997).

In addition to soil factors per se, the external factor of fertilizer applications determines the amount of Se uptake in plants either via competition for adsorption in soils and for plant uptake between Se and relevant anions released from fertilizer or via the dilution effect from the increase of plant yield (Gissel-Nielsen *et al.*, 1984). Soil application of phosphate fertilizer tends to increase Se plant uptake, whereas application of sulphate fertilizer can increase or decrease Se uptake (see above). On the other hand, application of mineral N fertilizer tends to decrease Se plant uptake, due to the dilution effect from the increase of plant yield (Gissel-Nielsen (1974) *in* Gissel-Nielsen *et al.*(1984*)*) and (Gissel-Nielsen, 1975).

1.5 Soil testing methods to determine Se bioavailability in soils

Soil extractions using water or weak electrolyte solutions, in combination with chemical speciation analyses (see Section 1.2.2 for the speciation techniques), have been employed to determine the level of bioavailable Se in soils (soil testing). The study of Weng *et al.* (2011) has shown that Se associated with small dissolved organic matter (DOM) and soluble S in 0.01 M $CaCl_2$ extraction can describe most of the variability of Se uptake in grass grown on low Se grassland soils with predominantly organic Se. Moreover, the study of Stroud *et al.* (2010a) showed that total soil Se in combination with soluble S and Se in 0.016 M KH_2PO_4 soil extraction describe most of Se uptake in wheat grain grown on low Se soils with up to 70% of soluble Se as inorganic selenite. Similarly, extractable Se in 0.1 M KH_2PO_4 is highly correlated to Se uptake in tea plants grown on seleniferous soils (Zhao *et al.*, 2005), which may be dominated by inorganic Se. These studies indicate that different soil testing methods used result in different parameters related to bioavailability of Se for plant uptake, reflecting factors influencing Se bioavailability in soils for plant uptake.

The variability of the results of soil testing can be influenced by many factors including the range of soil properties, soil sample pre-treatment, extraction method employed to the soils and the status of other elements in the corresponding soil extraction. In addition to correlating the amount of the element with plant uptake, inclusion of soil properties that influence the availability of the element in soils, such as pH and (dissolved) organic matter content, can increase the prediction of bioavailability of the elements for plant uptake (McLaughlin *et al.*, 2000). Soil sample pre-treatment applied before soil extractions, such as soil drying (moist soils vs. dried soils) and soil storage (low temperature vs. room temperature), influences the solubility of Se and possibly the chemical forms of Se in the soil samples due to the change of oxidation condition of the samples (Martens and Suarez, 1997). Drying the soil samples at high temperature can increase the solubility of Se either via organic matter decomposition (for soils with predominantly organic Se) or via oxidation of Se (change of organic Se or elemental Se^0 or selenite into selenate) (Martens and Suarez, 1997; Zhang and Moore, 1997). Moreover, extraction methods using certain extraction solutions (water vs. electrolyte solutions) result in different amount and predominant species extracted from the soils, depending on soil properties (Ros *et al.*, 2014). Therefore, the applicability of soil extractions (soil testing) to mimic bioavailable Se for crop uptake often shows inconsistent results (Ros *et al.*, 2014). Considering all of these influencing factors, validation of soil testing methods on a large number of soil types and plants is necessary.

1.6 Selenium fertilization: a strategy to increase Se content in crops

In general, Se content in non-accumulator crops, such as cereals and grasses, are low, mainly due to low availability of Se in soils. Strategies to increase Se content in food crops and fodder crops, including Se fertilization and plant genetic improvement, have been proposed (Broadley *et al.*, 2006; Hawkesford and Zhao, 2007; Zhu *et al.*, 2009). Selenium fertilization either via soil application or foliar application is a short term (temporary) solution to increase Se content in crops, whereas selection or breeding of crop varieties with enhanced Se accumulation characteristics is an option for a longer term solution (Broadley *et al.*, 2006; Hawkesford and Zhao, 2007). Selenium (as selenate) fertilization on agricultural fields has been applied in some countries with low Se content in their soils and agricultural products, such as Finland and New Zealand (Watkinson, 1983; Varo *et al.*, 1988; Yläranta, 1990), whereas the possible plant genetic improvement to increase Se uptake and accumulation in crops is still under research.

In general, research on Se fertilization, especially via soil application, has been performed intensively both on field scale and pot experimental scale. However, the application of Se fertilizer in agricultural fields, especially in low Se soils, has not been adopted widely. Selenium (as selenite) supplementation via Se pills or feeds or injection is a common way to provide adequate Se intake by animals (Watkinson, 1983; Andrieu and Wilde, 2008). These techniques are convenient for a small number of animals, but less effective for a large number of animals (Watkinson, 1983). Therefore, application of the minimum amount of Se fertilizer annually on grazed pastures can be an option to prevent Se deficiency in animals (Watkinson, 1983). Moreover, Se fertilization can correct soil Se deficiency for a certain period of time (Watkinson, 1983). However, Se fertilization on agricultural fields needs to be considered carefully. For example, in UK Se fertilization on grasslands is not favoured due to a lack of knowledge about Se fertilization recommendations, the risks of toxicity in crops, the risks for contamination of surface waters and ground waters, and temporarily-lived benefit (i.e. Se concentrations in crops decline with time) (Rimmer *et al.*, 1990). It is important to perform field experiments on different cropping systems and climate conditions to derive an optimum and reliable Se application rate (Hawkesford and Zhao, 2007).

On the other hand, Se fertilization (as multi-nutrient NPK fertilizers enriched with selenate) has been applied successfully on agricultural fields in Finland since 1984 to increase Se content in foods and Se intake in animals and humans (Yläranta, 1990). Selenium fertilization (as selenate prills or superphosphate fertilizers enriched with selenate) has also been practiced on pastures in New Zealand to overcome Se deficiency in animals (Watkinson, 1983). In both countries, a soil application of ≤ 10 g Se ha^{-1} has been applied annually (Watkinson, 1983; Yläranta, 1990; Hartikainen, 2005). In Finland, the effects of Se application rates are monitored regularly by analysing Se content in agricultural soils, plants, waters, plant and animal food products, feeds and human sera (Hartikainen, 2005). The results of this monitoring are used to adjust Se application rates when it is necessary to avoid Se toxicity in crops, forages, surface waters and ground waters (Hartikainen, 2005).

In addition to soil application, foliar application of Se fertilizer (as selenate or selenite) can be an alternative to avoid Se loss in soils due to immobilization and leaching. However, there are several challenges of foliar application under field conditions: Se uptake by plants depends on spraying conditions, stage of plant growth, and climate conditions during and after spraying; difficulties to apply an accurate dosage of a small amount of Se on leaves; and extra cost for operation of foliar application (Aspila, 2005). In general, a smaller application rate of Se is used in foliar application than that of soil application to reach similar Se concentrations in plants (Gissel-Nielsen *et al.*, 1984). On the other hand, soil application of Se fertilizer is a rather safe method because Se is mixed into the commercial macronutrient fertilizers and the level of Se applied to the soils is well controlled (Aspila, 2005).

The effectiveness of Se fertilization to increase Se content in plants depends on Se species used, fertilizer dose, application techniques (foliar vs. soil application, see above), timing, agronomic management options and properties of agro-ecosystem (Ros *et al.*, 2014). In general, soil or foliar fertilization with selenate (mixed into commercial fertilizers) is more effective to increase Se content in plant than that of selenite because selenate is more readily available for plant uptake than selenite (see Section 1.3 and 1.4). However, the residual effect of selenate application decreases more rapid than that of selenite over time, depending on soil properties, fertilizer management and climatic condition (Ros *et al.*, 2014). On the other hand, soil application of organic Se in the forms of Se-yeast and Se-methionine at similar concentration as inorganic selenate results in hardly any effects on Se uptake in wheat, possibly due to that the organic Se was immobilized by the soil microorganisms before the plant root took it up (Eich-Greatorex *et al.*, 2007). It is likely that a higher dose of organic Se than that of inorganic Se is necessary to achieve the same Se plant uptake (Eich-Greatorex *et al.*, 2007). In general, an increase of Se content in plants is linear to the amount of Se fertilizer applied due to a small effect of Se application on crop yields (Ros *et al.*, 2014). Previous studies suggested that the recommendation of Se (as selenate) fertilization for grassland and arable land systems is 3 up to 10 g ha^{-1} (Watkinson, 1983; Yläranta, 1990; Hartikainen, 2005; Stroud *et al.*, 2010a; Ros *et al.*, 2014). In relation to timing, use of slow or fast release of Se fertilizer following the crop growth can be an option in order to minimize the loss in the regions with high environmental risk (Ros *et al.*, 2014). In relation to agronomic management, applications of inorganic fertilizers (sulphate, phosphate and nitrogen fertilization, see Section 1.4) and organic fertilizers (manure, crop residue), liming, soil compaction, irrigation, and formation of waterlogged condition (on paddy soils) can all influence the plant uptake of Se from fertilizer (Ros *et al.*, 2014). Organic fertilization can either decrease or increase Se uptake from Se fertilizer applied, depending on soil pH, soil microbial activity (to reduce Se), and the competition for the adsorption on soil mineral surfaces between organic anions from organic fertilizer and Se from Se fertilizer (see Section 1.4) (Ajwa *et al.*, 1998; Øgaard *et al.*, 2006; Funwie, 2012). Application of lime in the form of $CaCO_3$ increased Se plant uptake in the soils applied with selenite (de la Luz Mora *et al.*, 2008), whereas application of lime in the form of gypsum ($CaSO_4$) decreased Se uptake in plants with selenate application due to the increase of S uptake, especially in S-deficient crops (Dhillon and Dhillon, 1991, 1997; Adams *et al.*, 2002; Stroud *et al.*, 2010b). Both soil compaction and irrigation decreased Se uptake in wheat grains, in which soil compaction impedes root growth and restricts nutrient (including Se) uptake, whereas irrigation leads to increased grain yield, therefore dilution of Se in grains, competition between sulphate from the irrigation water and Se, and leaching of Se (Zhao *et al.*, 2007). In relation to agro-ecosystem properties, the effectiveness of Se fertilization is strongly affected by local agro-ecosystem properties, such as climate and bioavailable Se pools in soil, but only slightly affected by soil properties, such as acidity and soil organic matter content, and total Se content in soil (Ros *et al.*, 2016).

1.7 Objectives and outline of the thesis

The general objective of this PhD thesis is to study the speciation, bioavailability and plant uptake of Se and their controlling factors and mechanisms in Dutch agricultural soils (both grassland and arable land). Moreover, the effects of fertilization methods (i.e. NPK, NPK plus cattle slurry, and selenate fertilization) on bioavailability and plant uptake of Se are studied. Specifically, the objectives of this thesis are:

i. To understand the effects of soil sample pre-treatment (i.e. drying and rewetting) on soluble Se concentration and speciation in 0.01 M $CaCl_2$ extraction, and the mechanisms leading to the deviation between moist and dried samples (Chapter 2). A 0.01 M $CaCl_2$ extraction (soil to solution ratio 1:10) has been proposed to estimate total soluble elements in soil solution and to evaluate bioavailability of the elements in soils (Houba *et al.*, 2000).

ii. To obtain a detailed understanding of Se speciation in Dutch agricultural soils and the key influencing factors (Chapter 3).

iii. To obtain more insight of the amount, chemical forms and pools of Se extracted in several commonly used single step extractions (Chapter 3).

iv. To identify the major soil parameters that control bioavailability and plant uptake of Se under controlled conditions and field conditions for Dutch agricultural soils with a relatively low Se content and predominantly organic Se, and to explore the mechanisms that control the bioavailability and plant uptake of Se in the soils (Chapter 4 and 5).

v. To study the effect of NPK fertilization on Se plant uptake and to identify the major soil property that influences the uptake under NPK fertilization (Chapter 5).

vi. To study the effects of Se (as selenate) fertilization on Se plant uptake on soils applied with N plus cattle slurry or NPK (Chapter 6).

To reach the objectives mentioned above, the approaches that are used in each chapter of this thesis are described below.

In Chapter 2, soluble Se concentrations in fresh-moist soil samples and (40^oC) oven-dried soil samples were measured to study the effects of soil drying and rewetting on soluble Se concentrations in 0.01 M $CaCl_2$ extraction. To understand the mechanisms and factors leading to the changes of Se concentrations upon drying, Se speciation in 0.01 M $CaCl_2$ extraction both in moist samples and dried samples was analysed using an anion exchange Donnan Membrane Technique

(DMT). In addition, dissolved organic C (DOC) concentrations in the corresponding moist and dried samples were also measured. Moreover, fractionation of dissolved organic matter (DOM) and Se was conducted using DAX-8 resin to investigate the relationship between Se and DOC in each fraction of DOM.

In Chapter 3, three single step extractions, i.e. ammonium oxalate – oxalic acid extraction (pH 3), 1 M NaOCl (pH 8) oxidation-extraction and aqua regia digestion, were performed to determine Se speciation in soils (i.e. inorganic, organic and residual Se). The inorganic Se content in the soils was determined by analysing Se species in ammonium oxalate soil extracts using a High Performance Liquid Chromatography (HPLC) system with an anion exchange column coupled online with High Resolution ICP-MS. The organic Se content in the soils was determined using 1 M NaOCl (pH 8) oxidation-extraction. Total Se content in the soils was measured using aqua regia digestion, and the residual Se was calculated as the difference between total Se content and the sum of inorganic and organic Se. The fractions of inorganic Se, organic Se and residual Se relative to total Se content were calculated. In addition, seven single step extractions, i.e. 0.01 M $CaCl_2$, 0.43 M HNO_3, hot water, ammonium oxalate – oxalic acid (pH 3), 0.1 M NaOH, 1 M NaOCl (pH 8) and aqua regia, were conducted to study different Se pools and their speciation and extractability in soils. Moreover, fractionation of soil organic matter and Se in 0.1 M NaOH extraction was conducted to investigate the distribution of Se among organic matter fractions.

In Chapter 4, a pot experiment using nineteen soil samples taken from different fields of arable land (potato fields) was conducted on summer wheat (*Triticum aestivum* L.). The soil samples received mineral NPK fertilizers, which have been applied on the fields before soil sampling. No Se fertilizer was applied to the soils. Selenium in wheat shoots and soil parameters, including basic soil properties, C:N ratio, inorganic selenite content, and Se and organic C in different soil extractions (0.01 M $CaCl_2$, 0.43 M HNO_3, hot water, ammonium oxalate, aqua regia) were analysed. Regression analysis was performed to identify soil parameters that are related to Se uptake in wheat shoots grown under controlled conditions. In addition, the mechanisms and factors that control the bioavailability and plant uptake were investigated.

In Chapter 5, two field experiments without and with NPK application were conducted in 2011 and 2012, respectively. The field experiment in 2011 was conducted on 21 grasslands in order to identify the soil parameters that determine Se bioavailability and uptake in grass grown under field conditions, for low Se soils with predominantly organic Se. The plots did not receive any mineral NPK and Se fertilizers and manure during that season, except for 3 plots that received mineral N fertilizer. Regression analysis was performed to identify soil parameters that determine Se uptake in grass. The field experiment in 2012 was conducted on 15 grasslands located at the same fields as used in the field experiment 2011. The experiment was aimed to study the effect of NPK fertilization on Se uptake in grass and to identify the major soil properties that influence the Se uptake reaction to NPK fertilization. Each plot received similar amounts of mineral NPK fertilizers. No Se fertilizer was applied to the fields. To evaluate the effect of NPK fertilization on Se uptake in grass, Se uptake in plots without NPK application was compared with that in plots with NPK application from the corresponding fields in 2011 and 2012.

In Chapter 6, two field experiments with Se fertilization (as mineral N fertilizer enriched with sodium selenate) were conducted in 2007 and 2012. In 2007, the field experiments were conducted on 20 grasslands consisting of different soil types. Each grassland field consisted of a plot with mineral N plus cattle slurry applications (control plot) and a plot with Se plus N plus cattle slurry applications (Se plot). The dosages of Se, mineral N, and cattle slurry varied among the fields following the scheme of individual farmers. In 2012, the field experiments were performed on 26 grasslands with different soil types. Each grassland field consisted of a plot with mineral NPK application (control plot) and a plot with Se plus NPK applications (Se plot). Similar amounts of Se, N, P and K fertilizers were applied on the corresponding plots. To evaluate the effect of Se fertilization on Se uptake in grass, Se uptake in plots without Se application (control plots) was compared with that in plots with Se application from the corresponding plots (Se plots).

In Chapter 7, the major findings in each chapter of this thesis are discussed in a broader and more general context.

Chapter 2

Drying Effects on Selenium and Copper in 0.01 M Calcium Chloride Soil Extractions

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Published as 'Drying effects on selenium and copper in 0.01 M calcium chloride soil extractions' in Geoderma, 2015, 255-256: 104-114

Abstract

The study aimed to understand the effects of drying and rewetting the soils on soluble selenium (Se) and copper (Cu) concentrations in 0.01 M $CaCl_2$ soil extraction, and the mechanisms leading to these changes. Soluble Se and Cu concentrations were measured in the extractions of 58 soil samples taken from grassland and arable land in the Netherlands before and after oven drying at 40^{o}C. Selenium speciation and dissolved organic matter (DOM) fractionation were performed using Donnan Membrane Technique (DMT) and DAX-8 resin. Drying and rewetting the soils increased soluble Se and Cu concentrations in $CaCl_2$ extraction. The increase of both elements was associated with the increase of dissolved organic carbon (DOC). Compared to the magnitude of DOC increase (1.3 to 11 fold), the increase of soluble Se (1.3 to 34 fold) was more pronounced, whereas the increase of soluble Cu (1.1 to 4.6 fold) was less strong. The DMT analysis confirmed the dominance of colloidal sized DOM-associated Se both in the moist and dried soil extractions, showing that the colloidal sized organic Se is mainly responsible for the increase of total soluble Se upon drying. The Se to DOC ratio increased upon drying in most of the soils, especially in grassland soils and maize field soils, indicating enrichment of Se in DOM after drying. No consistent difference in Se to DOC ratio was found between the fulvic acids (FA) and hydrophilic (Hy) fraction of DOM after drying. Fractionation of DOM showed that Hy and FA fraction accounted for respectively 74% and 26% of total DOC in the dried soils, which corresponds to the Se fraction in Hy and FA of DOM. The decrease of SUVA (0.3 to 1.0 fold) after drying implied a decrease of FA:Hy ratio in DOM and a decrease of average DOM aromaticity. Upon drying, a positive correlation between the change of Cu loading on DOC (Cu:DOC ratio) and the change of aromaticity (SUVA) of DOM was found, showing that the increase of Cu complexed with (mainly) aromatic DOM explains soluble Cu increase upon drying. The decrease of Cu loading on DOC upon drying was attributed to a decrease of the aromaticity and affinity to Cu of the DOM. Colloidal sized organic Se and Cu-DOM complex are the major species of Se and Cu, respectively, in the extractions of moist and dried soils, which may not be directly available for plant uptake.

2.1 Introduction

Selenium (Se) and copper (Cu) are essential micronutrients for animals and humans and play important roles in physiological and metabolic processes of cell systems. Therefore, an adequate level of Se and Cu in fodder, crops, or animal products, such as beef and milk, are necessary to sustain animal and human requirements on Se and Cu intake. In dairy farming systems, Se and Cu are usually added in the feed in order to increase the intake by dairy cattle. Application of Se or Cu fertilizers to grass or other fodder crops is another way to increase the intake of Se and Cu by dairy cattle (through grass and fodder). However, an accurate Se or Cu fertilization recommendation based on soil analysis is essential because the discrepancy between deficiency and toxicity levels of these micronutrients in soil is relatively small. For example, in China, the total Se content in soil for Se deficiency level is < 0.125 mg kg^{-1}, whereas the Se excess level is > 3 mg kg^{-1} (Tan *et al.*, 2002). In addition, not the total amount of Se and Cu, but the available amount in soils, is the key factor determining the amount of Se or Cu entering a food chain (crops, animals and humans). The species of Se and Cu in soil solution that can be directly taken up by plants is mainly present as inorganic ions, i.e. SeO_4^{2-} (selenate), $HSeO_3^-$ (selenite) and Cu^{2+}, respectively.

Chemical extraction is a common approach to estimate the bioavailable level of elements in soils. The extractions are usually performed on air-dried or 40°C oven-dried soils using water or various chemical solutions containing mild salt, chelate, acid or base. The reasons to work with dried soils are (1) it makes the methodology more standardized and the results for different soils more comparable and (2) the dried soils are easier to handle and can be stored for a longer time without too much change in the soil properties compared to the moist soils. However, drying the soils may lead to changes in their elemental status and properties, thus it raises the question if the results obtained on the dried samples are representative to field conditions and can be used to determine fertilizer recommendations. Van Erp *et al.* (2001) suggested that a decision to use moist or dried soils for a soil testing to evaluate the bioavailability of an element in the field should be deduced from the relationship between the concentrations of the element extracted from the moist or dried soils and crop response. However, for Se and Cu, it has been found that total concentration of soluble element extracted from dried soil is poorly correlated to plant uptake (Jarvis and Whitehead, 1981; Wang and Sippola, 1990). Taking into account the changes of the concentration and chemical forms (speciation) of the soluble element upon drying the soils may help us to understand the relevance of soluble Se and Cu concentration measured in dried soils for plant uptake.

Several studies have been carried out to understand the effects of drying on total soluble concentrations of micronutrients (Cu, Se, Zn, Fe, Mn) in soils or sediments using water or mild salt extractions (Bartlett and James, 1980; Haynes and Swift, 1991; Zhang and Moore, 1997; Tack *et al.*, 2006; Klitzke and Lang, 2007; Claff *et al.*, 2010; Koopmans and Groenenberg, 2011). Some of these studies showed that drying the samples increased total soluble Cu in soils (Haynes and Swift, 1991; Tack *et al.*, 2006; Klitzke and Lang, 2007; Claff *et al.*, 2010; Koopmans and Groenenberg, 2011). It has been concluded that the increase of total soluble Cu after drying is related to the increase of solubility of organic matter (Tack *et al.*, 2006; Klitzke and Lang, 2007; Koopmans and Groenenberg, 2011), hence, to the increase of soluble Cu-DOM (dissolved organic matter) complexes (Klitzke and Lang, 2007; Koopmans and Groenenberg, 2011); and not related to the changes of the degree of hydration and crystallization of oxides in soils (Tack *et al.*, 2006). Compared to those on Cu, studies about comparison between total soluble Se in moist and dried soil samples are still rare. Zhang and Moore (1997) found that soluble Se in sediments increased upon drying. The increase of total soluble Se in sediment parallels with the increase of drying temperature, which has been attributed to the release of organic Se from organic matter decomposition and Se oxidation into selenate (Zhang and Moore, 1997).

A 0.01 M calcium chloride ($CaCl_2$) extraction (soil to solution ratio 1:10) is a standard method to determine total soluble elements in soil solution and to evaluate bioavailability of the elements in soils (Houba *et al.*, 2000). The objective of this study is to understand the effects of drying and rewetting of soil samples on soluble Se and Cu concentrations measured in 0.01 M $CaCl_2$ extraction, and the mechanisms leading to the deviation between the moist samples and samples dried at 40°C under forced air ventilation. These results can help us to better understand the relevance of soluble Se and Cu measured in the extraction using dried soils to bioavailability assessment.

2.2 Materials and methods

2.2.1 Soil samples

Soil samples were taken from 27 grassland fields (top soil 0 to 10 cm), 15 maize fields (top soil 0 to 20 cm) and 16 potato fields (top soil 0 to 20 cm) in the Netherlands. The soil samples from grassland and maize field were taken in Spring 2011 and the soil samples from potato field were taken in Spring 2013. The soil samples consisted of sandy, clay, clay-peat and peat soils (Table 1). After removing the plant residuals and gravels, sieving with 5 mm sieve and homogenization, part of the soil samples was stored in the cool room at 2 to 4°C for further analyses (moist samples). Before analyses, soil moisture content of the moist soils was measured by drying subsamples of the soils at 105°C for 48 h. Soon after the samples were taken from the field, a part of the moist samples was oven-dried at 40°C under forced air ventilation for 24 to 48 h, ground, and sieved with 2 mm sieve for further analyses (dried samples). Little amount of particles between 5 mm and 2 mm (plant residues, large soil particles) was removed when sieving the dried soils over 2 mm sieve and this effect on DOC, Se and Cu extracted in 0.01 M $CaCl_2$ is expected to be small. The dried soil samples were stored under room temperature. Basic soil properties and total Se and Cu contents (aqua regia) of the soils were measured using dried soil samples (Table 1 and 2).

Table 1. Basic soil properties (mean ± standard deviation).

Soil type	pH[a]	Organic matter %	Clay %	CEC[b] mmol(+) kg^{-1}
Grassland (n = 27)				
Sand (n = 13)	5.6 ± 0.9	6.4 ± 2.7	3.0 ± 2.1	73.2 ± 26.2
Clay (n = 7)	6.7 ± 0.7	6.3 ± 3.7	20.0 ± 10.0	198.9 ± 93.2
Clay-peat (n = 6)	5.2 ± 0.3	31.0 ± 17.3	44.8 ± 14.6	521.3 ± 108.9
Peat (n = 1)	5.1	25.5	6.3	247.0
Maize field (n = 15)				
Sand (n = 5)	5.1 ± 0.3	7.7 ± 2.2	2.8 ± 0.9	84.4 ± 41.3
Clay (n = 3)	6.5 ± 0.9	5.9 ± 2.0	34.7 ± 10.2	264.7 ± 49.1
Clay-peat (n = 6)	5.2 ± 0.7	25.2 ± 12.5	26.2 ± 11.5	343.8 ± 135.4
Peat (n = 1)	4.7	26.5	10.7	214.0
Potato field (n = 16)				
Sand (n = 5)	5.5 ± 1.0	6.2 ± 4.9	2.8 ± 1.5	85.6 ± 65.2
Clay (n = 11)	6.8 ± 0.8	3.0 ± 1.4	20.5 ± 7.0	161.2 ± 63.2

[a] Measured in 0.01 M $CaCl_2$ extraction at soil to solution ratio 1 : 10.

[b] Cation exchange capacity.

2.2.2 0.01 M $CaCl_2$ extraction

On both the moist and dried soil samples, a 0.01 M $CaCl_2$ extraction (soil to solution ratio 1:10) was performed using the procedure proposed by Houba *et al.* (2000). The time span between the field sampling and the 0.01 M $CaCl_2$ extraction is 11 days for moist soil samples and 11 to 19 days for dried soil samples. Extraction of dried soils was conducted by weighing 3.0 g dried soils into 50 mL plastic bottles and adding 30 mL 0.01 M $CaCl_2$ solution. Extraction of moist soils was performed by weighing the moist soil samples that are equivalent to 3.0 g dry weight, after corrections for soil moisture contents, into 50 mL plastic bottles. Subsequently, 15 mL 0.02 M $CaCl_2$ solution was added to each soil sample. Thereafter appropriate amount of ultrapure water (UPW) was added to top to a final volume of 30 mL and a final concentration of 0.01 M $CaCl_2$, taking into account of soil moisture present. Soil suspensions were shaken using reciprocating horizontal shaker for 2 h at constant temperature of 20 ± 1°C. pH of the suspensions was measured and the suspensions were centrifuged at 3000 rpm for 10 minutes and filtered with a 0.45 μm pore-size syringe cellulose acetate filter (Whatman Aqua 30/0.45 CA). Total soluble Se and Cu concentrations in the filtrate were measured after acidifying the filtrate to a final concentration of 0.14 M HNO_3. Concentration of dissolved organic C (DOC) in the filtrate was also measured. The concentration of DOC in the filtrate was corrected using the DOC concentration measured in blank (no soil added) of 0.01 M $CaCl_2$ extraction (due to small amount of DOC released from bottles and filter membranes). All the solutions used in this study were prepared using UPW with an electric conductivity resistance of 18.2 mΩ.

Since Se concentrations measured in the samples are relatively low, to control the quality of the data, the detection and determination limits were determined from the blanks (no soil added) of 0.01 M $CaCl_2$ extraction in each batch of the extractions. The detection limits were calculated as 3 times of the standard deviations of measurements of the blanks of each batch, whereas the determination limits were calculated as 3.3 times of the detection limits. The detection limits thus derived are 0.01–0.06 µg L^{-1} for Se, 0.04–0.18 µg L^{-1} for Cu and 0.15–0.81 mg L^{-1} for DOC, whereas the determination limits are 0.05–0.21 µg L^{-1} for Se, 0.14–0.62 µg L^{-1} for Cu and 0.50–2.70 mg L^{-1} for DOC. Concentrations of Se, Cu and DOC in the soil extractions were compared to the detection and determination limits of the same batch to determine the reliability of these data.

To evaluate changes in dissolved organic matter (DOM) quality or composition due to the drying, ultraviolet (UV) absorbance in the 0.01 M $CaCl_2$ extractions of the moist and dried soils was measured at 254 nm wavelength. Specific UV absorbance (SUVA) of the DOM in the samples, which was used as an estimation of aromaticity of DOM (Weishaar *et al.*, 2003), was calculated using the following equation (Amery *et al.*, 2008):

$$\mathrm{SUVA} = A_{254} * 1000/[\mathrm{DOC}]$$

where *SUVA* is the specific UV absorbance of DOM (L g^{-1} cm^{-1}); A_{254} is the total UV absorbance at 254 nm (cm^{-1}); and *[DOC]* is DOC concentration (mg L^{-1}). Absorbance contributions from soluble Fe^{3+} and NO_3^- were ignored because the concentrations of both compounds were very low in the soil extractions. The concentrations of Fe^{3+} were ≤ 0.5 mg L^{-1} (Table A.1), which would have added only

0 to 0.04 cm^{-1} to the UV absorbance at 254 nm (Weishaar *et al.*, 2003). The concentrations of NO_3^- measured in several moist and dried soil samples selected from the sample set were < 40 mg L^{-1} (Table A.2). Weishaar *et al.* (2003) suggested that NO_3^- absorbance is not measurable at 254 nm if the concentration is < 40 mg L^{-1}.

Since the soil samples were taken from different land uses, their results are presented separately. Differences in land uses may lead to, among others, differences in the quality of soil organic matter and its vulnerability to disturbances due to soil tillage. The drying effects are expressed as ratios of the soluble concentration in dried to that in moist soils for each sample. The average of the ratios was then calculated for each soil type from grassland, maize field, and potato field, respectively. About 5 out of 58 samples were not included in the comparison for Se because their moist samples contained Se concentrations that were below the detection limit. Two samples were not included in the comparison for DOC because DOC concentrations in their moist samples were below the determination limit.

2.2.3 Size fractionation of Se and DOM using Donnan Membrane Technique (DMT)

The anion exchange DMT was used to separate the colloidal-sized organic Se (1 nm–0.45 µm) from the inorganic Se ions (selenate, selenite) and very small organic Se molecules (< 1 nm). Procedure of DMT analysis used was the same as that used by Vega *et al.* (2010). Before use, the positively charged anion exchange membranes (BDH 55164 2S) were cleaned using the following steps: 0.1 M HCl (2 times); UPW (2 times); concentrated acceptor solution (0.5 M $CaCl_2$) (2 times); and background or acceptor solution (0.01 M $CaCl_2$) (3 times). Each step was done by shaking the membranes in the corresponding solution for at least one hour. All the pump tubes of the DMT system were cleaned successively with 0.1 M HNO_3, UPW, 0.01 M $CaCl_2$, and UPW. Each step lasted for a few hours. The other instruments (i.e. DMT cells, glassware, and bottles) were cleaned with 0.1 M HNO_3 (2 times) and UPW (2 times) and dried in air. Every cleaning step was done for at least a few hours.

Three dried soil samples from grassland (one sand, one clay and one clay-peat soil) chosen from the sample set were analysed using DMT. The moist soil sample corresponding to the chosen clay-peat soil was also analysed using DMT, whereas the moist soil samples of the chosen sand and clay soils had too low Se and DOC concentrations in the 0.01 M $CaCl_2$ extracts, therefore were not included. The DMT analysis was conducted in duplicate for each sample. About 300 mL of 0.01 M $CaCl_2$ soil extract was prepared for each sample following a similar extraction procedure described in Section 2.2.2, but without filtration before used for DMT analysis. After centrifugation, the supernatants were used as the donor solutions in the DMT analysis. About 15 mL of blank 0.01 M $CaCl_2$ was used as the acceptor solution for all samples. Sodium azide (NaN_3) was added to the donor and acceptor solution to a final concentration of 1 mM in order to stop microorganism growth. After that, pH of the donor and acceptor solutions was readjusted to the initial pH using NaOH and HNO_3. After all the instruments and the solutions were ready, the DMT cells were constructed. The pumps, tubes, cells, donor solutions and acceptor solutions were connected.

During the DMT analysis, the donor and acceptor solutions were circulated continuously at 2.5 mL minute^{-1} flow rate using peristaltic pumps (Gilson Miniplus III). The experiment was conducted in the conditioned room (20 ± 1^{o}C). Around 11 mL of samples from both the donor and acceptor solutions were taken at the beginning of the experiment (at 0 h), and at 8 h, 3 and 6 days. After each sampling, the acceptor solution was refilled with blank 0.01 M $CaCl_2$. The samples of the donor solution were filtered with 0.45 µm pore-size syringe cellulose acetate filter (Whatman Aqua 30/0.45 CA), whereas the samples of acceptor solution were not filtered. The first 5 mL filtrate of donor solution was discarded. In these samples, pH, concentrations of Se, DOC and Cl were measured. Concentration of Cl was measured because Cl was used as a reference ion in the DMT analysis. The subsamples from the donor and acceptor solutions were taken at the different time steps in order to see the change with time of Se and DOC concentrations both in the donor and acceptor solutions. The results of DMT analysis in this study were presented based on the results on day 6 when Se and DOC in the acceptor solutions reached equilibrium concentrations. The determination limits in the DMT experiment are 0.04–0.05 µg L^{-1} for Se and 0.52–1.25 mg L^{-1} for DOC.

2.2.4 Fractionation of DOM and Se using DAX-8 resin

Fractionation of DOM and Se using acidification and DAX-8 resin was conducted to evaluate the contribution of organic molecules with different hydrophobicity to the increase of DOC and soluble Se upon drying. In addition, the experiment aimed to understand to which fraction of DOM, the organic Se is mostly associated. The fractionation was performed on ten dried soils from grassland (including sandy, clay, clay-peat, peat soils) and eight dried soils from potato field (including sandy and clay soils) selected from the sample set. The fractionation was not conducted in the corresponding moist soils because the total concentrations of DOC and Se in many samples are low, i.e. < 5 mg DOC L^{-1} and < 0.20 µg Se L^{-1}, respectively (Table 2). Therefore it is expected that in many moist soil samples the DOC and Se concentrations after fractionation may come below the determination limits. The determination limits in the fractionation experiment are 0.10–0.17 µg L^{-1} for Se and 1.07–3.98 mg L^{-1} for DOC.

The DOM in the dried soil samples was fractionated into humic acids (HA), fulvic acids (FA), hydrophilic acids (Hy), and hydrophobic neutral organic matter (HON) according to the rapid batch technique of Van Zomeren and Comans (2007). For each soil sample, about 65 mL of 0.01 M $CaCl_2$ soil extract was adjusted to pH 1.0 to 1.5 with 6 M HCl and let stand overnight to precipitate HA fraction. Then the extracts were centrifuged at 3500 rpm for 20 minutes. We did not find precipitated HA pellet in all samples, therefore HA fraction in DOM is considered negligible. Subsamples were taken from the extract for analysis, representing the sum of FA, Hy, and HON fractions. To 35 mL of the remaining extract about 7.0 gram of DAX-8 resin was added to adsorb FA and HON, shaken at 220 rpm for 1 h, and filtered over 0.45 µm pore-size syringe cellulose acetate filter (Whatman Aqua 30/0.45 CA). Subsamples were taken from the filtrate for analysis, representing the Hy fraction. About 25 mL 0.1 M KOH was added to the resin to desorb FA from the resin, shaken at 220 rpm for 1 h, and filtered over 0.45 µm pore-size syringe cellulose acetate filter (Whatman Aqua 30/0.45 CA). Subsamples were taken from the filtrate for analysis, representing the FA fraction. The filters (Whatman Aqua 30/0.45 CA) were washed with UPW before use to reduce DOC release. The UV absorbance of the FA and Hy fractions of DOM were measured at 254 nm without pH adjustment. Before the fractionation, subsamples of the initial $CaCl_2$ extractions were also analysed. In all the samples, DOC and Se concentrations were measured. The results showed that about only 5% of DOC was present as HON fraction and the amounts of HON fraction derived were mostly very low and have large uncertainties. Therefore, for a better accuracy, the data presented in this study did not make distinction between FA and HON fractions. Total FA and HON fraction was derived from the difference between the total DOC measured in the soil extracts after the acidification step (representing the sum of FA, Hy and HON fraction) and the DOC in the Hy fraction. The FA and HON fraction is dominated by the FA fraction and presented in this study as total FA fraction.

2.2.5 Chemical analysis

Concentrations of Se and Cu were measured on High Resolution ICP-MS (Thermo Scientific Element2). Concentrations of DOC were analysed using a segmented flow analyser (SFA) (3039 Skalar). pH was measured using a pH meter (Radiometer Analytical PHM210) with a glass electrode (Beckman 511084). UV absorbance was measured using a UV-VIS spectrophotometer (Beckman Coulter or Genesys 10S Thermo Scientific). Concentration of Cl was measured using a Foss-Tecator Fiastar 5000 (continuous flow system).

2.3 Results and discussion

2.3.1 Total and soluble Se and Cu

Total Se (aqua regia) of Dutch agricultural soils ranges from 0.12 to 1.97 mg kg^{-1} (Table 2). In general, the total Se content is relatively low in the sandy soils and relatively high in the clay-peat and peat soils. Gupta and Gupta (2000) reported that in general, a total soil Se less than 0.6 mg kg^{-1} is considered deficient. Around 72% of the measured soils (n = 58) contain less than 0.6 mg Se kg^{-1}, indicating that the agricultural soils in the Netherlands have a relatively low Se level and may face Se deficiency problem. The soils that have a total Se more than 0.6 mg kg^{-1} are characterized with a high organic matter content, such as peat and clay-peat soils (Table 1 and 2).

Concentrations of Se in 0.01 M $CaCl_2$ extraction are also low, i.e. 0.03 to 0.48 µg L^{-1} in moist soils and 0.20 to 4.02 µg L^{-1} in dried soils (Table 2). Total soluble Se in moist soils accounts for 0.04 to 2.1% (average 0.3%), whereas in dried soils 0.3 to 2.9% (average 1.4%), of the total Se (aqua regia). For the moist soils, soluble Se concentrations in 9% of the samples (n = 58) are below the detection limit; 53% are in between the detection and determination limits; and 38% are above the determination limit of the High Resolution ICP-MS analysis. Because Se concentrations measured in the moist samples are relatively low, in this work we included values above the detection limits as an estimation of Se concentrations in the moist samples. However, we should keep in mind that Se concentrations that are below the determination limit contain relatively large uncertainty compared to the Se concentrations that are above the determination limit. On the other hand, total soluble Se in dried samples is higher than in the moist samples and all dried samples have soluble Se concentrations above the determination limit. Seven soil samples, which were analysed in duplicate, showed that the Se concentrations measured in the dried soils have a better reproducibility (variation coefficient 6%) compared to those in the moist soils (variation coefficient 14%), due to the relatively high Se concentration in the dried soils compared to that in the moist soils.

Total Cu (aqua regia) in Dutch agricultural soils is in the range of 6 to 76 mg kg^{-1} (Table 2). In general, the total Cu content is relatively low in the sandy soils and relatively high in the clay-peat soils. Compared to the background levels of Cu

Table 2. Total Se and Cu in soils (aqua regia) and total soluble Se, Cu, DOC and SUVA of DOM measured in 0.01 M $CaCl_2$ extraction of moist and dried soils presented as the mean and (range) per soil type. (Each soil sample was analysed without replicate, except for seven selected samples that were analysed in duplicate.)

Soil type	Land use[a]	Total Se (aqua regia) mg kg^{-1}	Total Cu (aqua regia) mg kg^{-1}	Se-$CaCl_2$ µg L^{-1}		Cu-$CaCl_2$ µg L^{-1}		DOC mg L^{-1}		SUVA DOM L g^{-1} cm^{-1}	
				Moist soil	Dried soil	Moist soil	Dried soil	Moist soil	Dried soil	Moist soil	Dried soil
Sand (n = 13)	GL	0.23 (0.12 – 0.37)	10.04 (6 – 15)	0.05 (0.03 – 0.07)[b]	0.37 (0.21 – 0.59)	1.85 (1.03 – 2.71)	4.48 (2.44 – 8.88)	3.22 (1.52 – 5.42)[c]	13.65 (5.53 – 21.63)	39 (32 – 54)[c]	20 (14 – 27)
Clay (n = 7)	GL	0.34 (0.20 – 0.56)	13.84 (11 – 16)	0.08 (0.05 – 0.11)	0.58 (0.39 – 0.77)	1.41 (0.90 – 2.14)	5.14 (3.16 – 7.21)	2.55 (1.72 – 3.72)[c]	16.04 (5.93 – 35.43)	37 (29 – 46)[c]	19 (14 - 23)
Clay-peat (n = 6)	GL	1.48 (1.16 – 1.97)	59.60 (47 – 76)	0.11 (0.07 – 0.16)	2.31 (1.07 – 4.02)	4.89 (3.11 – 7.34)	11.57 (8.49 – 14.99)	10.87 (6.52 – 20.71)	66.76 (39.88 – 93.93)	28 (18 – 28)	12 (8 – 58)
Peat (n = 1)	GL	1.14	14.42	0.05	1.37	1.17	2.61	6.52	50.03	28	12
Sand (n = 5)	MF	0.30 (0.20 – 0.39)	8.80 (6 – 12)	0.05 (0.04 – 0.07)[b]	0.45 (0.26 – 0.71)	1.58 (0.96 – 1.94)	2.44 (1.70 – 3.31)	4.45 (3.27 – 7.52)	14.38 (11.18 – 17.48)	41 (33 – 46)	22 (19 – 27)
Clay (n = 3)	MF	0.47 (0.25 – 0.73)	16.26 (13 – 18)	0.16 (0.11 – 0.22)	0.59 (0.37 – 0.83)	2.49 (1.57 – 3.92)	4.86 (3.34 – 6.20)	4.37 (2.92 – 5.57)	15.68 (9.58 – 23.48)	33 (30 – 36)	20 (17 – 25)

Table 2. Continued.

Soil type	Land use[a]	Total Se (aqua regia) mg kg^{-1}	Total Cu (aqua regia) mg kg^{-1}	Se-CaCl$_2$ µg L^{-1}		Cu-CaCl$_2$ µg L^{-1}		DOC mg L^{-1}		SUVA DOM L g^{-1} cm^{-1}	
				Moist soil	Dried soil	Moist soil	Dried soil	Moist soil	Dried soil	Moist soil	Dried soil
Clay-peat (n = 6)	MF	0.96 (0.43 – 1.20)	22.25 (13 – 38)	0.11 (0.04 – 0.21)	0.74 (0.30 – 1.00)	2.40 (1.48 – 2.93)	4.55 (3.27 – 7.08)	11.27 (6.02 – 20.01)	35. 13 (23.18 – 53.88)	34 (25 – 39)	22 (15 – 34)
Peat (n = 1)	MF	1.09	11.66	0.13	1.13	2.02	2.68	12.31	47.38	42	19
Sand (n = 5)	PF	0.39 (0.19 – 0.69)[d]	8.29 (7 – 12)[d]	0.07 (0.06 – 0.08)	0.26 (0.20 – 0.35)	1.65 (1.05 – 2.17)	3.11 (2.05 – 4.24)	2.70 (1.80 – 3.70)	7.09 (4.64 – 9.04)	38 (34 – 46)	29 (24 – 37)
Clay (n = 11)	PF	0.35 (0.22 – 0.73)[d]	21.77 (6 – 45)[d]	0.19 (0.11 – 0.48)	0.42 (0.25 – 0.60)	2.83 (1.42 – 6.49)	5.27 (2.77 – 13.52)	2.69 (1.60 – 4.60)	6.54 (3.54 – 18.44)	28 (16 – 35)	24 (11 – 29)

[a] Land use consists of grassland (GL), maize field (MF) and potato field (PF).

[b] Samples that have Se concentrations below the detection limit are excluded from the table.

[c] Samples that have DOC concentrations below the determination limit are excluded from the table.

[d] Including the samples that have total Se (aqua regia) below the determination limit.

calculated for each soil with an empirical relation derived in the past including corrections for clay and soil organic content (Lexmond and Edelman, 1987), most of the soils (about 93% of total 58 samples) have a total Cu content close to or below the background values. About 7% of the soils, which are mainly clay and clay-peat soils, have a total Cu above the Dutch background values.

In contrast to Se, no problem related to the detection limit was encountered for measuring soluble Cu in moist and dried samples using the High Resolution ICP-MS. Total soluble Cu in moist and dried samples of 0.01 M $CaCl_2$ soil extraction measured in this study are above the determination limit. Seven soil samples, which were analysed in duplicate, showed that the Cu concentrations measured in moist and dried soils have good reproducibility with variation coefficients of 3%.

2.3.2 Drying effect on concentration and properties of DOC

Concentrations of DOC in 0.01 M $CaCl_2$ soil extractions of grassland and arable land soils are higher in the dried soils than in the moist soils (Table 2 and Fig. 1A). Upon drying, DOC increased on average for each soil type 5 to 7.7 fold in the grassland soils, 3.5 to 4 fold in the maize field soils, and 2.5 to 2.7 fold in the potato field soils (Fig. 1A). The increase of DOC concentrations due to drying is stronger for grassland soils compared to arable land soils, which cannot be explained by the difference in the basic soil properties (Table 1). The SUVA of DOM at 254 nm wavelength is in general lower for the dried soils than for the moist soils (Table 2 and Fig. 1B). The decrease of SUVA is stronger for grassland soils (48 to 58% decrease) than for maize field soils (36 to 54% decrease) and potato field soils (16 to 23% decrease) (Fig. 1B), which is in line with a stronger increase of DOC in grassland soils than that in maize and potato field soils upon drying (Fig. 1A).

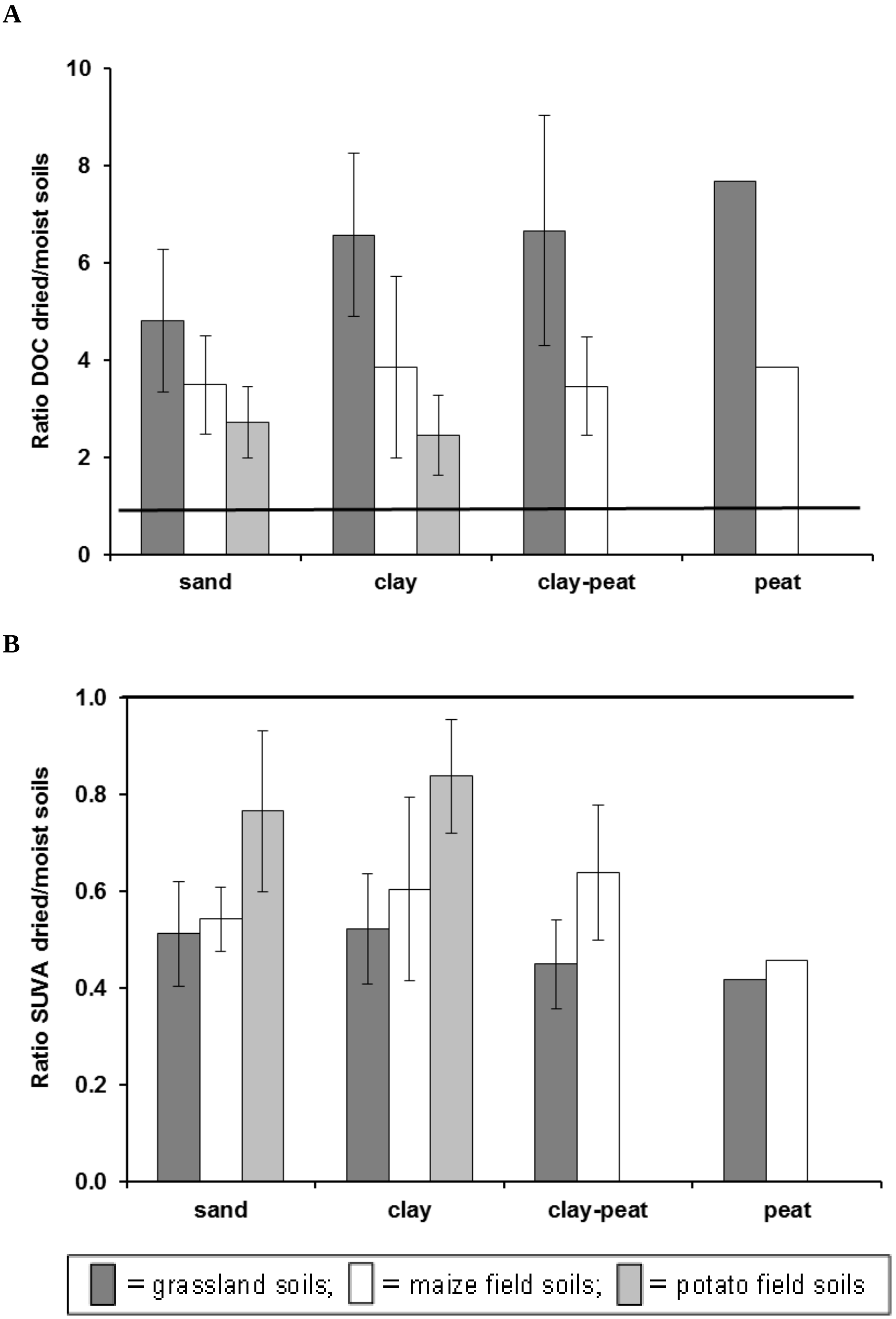

Fig. 1. Drying effect on DOC and SUVA of DOM in 0.01 M $CaCl_2$ extraction. A = DOC concentration; B = SUVA of DOM at 254 nm wavelength. The horizontal line

indicates a ratio = 1. (Results from all soil samples from grassland, maize field, and potato field are included (Table 1), except for DOC 2 soil samples are not included due to too low DOC concentrations in the moist samples.)

The fractionation of DOM in the dried soil extracts from grassland and arable land showed that the Hy fraction contributes on average 74% and FA fraction (including a small amount of HON fraction) contributes 26% to the total DOC concentrations (Table 3). We did not find measureable HA fraction in our samples. The high calcium concentration, i.e. 0.01 M $CaCl_2$, used in the extraction may have reduced the solubility of HA. A complete coagulation of natural high molecular weight HA in soil starts when Ca concentration exceeds 0.0075 to 0.01 M (Römkens and Dolfing, 1998; Weng *et al.*, 2002).

The SUVA of the Hy DOM fraction (2-6 L g^{-1} cm^{-1}) in the dried soil extracts from grassland and arable land is much lower compared to the SUVA of FA fraction (20-53 L g^{-1} cm^{-1}) and total DOM (8-28 L g^{-1} cm^{-1}) (Table 3), which reflects the lower aromaticity of the Hy fraction compared to the FA fraction. From these results we can deduce that UV absorbance of the dried soil extracts is originated to a large extent from the FA fraction, and SUVA of the soil extracts will vary close to proportionally to the percentage of FA over DOC. The SUVA of total DOM in the moist samples are higher (on average per soil type 28-42 L g^{-1} cm^{-1}) than that in the dried samples (12–29 L g^{-1} cm^{-1}) (Table 2) and close to the SUVA of the FA fraction measured in the dried samples (Table 3), suggesting dominance of FA fraction in DOM in the moist samples.

The results of the DMT experiment show that in the dried samples < 4% of DOC had passed the anion exchange membrane (Table 4). Simple organic acids, e.g. citrate, can pass the anion exchange membrane in the DMT and be detected in the acceptor solution (Van der Stelt, 2007), whereas colloidal-sized organic molecules, such as HA and FA cannot (Van der Stelt, 2007; Weng *et al.*, 2011). The results indicate that most of the DOC fractions in the dried samples, including the Hy

Table 3. Fractionation of Se and DOC species in 0.01 M $CaCl_2$ extraction of dried soils selected from grassland and potato field samples.

Soil type (sample)	pH	Total Se μg L^{-1}	Se in DOM fractions %		Total DOC mg L^{-1}	DOC fractions %		Ratio Se:DOC μg g^{-1}		SUVA L g^{-1} cm^{-1}		
			Hy[a]	FA[b]		Hy[a]	FA[b]	Hy[a]	FA[b]	Hy[a]	FA[b]	Total DOM
Grassland												
Sand 1 (soil 6)[c]	6.99 ± 0.11	0.50 ± 0.02	58 ± 3	42 ± 3	19.4	61 ± 1	39 ± 1	25 ± 2	28	4 ± 2	43	22
Sand 2 (soil 11)	5.35	0.55	62	38	24.9	65	35	21	25	2	47	18
Sand 3 (soil 23)	5.37	0.51	69	31	13.9	74	26	34	44	6	33	17
Clay 1 (soil 5)	5.84	0.53	54	46	20.4	75	25	18	49	4	26	17
Clay 2 (soil 8)	6.10	0.86	61	39	44.3	76	24	16	32	3	39	17
Clay 3 (soil 25)	7.12	0.36	73	27	13.0	70	30	29	26	4	32	20
Clay-peat 1 (soil 9)[c]	5.34 ± 0.01	1.42 ± 0.03	55 ± 2	45 ± 2	70.3 ± 2.0	79	21	14	44 ± 2	2	29 ± 5	11
Clay-peat 2 (soil 10)	5.55	1.60	58	42	70.5	76	24	17	40	2	31	15
Clay-peat 3 (soil 27)	5.02	3.58	92	8	127.0	81	19	32	11	4	24	14
Peat (soil 16)	5.17	1.37	83	17	58.8	78	22	25	18	3	26	12
Average ± SD[d]			**67 ± 13**	**33 ± 13**		**74 ± 6**	**26 ± 6**	**23 ± 7**	**32 ± 12**	**3 ± 1**	**33 ± 8**	**16 ± 3**
Potato field												
Sand 1 (soil no 1)	4.70	0.22	67	33	7.2	62	38	33	26	3	53	28
Sand 2 (soil no 6)	5.06	0.27	55	45	8.9	86	14	19	96	4	35	19
Sand 3 (soil no 5)	5.21	0.36	72	28	7.9	73	27	45	47	4	20	20
Sand 4 (soil 21)[c]	4.99 ± 0.01	0.39 ± 0.01	70 ± 3	30 ± 3	10.9 ± 0.1	68 ± 2	32 ± 2	37 ± 2	34 ± 6	5 ± 0.1	47 ± 4	22 ± 0.5
Clay 1 (soil no 9)	5.46	0.44	69	31	11.4	71	29	38	41	3	42	18
Clay 2 (soil no 18)	6.40	0.53	74	26	8.4	69	31	67	53	4	43	22
Clay 3 (soil no 14)	4.94	0.53	74	26	22.3	82	18	21	35	3	24	8
Clay 4 (soil no 11)[c]	7.21	0.63 ± 0.04	83 ± 2	17 ± 2	7.3 ± 0.3	77 ± 5	23 ± 5	93 ± 6	66 ± 5	3 ± 0.3	38 ± 9	18 ± 0.1
Average ± SD[d]			**70 ± 8**	**30 ± 8**		**73 ± 8**	**27 ± 8**	**44 ± 25**	**50 ± 23**	**4 ± 0.6**	**38 ± 11**	**19 ± 5**

[a] Hydrophilic acids; [b] Fulvic acids.

[c] The samples were analysed in duplicate, whereas other samples were analysed without replicate.

[d] Standard deviation.

Table 4. Se and DOC speciation in 0.01 M $CaCl_2$ extraction of three dried soils and one moist soil selected from grassland samples obtained from DMT experiment. (The values presented in the table are the mean ± standard deviation of duplicate.)

Dried soils (n = 3)												
	$CaCl_2$ extraction (donor)						acceptor		Se and DOC speciation			
Soil type (sample)	pH		Se µg L^{-1}		DOC mg L^{-1}		Se µg L^{-1}	DOC mg L^{-1}	Colloidal organic Se	Sub-nanometer Se	Colloidal DOC	Sub-nanometer DOC
	0 day	6 day	0 day	6 day	0 day	6 day	6 day	6 day				
Sand (soil 23)	5.16 ± 0.02	5.25 ± 0.03	0.50 ± 0.01	0.46	12.60 ± 0.57	11.70 ± 0.14	0.05^a	0.55 ± 0.28^a	≈100%	0%	≈100%	0%
Clay (soil 25)	7.14 ± 0.08	6.98 ± 0.07	0.36 ± 0.02	0.28	9.30 ± 0.14	6.60 ± 0.85	0.03^a	0.60 ± 0.07^a	≈100%	0%	≈100%	0%
Clay-peat (soil 27)	5.10 ± 0.03	5.22 ± 0.01	3.89 ± 0.02	3.69 ± 0.09	117.60 ± 1.98	114.90 ± 2.97	0.11	4.15 ± 0.42	97%	3%	96%	4%

Moist soil (n = 1)												
	$CaCl_2$ extraction (donor)						acceptor					
Soil type (sample)	pH		Se µg L^{-1}		DOC mg L^{-1}		Se µg L^{-1}	DOC mg L^{-1}	Colloidal organic Se	Sub-nanometer Se	Colloidal DOC	Sub-nanometer DOC
	0 day	6 day	0 day	6 day	0 day	6 day	6 day	6 day				
Clay-peat (soil 27)	5.67 ± 0.13	5.27 ± 0.05	0.14	0.13 ± 0.04	16.60 ± 2.55	16.20 ± 0.85	n.d.^{b}	0.90 ± 0.07^a	≈100%	0%	≈ 100%	0%

[a] Below or close to the determination limit.

[b] Not detected.

fraction, are colloidal in size (1 nm to 0.45 µm). In the moist sample of the selected clay-peat soil, hardly any DOC passed the anion exchange membrane, showing that all DOC in this moist soil are also colloidal in size (Table 4). Our result is similar to that of Koopmans and Groenenberg (2011) that hardly any simple organic acids (< 1 nm in size), such as citrate and oxalate, were found in their moist and dried soil extractions.

A large fraction of the increase of DOC concentrations after soil drying has been attributed to microbial cell lysis induced by osmotic shock upon rewetting the dried soils (Salema *et al.*, 1982; Kieft *et al.*, 1987; Zsolnay *et al.*, 1999), which produces hydrophilic (Hy) or non-humic DOM (Zsolnay *et al.*, 1999; Koopmans and Groenenberg, 2011). Koopmans and Groenenberg (2011) and Kieft *et al.* (1987) reported that bacterial biomass may contribute 20 to 62% of the increase of DOC concentrations in their soil samples upon drying. The dominance of the colloidal sized Hy fraction in the dried soil samples and the decrease of SUVA after drying found in our soil samples are in line with the hypothesis that the major source of DOC increase after drying is probably due to microbial cell lysis. The study of Zsolnay *et al.* (1999) showed that the humification index of fumigated soil extracts is lower (around 2) than that of fulvic acids (around 13 to 16), indicating that DOM derived from microbial cell lysis is less aromatic than soil organic matter. Additional sources of increased DOC can come from release of soil organic C into soil solution, mainly fulvic acid (FA) fraction and slightly humic acid (HA) fraction (Koopmans and Groenenberg, 2011), due to disruption of organo-mineral complexes (Haynes and Swift, 1991) and decreased binding of those fractions to metal (hydr)oxides (Christ and David, 1994). However, in our study we cannot differentiate clearly the sources of DOC increase upon drying using the current data.

2.3.3 Drying effect on soluble Se

Drying the soils at 40^{o}C increased in general the concentrations of Se extracted using 0.01 M $CaCl_2$ (Table 2 and Fig. 2A). After drying, the increase of soluble Se concentrations in grassland soils is in the range of 8 to 26 fold, in maize field soils 4 to 9 fold, and in potato field soils 2 to 4 fold (Fig. 2A). Generally, the increase of soluble Se concentrations due to drying is stronger in grassland soils than in arable land soils (Fig. 2A), which corresponds to the trend of DOC increase upon drying (Fig. 1A). In general, soluble Se in moist soils is poorly correlated with that in dried soils (Fig. 3). A better correlation exists for grassland soils ($R^2 = 0.46$, $p < 0.001$) and potato field soils ($R^2 = 0.56$, $p < 0.001$) than for maize field soils ($R^2 = 0.19$, $p > 0.05$), which is due to a larger range of the Se concentration in the grassland soils and potato field soils than in the maize field soils.

Selenium speciation analysis using DMT showed that although the total soluble Se in the $CaCl_2$ extracts of three dried soils and one moist soil (clay-peat soil) are above the determination limit, Se concentrations in the acceptor solution are only < 3% of the total soluble Se, indicating that a major part of total soluble Se is present as colloidal sized Se (Table 4). The colloidal sized Se (1 nm to 0.45 µm) is expected to be Se associated with the colloidal sized DOC that cannot pass through the DMT membrane. We hardly found measurable sub-nanometer Se molecules that can pass through the DMT membrane and enter the acceptor solution in the dried and moist samples (Table 4). The sub-nanometer Se compounds were not measurable either in a preliminary test using High Performance Liquid Chromatography (HPLC) with an anion exchange column (data not shown). The sub-nanometer Se molecules include inorganic selenate, selenite, or sub-nanometer organic Se. In the dried clay-peat soil around 3% of sub-nanometer Se measured in the acceptor is probably present as sub-nanometer organic Se associated with sub-nanometer Hy DOC. Previous DMT analysis using other dried soil samples from grassland has also shown that colloidal sized organic Se accounts for 67 to 86% of total soluble Se in $CaCl_2$ soil extracts, whereas selenate, selenite and small organic Se molecules account for the remaining 15 to 33% (Weng *et al.*, 2011). The results of DMT analysis indicate that the increase of total soluble Se upon drying is mainly due to the increase of colloidal sized organic Se.

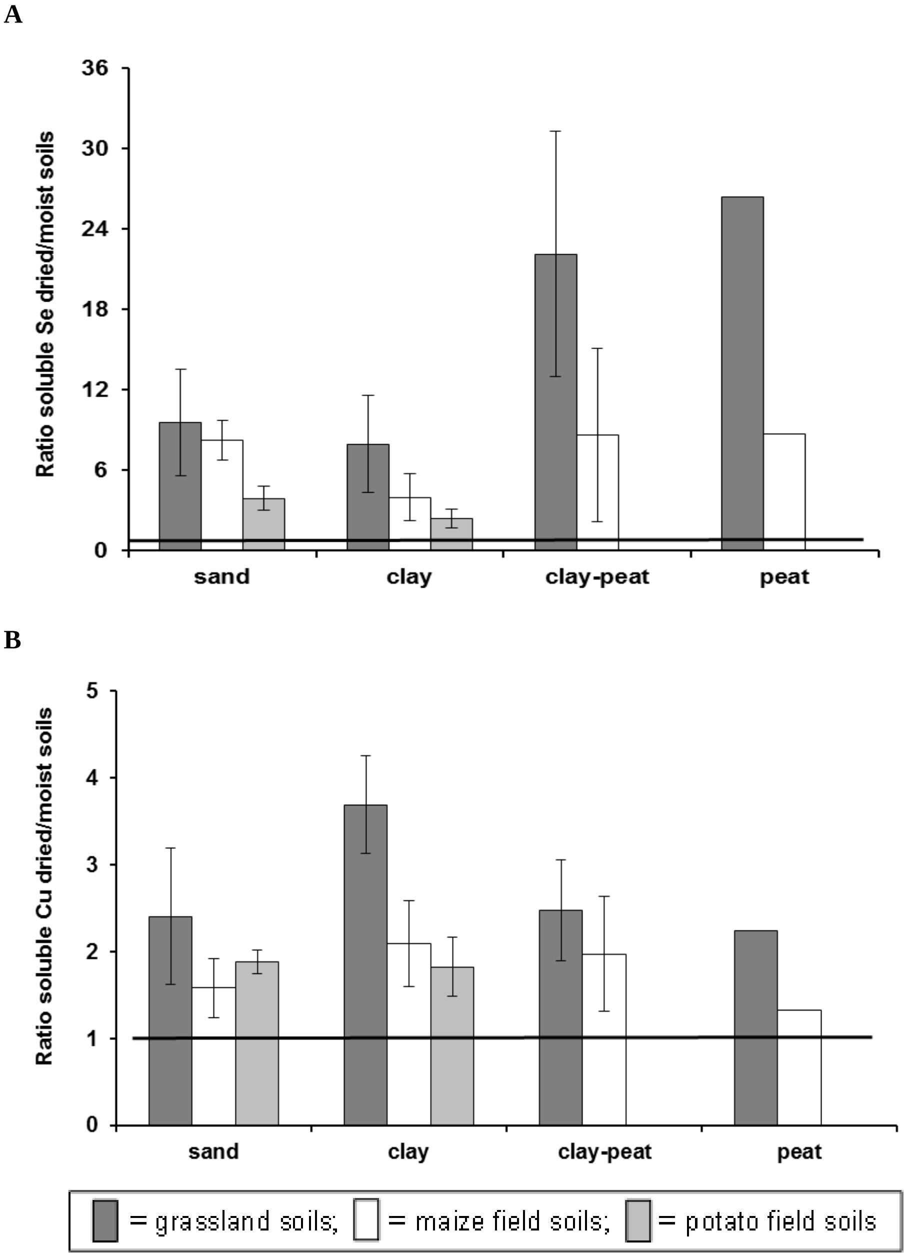

Fig. 2. Drying effect on soluble Se and Cu concentrations in 0.01 M $CaCl_2$ extractions. A = Se; B = Cu. The horizontal line indicates a ratio = 1. (Results from all soil samples from grassland, maize field, and potato field are included (Table 1),

except for soluble Se, 5 soil samples are not included due to too low concentrations in the moist samples.)

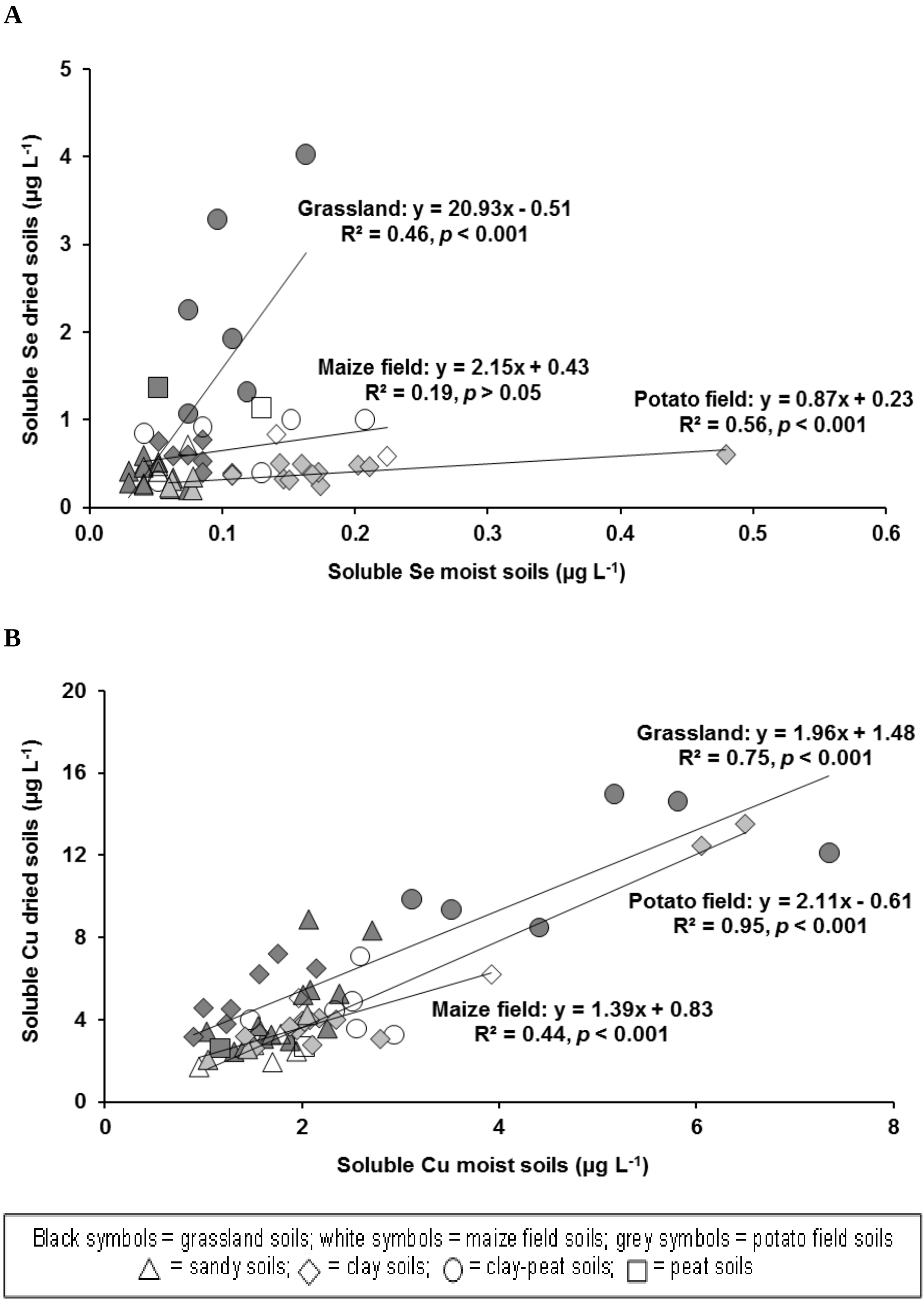

Fig. 3. Correlation between soluble Se or Cu concentration in 0.01 M $CaCl_2$ extraction of moist soils and dried soils. A = Se; B = Cu. (Results from all soil samples from grassland, maize field, and potato field are included (Table 1), except for soluble Se in moist soils, 5 soil samples are not included due to too low concentrations in the moist samples.)

A positive correlation between DOC and soluble Se concentration was found in the dried soil samples, except for soils from potato field (Fig. 4A), indicating that there is an association between Se and DOC upon drying. Similar relationship between DOC and soluble Se has been observed using other dried soil samples from grassland (Weng *et al.*, 2011). The absence of a correlation between DOC and soluble Se in the dried samples from potato field can be due to very low level and small range of DOC and soluble Se in the samples. A positive relationship is visible for the moist soils as well, but because of the much narrower range of both DOC and Se in the moist soils compared to those in the dried soils, the correlation is weaker for the moist soils (Fig. 4B). The positive correlation between DOC and Se in the dried soil samples supports the DMT results showing that the colloidal Se associated with DOC contributed to the increase of total soluble Se upon drying.

Both drying the soil and the presence of bivalent Ca^{2+} cation in the extraction will reduce the possible presence of fine mineral colloids in the extracts of the dried soils. In addition, a model calculation using the same sample set of soils as used in this study showed that soluble Fe in the $CaCl_2$ extracts of moist and dried soils can be mainly attributed to Fe-DOM complexes (data not shown). Therefore, the colloidal Fe-(hydr)oxides is probably absent or in a very small amount in the soil extracts. It is not very likely that the inorganic Se bound to colloidal metal (hydr)oxides contributes to a great extent to the increase of total soluble Se in the 0.01 M $CaCl_2$ soil extracts upon drying.

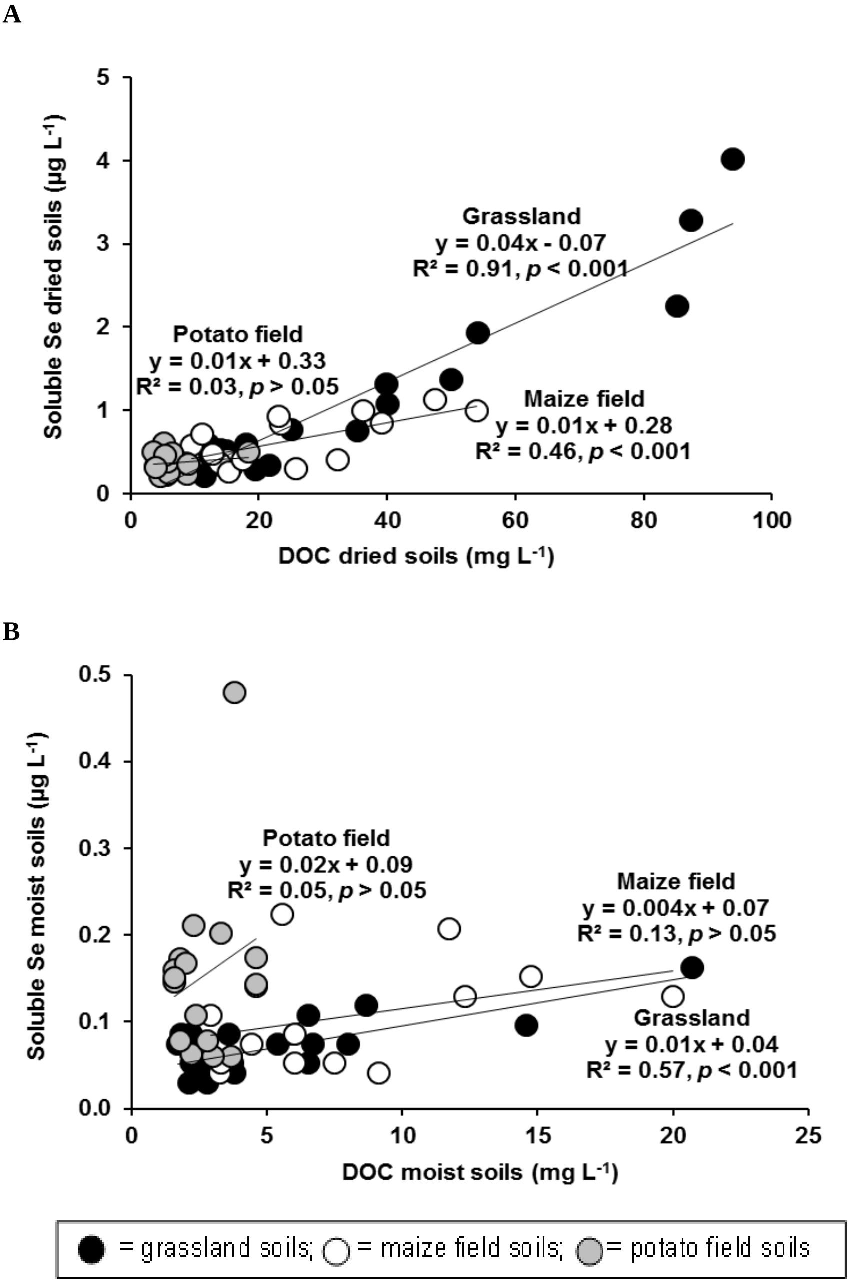

Fig. 4. Correlation between DOC and soluble Se in 0.01 M $CaCl_2$ extraction of dried soils and moist soils. A = DOC and soluble Se in dried soils; B = DOC and soluble Se in moist soils. (Results from all soil samples from grassland, maize field,

and potato field are included, except for moist soils, 7 soil samples are not included due to too low DOC and Se concentrations in the moist samples.)

Although the positive relationship between Se and soil organic matter has been observed in many studies, the chemical nature of this association remains unclear. Adsorption to the reactive groups on soil organic matter (directly or indirectly via metal ions) or as part of the structure of organic matter molecules are two possible mechanisms for Se association with soil organic matter (Gustafsson and Johnsson, 1994; Qin *et al.*, 2012). A study carried out by Gustafsson and Johnsson (1994) suggests that Se incorporated into soil organic matter through microbial activity is the major mechanism for Se-organic matter association.

In the DOM fractionation analysis, distribution of Se in the FA and Hy fraction was analysed as well. Around 54 to 92% (on average 68%) of total soluble Se in dried soil extraction is in the Hy DOM fraction, whereas the rest is mainly in the FA fraction, which corresponds to the ratio of Hy to FA fraction in DOM (Table 3). The Se content in DOC fractions equals 23 ± 7 µg g^{-1} in the Hy and 32 ± 12 µg g^{-1} in the FA fraction of DOM in the grassland soils, and 44 ± 25 µg g^{-1} in the Hy and 50 ± 23 µg g^{-1} in the FA fraction of DOM in the potato field soils. No consistent difference of Se content in DOC was found between the Hy and FA fractions in the dried soils. The ratio of Se to DOC in the Hy and FA fraction is in general somewhat higher in the potato field soils compared to the grassland soils (Table 3), which is in line with the ratio of total soluble Se to DOC in the extraction of dried soils from potato field (64 ± 33 µg g^{-1}) and grassland (34 ± 13 µg g^{-1}) (Table A.3).

On average, the ratio of total soluble Se to DOC in the $CaCl_2$ extraction of dried soils is higher (i.e. 34 ± 13 µg g^{-1} for grassland and 30 ± 16 µg g^{-1} for maize field) than that of the moist soils (i.e. 18 ± 12 µg g^{-1} for grassland and 17 ± 11 µg g^{-1} for maize field), except for soil samples from potato field (Table A.3). No significant difference in the ratio of total soluble Se to DOC in the dried soils (64 ± 33 µg g^{-1}) and moist soils (62 ± 35 µg g^{-1}) from potato fields was found (Table A.3). In general, total organic matter content, DOC concentration and the increase of DOC after drying in the soil samples from grassland and maize field are higher than those in the soil samples from potato field (Table 1 and 2; Fig. 1A). The enrichment of Se in DOM in grassland and maize field soils upon drying indicates that a higher amount of Se-containing organic compounds was extracted in the dried soils than in the moist soils from grassland and maize field. Since Se to DOC ratio in different DOM fractions in the moist soils could not be measured due to the too low Se and DOC concentrations, it is unclear if Se is enriched similarly in both the FA and Hy DOM fractions upon drying.

Zhang and Moore (1997) also found increases of soluble Se in two wetland sediments extracted with 0.25 M KCl after drying the samples at 42°C, however, the increases are less pronounced (i.e. 2 fold) compared to those found in our study (i.e. 2 to 26 fold; Fig. 2A). They found that the concentrations of DOC are 6 times higher after drying the sediments, whereas the increases of DOC in our soils are 2.5 to 7.7 times (Fig. 1A). On average total Se content (4 mg kg^{-1}) and soluble Se (69 µg kg^{-1} in moist sediments; 118 µg kg^{-1} in dried sediments) measured in their study are higher compared to our soil samples (on average total Se (aqua regia) 0.5 mg kg^{-1}; soluble Se 1.1 µg kg^{-1} in moist soils and 7 µg kg^{-1} in dried soils). On the other hand, the average total C in the sediments is 2 times lower compared to our samples. It might imply that the increase of soluble Se in the low Se high organic matter soils is more pronounced than those in the high Se low organic matter soils upon drying. The stronger increase of soluble Se after drying found in our current work compared to those found by Zhang and Moore (1996) can thus be attributed to a more significant contribution of organic Se to total soluble Se in low Se high organic matter soils (samples in this study). Other studies also found that soluble Se in different extractions is mainly present as organic Se, especially in soils with low Se content, i.e. ≤ 1 mg Se kg^{-1} (Yamada and Hattori, 1989; Stroud *et al.*, 2012).

2.3.4 Drying effect on soluble Cu

Upon drying, the increase of soluble Cu in grassland soils is 2.2 to 3.7 fold, in maize field soils 1.3 to 2 fold, and in potato field soils 1.8 to 1.9 fold (Fig. 2B). Similar to Se, generally the increase of soluble Cu concentrations due to drying is stronger in grassland soils than in arable land soils (Fig. 2A and B). Compared to Se, Cu concentrations in the moist and dried soils are better correlated, especially for grassland soils ($R^2 = 0.75$, $p < 0.001$) and potato field soils ($R^2 = 0.95$, $p < 0.001$) (Fig. 3B).

Comparison between the increase of soluble Cu and DOC upon drying (Fig. 2B and 1A) showed that the increase of DOC is on average about 2 times stronger than the increase of total soluble Cu both in grassland soils and maize field soils, whereas it is about 1.4 times stronger in potato field soils. This result is different from those found by Koopmans and Groenenberg (2011), that the level of DOC increase is only slightly higher (i.e. 1.7 fold) than the level of increase of total soluble Cu (i.e. 1.6 fold) after drying. Samples used in the work of Koopmans and Groenenberg (2011) are sandy soils from a forest area, which was formerly an infiltration field of domestic and industrial wastewaters. The difference between the results of our study and theirs may be related to the difference in the quality of soil organic matter. Their soils are mainly enriched with organic matter coming from previous domestic and industrial wastewater. No SUVA of DOM data were reported in the work of Koopmans and Groenenberg (2011), but more Hy fraction and less FA fraction of DOM were found in our soils after drying (on average 74% Hy and 26% FA, Table 3) compared to those in their soils (on average 61% Hy and 38% FA).

In general, loading of Cu on DOC is lower in the dried soils (on average 5 ± 3 mmol kg^{-1} in grassland soils, 3 ± 2 mmol kg^{-1} in maize field soils and 11 ± 5 mmol kg^{-1} in potato field soils) than that in the moist soils (on average 9 ± 4 mmol kg^{-1} in grassland soils, 5 ± 3 mmol kg^{-1} in maize field soils and 15 ± 9 mmol kg^{-1} in potato field soils) (Table A.3). After drying, the loading of Cu on DOC decreased on average 49% in grassland soils, 44% in maize field soils and 28% in potato field soils (Table A.3), which corresponds to the decrease of SUVA of DOM upon drying, i.e. on average 50% in grassland soils, 41% in maize field soils, and 18% in potato field soils (Fig. 1B). Koopmans and Groenenberg (2011) have shown that drying has little effect on free Cu^{2+} concentration. Our data showed that the pH of grassland soils slightly increased upon drying (i.e. on average 0.15 pH unit), whereas the pH of maize and potato field soils did not change on average (Fig. A.1). Apparently, the lower Cu to DOC loading observed after drying can be attributed to a decrease in DOM affinity for Cu in the dried soils compared to that in the moist soils. As discussed above, the extra DOM produced upon drying and rewetting the soils is mainly Hy fraction that has a low aromaticity (see Section 2.3.2), which may explain its low affinity for Cu. The change of Cu loading on DOC upon drying shows a positive correlation with the change of SUVA of DOM upon drying (Fig. 5). The correlation is stronger in arable land soils ($R^2 = 0.75$, $p < 0.001$ in maize field soils; $R^2 = 0.87$, $p < 0.001$ in potato field soils) than that in grassland soils ($R^2 = 0.55$, $p < 0.001$). A positive correlation between the Cu binding affinity and SUVA of DOM has been found in several studies (Amery *et al.*, 2007; Amery *et al.*, 2008; Baken *et al.*, 2011). Taking into account both the increase of DOC (on average 4.3 fold; Fig. 1A) and change of SUVA (on average 60%; Fig. 1B), the average 2.3 times increase of soluble Cu upon drying (Fig. 2B) nearly coincides with the increase of the total aromatic fraction of DOM (i.e. around 2.6 fold). After soil drying, the increase of total soluble Cu corresponds much better to the increase of humic fractions of DOM (i.e. FA plus HA) than to the increase of Hy fraction (Koopmans and Groenenberg, 2011). Copper binds strongly to humic substances (Temminghoff *et al.*, 1997; Koopmans and Groenenberg, 2011) and much less to the Hy or non-humic fraction (Amery *et al.*, 2007). The loading of Cu on humic fractions of DOM does not change due to soil drying, whereas the loading of Cu on total DOM decreases upon drying (Koopmans and Groenenberg, 2011). The increase of Cu solubility upon drying apparently does not correspond to the increase of organic matter derived from lysis of microbial cells, but mainly relates to disruption and decomposition of soil organic matter (Haynes and Swift, 1991).

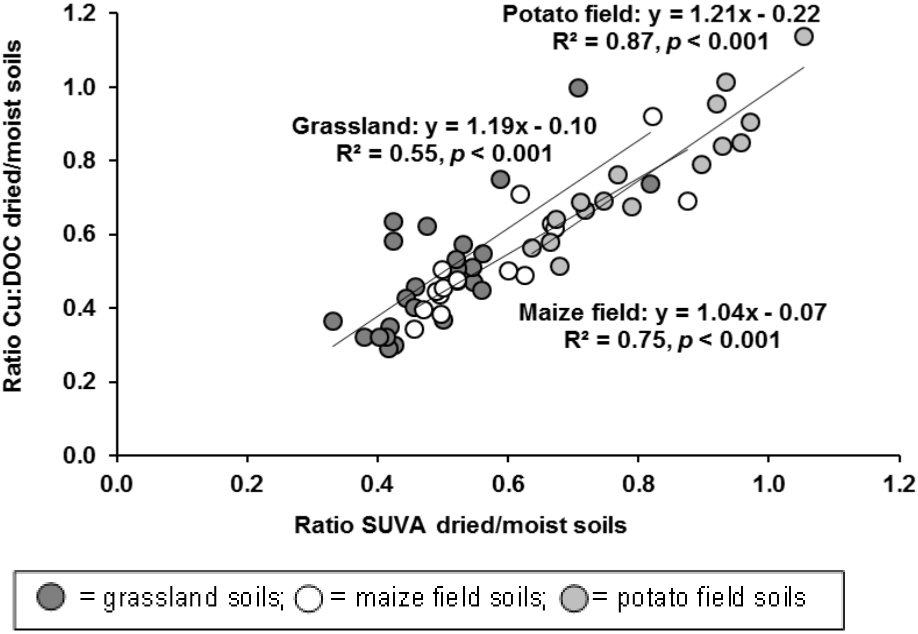

Fig. 5. Correlation between drying effect on Cu loading on DOC (ratio Cu:DOC) and drying effect on SUVA of DOM. (Results from all soil samples from grassland, maize field, and potato field are included (Table 1), except 2 soil samples are not included due to too low DOC concentrations in the moist samples.)

2.3.5 Relevance of Se and Cu in $CaCl_2$ extraction of dried soils for bioavailability prediction

Soluble Se and Cu both in the moist and dried soils are mainly present as colloidal sized organic Se and Cu-DOM complexes, respectively, which in principle cannot be taken up directly by plants. Therefore, neither total soluble Se and Cu in 0.01 M $CaCl_2$ extraction of moist soils nor dried soils can be used directly as the sole indicator to predict Se and Cu concentrations available for plant uptake. In the case of solution-diffusion controlled plant uptake, soluble Cu concentration in the moist and dried soils can be a good predictor for Cu plant uptake, if the Cu-DOM complexes dissociate fast enough (labile) at the root surface, releasing free Cu^{2+}. But for Se, even when solution-diffusion is controlling Se plant uptake, soluble Se may not be correlated to plant uptake, unless the soluble organic Se can be mineralized fast at the root surface and release inorganic Se or (small organic Se) that are directly available for plant uptake.

2.4 Conclusions

Oven-drying the soil samples at 40°C increased soluble Se and Cu concentrations in 0.01 M $CaCl_2$ extraction. The increase is more significant for Se (1.3 to 34 fold) than for Cu (1.1 to 4.6 fold). In addition, oven-drying led to an increase of DOC concentrations (1.3 to 11 fold) and a decrease of SUVA of DOM (0.3 to 1.0 fold). Soluble Se and DOC in the moist and dried soil extractions are predominantly in the form of colloidal sized (1 nm to 0.45 μm) organic compounds. This finding indicates that the colloidal sized organic Se associated with DOM is mainly responsible for the increase of total soluble Se upon drying, which is supported by a positive correlation between total soluble Se and DOC after drying. Most of the Se (68%) and DOC (74%) in the dried soil extractions are in the hydrophilic (Hy) fraction, and to a less extent (32% Se and 26% DOC) in the fulvic acid (FA) fraction. The total Se to DOC ratio increased upon drying, especially in grassland soils and maize field soils, corresponding to a stronger increase in soluble Se than the increase of DOC. No consistent difference was found in the Se to DOC ratio between the Hy and FA fractions of DOM in the dried soils. Upon drying, a positive correlation between the change of Cu loading on DOC (Cu to DOC ratio) and the change of aromaticity (SUVA) of DOM was found, showing that the increase of soluble Cu upon drying is mainly associated with the increase of the aromatic fraction of DOM. The total Cu to DOC ratio decreased upon drying. This can be attributed to a lower aromaticity (SUVA) and lower Cu binding capacity of DOC after drying, correlating to a weaker increase in soluble Cu than the increase of DOC. In conclusion, the increase of DOC is the main factor controlling the increase of soluble Se and Cu in 0.01 M $CaCl_2$ extraction of Dutch agricultural soils upon drying. However, the effects of DOC increase on the increase of soluble Se and Cu depend not only on the amount of DOC, but also on the chemical composition and properties of DOC. Total soluble Se and Cu in the $CaCl_2$ extraction of both moist and dried soils cannot be used as a single indicator for Se and Cu directly available for plant uptake since the soluble Se and Cu are mainly present as colloidal sized organic Se and Cu-DOM complexes, respectively.

Appendices

Table A.1. Soluble Fe, Al and Mn concentrations in 0.01 M $CaCl_2$ extraction of moist and dried soils presented as the mean and (range) per soil type. (Each soil sample was analysed without replicate, except for seven selected samples that were analysed in duplicate.)

Soil type	Land use[a]	Fe (mg L^{-1})[b]		Al (mg L^{-1})[b]		Mn (mg L^{-1})[b]	
		Moist soil	Dried soil	Moist soil	Dried soil	Moist soil	Dried soil
Sand (n = 13)	GL	0.04 (0.01 – 0.06)	0.05 (0.01 – 0.12)	0.14 (0.01 – 0.34)	0.20 (0.03 – 0.45)	0.17 (0.0005 – 0.80)	0.61 (0.01 – 1.36)
Clay (n = 7)	GL	0.03 (0.02 – 0.05)	0.02 (0.003 – 0.04)	0.03 (0.01 – 0.05)	0.01 (0.01 - 0.02)	0.01 (0.0003 – 0.02)	0.09 (0.004 – 0.37)
Clay-peat (n = 6)	GL	0.20 (0.06 – 0.58)	0.20 (0.06 – 0.52)	0.44 (0.11 – 1.26)	0.26 (0.07 – 0.62)	0.95 (0.02 – 2.62)	2.00 (0.56 – 3.61)
Peat (n = 1)	GL	0.15	0.15	0.23	0.31	0.07	0.58
Sand (n = 5)	MF	0.05 (0.04 – 0.06)	0.07 (0.03 – 0.09)	0.23 (0.10 – 0.50)	0.25 (0.12 – 0.48)	0.17 (0.06 – 0.29)	0.45 (0.37 – 0.60)

Table A.1. Continued.

Soil type	Land use[a]	Fe (mg L^{-1})[b]		Al (mg L^{-1})[b]		Mn (mg L^{-1})[b]	
		Moist soil	Dried soil	Moist soil	Dried soil	Moist soil	Dried soil
Clay (n = 3)	MF	0.01 (0.013 – 0.014)	0.01 (0.004 – 0.022)	0.02 (0.01 – 0.03)	0.01	0.01 (0.001 – 0.03)	0.15 (0.02 – 0.32)
Clay-peat (n = 6)	MF	0.15 (0.07 – 0.30)	0.20 (0.03 – 0.30)	0.32 (0.11 – 0.62)	0.37 (0.01 – 0.57)	0.53 (0.005 – 2.08)	0.74 (0.03 – 2.02)
Peat (n = 1)	MF	0.18	0.26	0.73	0.81	2.99	3.14
Sand (n = 5)	PF	0.03 (0.005 – 0.05)	0.05 (0.01 – 0.07)	0.19 (0.01 – 0.33)	0.25 (0.01 – 0.45)	0.38 (0.001 – 1.15)	0.61 (0.01 – 1.28)
Clay (n = 11)	PF	0.01 (0.003 – 0.02)	0.01 (0.003 – 0.03)	0.02 (0.002 – 0.16)	0.03 (0.003 – 0.19)	0.06 (0.003 – 0.28)	0.13 (0.002 – 0.77)

[a] Land use consists of grassland (GL), maize field (MF) and potato field (PF).

[b] The soil samples that have soluble Fe, Al and Mn concentrations below the determination limit are excluded from the table.

Table A.2. Nitrate plus nitrite concentration in 0.01 M $CaCl_2$ extraction of moist and dried selected soils from grassland and potato field presented as the mean and (range) per soil type.

Soil type	Land use[a]	$NO_3 + NO_2$ (mg L^{-1})	
		Moist soil	Dried soil
Sand (n = 11)	GL	Not measured	1.76 (0.67 – 3.64)
Clay (n = 6)	GL	Not measured	5.12 (0.58 – 7.70)
Clay-peat (n = 4)	GL	Not measured	4.88 (0.61 – 7.38)
Sand (n = 5)	PF	9.26 (1.92 – 20.91)	9.01 (1.67 – 21.11)
Clay (n = 11)	PF	10.80 (5.08 – 20.01)	10.71 (5.08 – 19.81)

[a] Land use consists of grassland (GL), maize field (MF) and potato field (PF).

Table A.3. Ratio of total soluble Se to DOC and ratio of total soluble Cu to DOC in 0.01 M $CaCl_2$ extraction of moist and dried soils presented as the mean and standard deviation per soil type. (Each soil sample was analysed without replicate, except for seven selected samples that were analysed in duplicate.)

Soil type	Land use[a]	Ratio Se:DOC µg g^{-1}		Ratio Cu:DOC mmol kg^{-1}	
		Moist soil	Dried soil	Moist soil	Dried soil
Sand (n = 13)	GL	15 ± 4[b]	30 ± 12	10 ± 4[b]	6 ± 6
Clay (n = 7)	GL	32 ± 13[b]	44 ± 15	10 ± 6[b]	7 ± 4
Clay-peat (n = 6)	GL	11 ± 4	34 ± 6	8 ± 3	3 ± 2
Peat (n = 1)	GL	8	27	3	0.8
Average		**18 ± 12**	**34 ± 13**	**9 ± 4**	**5 ± 3**
Sand (n = 5)	MF	13 ± 4[b]	34 ± 18	6 ± 2	3 ± 0.9
Clay (n = 3)	MF	36 ± 5	41 ± 18	9 ± 2	6 ± 4
Clay-peat (n = 6)	MF	10 ± 5	22 ± 11	4 ± 2	2 ± 1
Peat (n = 1)	MF	11	24	3	0.9
Average		**17 ± 11**	**30 ± 16**	**5 ± 3**	**3 ± 2**
Sand (n = 5)	PF	27 ± 10	38 ± 7	10 ± 4	7 ± 2
Clay (n = 11)	PF	78 ± 30	77 ± 33	18 ± 10	13 ± 5
Average		**62 ± 35**	**64 ± 33**	**15 ± 9**	**11 ± 5**

[a] Land use consists of grassland (GL), maize field (MF) and potato field (PF).

[b] The moist soil samples that have Se and/or DOC concentrations below the detection limits are excluded from the table.

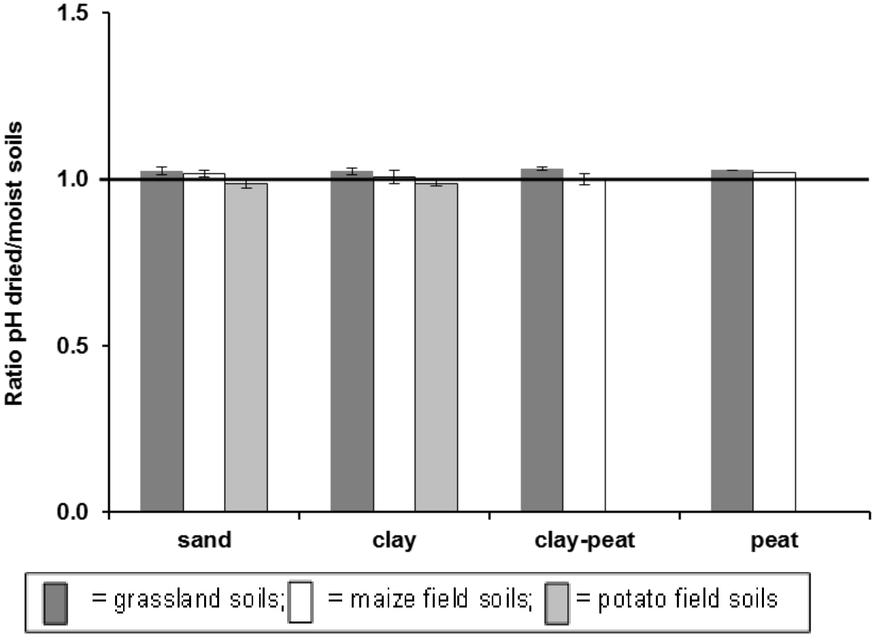

Fig. A.1. Drying effect on pH measured in 0.01 M $CaCl_2$ extraction. The horizontal line indicates a ratio = 1.

Chapter 3

Selenium Speciation and Extractability in Dutch Agricultural Soils

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Published as ‘Selenium speciation and extractability in Dutch agricultural soils’ in Science of the Total Environment, 2015, 532: 368-382

Abstract

The study aimed to understand selenium (Se) speciation and extractability in Dutch agricultural soils. Top soil samples were taken from 42 grassland fields and 41 arable land fields in the Netherlands. Total Se contents measured in aqua regia were between 0.12 and 1.97 mg kg^{-1} (on average 0.58 mg kg^{-1}). Organic Se after NaOCl oxidation-extraction accounted for on average 82% of total Se, whereas inorganic selenite (selenate was not measurable) measured in ammonium oxalate extraction using HPLC-ICP-MS accounted for on average 5% of total Se. The predominance of organic Se in the soils is supported by the positive correlations between total Se (aqua regia) and total soil organic matter content, and Se and organic C content in all the other extractions performed in this study. The amount of Se extracted followed the order of aqua regia > 1 M NaOCl (pH 8) > 0.1 M NaOH > ammonium oxalate (pH 3) > hot water > 0.43 M HNO_3 > 0.01 M $CaCl_2$. None of these extractions selectively extracts only inorganic Se, and relative to other extractions 0.43 M HNO_3 extraction contains the lowest fraction of organic Se, followed by ammonium oxalate extraction. In the 0.1 M NaOH extraction, the hydrophobic neutral (HON) fraction of soil organic matter is richer in Se than in the hydrophilic (Hy) and humic acid (HA) fractions. The organic matter extracted in 0.01 M $CaCl_2$, hot water and 0.1 M NaOH is in general richer in Se compared to the organic matter extracted in other extractions (HNO_3, ammonium oxalate, NaOCl, and aqua regia). Although the extractability of Se follows to a large extent the extractability of soil organic carbon, there is several time variations in the Se to organic C ratios, reflecting the changes in composition of organic matter extracted.

3.1 Introduction

Selenium (Se) is an essential micronutrient for humans and animals. Both Se shortage and surplus cause serious health problems. Selenium deficiency and toxicity show a regional pattern, which is related to the abundance and availability of Se in local soils, and consequently Se status in the regional food products. In dairy farming, Se intake by livestock is important in determining the health of livestock and the nutritional quality of its products, and further influences Se intake by humans. In agricultural fields with relatively low available Se, such as in Finland, Se fertilization has been applied to increase the amount of Se in grass and crops (Hartikainen, 2005). However, because the gap between Se deficiency and toxicity level is very small, for example in China the total Se content in soil for Se deficiency level is < 0.125 mg kg^{-1}, whereas the Se excess level is > 3 mg kg^{-1} (Tan *et al.*, 2002), therefore, an accurate assessment of Se availability in soil and the need and level of fertilization is essential. Up to now, the Se levels and chemical speciation in agricultural soils in the Netherlands have not been studied in detail yet and no reliable method is available for Se soil fertility evaluation and fertilization recommendation.

In soil, Se is present in both inorganic and organic forms. Inorganic Se exists in several oxidation states, ranging from +6 (selenate), +4 (selenite), 0 (elemental Se), to -2 (selenides) (Hartikainen, 2005). Under oxic condition, inorganic Se is present as selenate (SeO_4^{2-}), which is the most mobile Se form in soil and is weakly adsorbed on oxide surfaces (Haygarth, 1994). Selenate can be reduced into selenite (SeO_3^{2-}) at somewhat lower redox potential. Selenite has a higher affinity than selenate for adsorption to oxide surfaces, especially at low pH, thus reducing its availability for plants (Hartikainen, 2005). Under more strongly reducing conditions, selenite is reduced into elemental Se (Se^0) or selenides (Se^{2-}), which are insoluble and unavailable for plant uptake (Haygarth, 1994).

The chemical nature of organic-associated Se is not well understood yet. In environmental samples, organic Se species can be present as selenoamino acids, methylated compounds and selenoproteins (Pyrzyńska, 2002). Previous studies have reported different forms of soluble organic Se detected in soils, i.e. selenomethionine ($C_5H_{11}NO_2Se$), methane seleninic acids (CH_3SeOOH) and trimethyl selenium ion ($(CH_3)_3Se^+$) (Abrams and Burau, 1989; Yamada and Hattori, 1989; Abrams *et al.*, 1990a; Stroud *et al.*, 2012; Tolu *et al.*, 2014). In the solid organic matter, organic Se is present as Se associated with humic acids and fulvic acids, which may be in the forms of selenoamino acid incorporated into protein or peptides (Kang *et al.*, 1991b). Through microbial activity, organic Se can be transformed into inorganic forms upon mineralization, and vice versa inorganic Se, mainly selenite (+4) can be reduced and incorporated into soil organic matter (Gustafsson and Johnsson, 1994). In addition, plants or soil microorganisms can transform inorganic Se into volatile Se compounds, such as diselenides (RSeSeR) or dimethylselenide ($(CH_3)_2Se$) (Pyrzyńska, 2002; Hartikainen, 2005).

To be able to estimate (potentially) available Se for plant uptake, understanding the Se speciation in different soil phases (i.e. soil solution and soil solid phase) and key controlling factors is needed. In soil solution, Se is present as selenate and selenite anions as well as organic Se (Tolu *et al.*, 2011; Weng *et al.*, 2011; Stroud *et al.*, 2012). Soluble selenate and selenite are the major bioavailable chemical forms for plants, although some research showed that selenoamino acids such as selenomethionine and selenocystine can also be taken up (Abrams *et al.*, 1990b; Williams and Mayland, 1992; Kikkert and Berkelaar, 2013). For the anionic selenate and selenite, adsorption to metal (hydr)oxides and to the edges of clay minerals is the major mechanism that controls their solid solution distribution (Hamdy and Gissel-Nielsen, 1977; Vuori *et al.*, 1989). For most of organic Se, mineralization is required for the Se to become bioavailable. Regarding Se bioavailability, both the prevailing processes and controlling factors differ for soils in which either the inorganic or organic Se forms dominate. So far, no specific research related to Se speciation in Dutch agricultural soils has been done. We expect that the results of the current study can be used further in methodology development to assess Se availability in soils and can be embedded in Se fertilization recommendation.

Chemical soil extraction using various reagents is a commonly used approach to measure nutrient status in soils. For Se, both single step and sequential extractions have been proposed. Sequential extraction schemes fractionate Se into e.g. soluble Se, exchangeable Se, Se adsorbed to (hydr)oxides or carbonates, and Se bound to organic matter or humic compounds (Chao and Sanzolone, 1989; Tokunaga *et al.*, 1991; MacLeod *et al.*, 1998; Mao and Xing, 1999; Wang and Chen, 2003; Hagarová *et al.*, 2005). Single step extractions using (hot) water, mild salt solution or mild phosphate solution has been proposed to estimate soluble Se in soils (Yamada and Hattori, 1989; Johnsson, 1992; Keskinen *et al.*, 2009; Tolu *et al.*, 2011; Stroud *et al.*, 2012), whereas extractions using phosphate buffers and ammonium oxalate have been used to determine exchangeable Se (Alam *et al.*, 2000; Keskinen *et al.*, 2009; Tolu *et al.*, 2011) and Se adsorbed to metal oxides (Keskinen *et al.*, 2009), respectively. Extractions using NaOCl, 0.1 M NaOH, or a mixture of 0.1 M NaOH and 0.1 M $Na_4P_2O_7$ solution have been used to estimate Se associated with soil organic matter (Abrams *et al.*, 1990a; Kang *et al.*, 1991b; Tokunaga *et al.*, 1991; Gustafsson and Johnsson, 1992, 1994; Qin *et al.*, 2012). Relating the amount of Se extracted in different single extractions to bioavailability requires a detailed insight into the chemical forms extracted and the pools from which these forms originate.

The objectives of this study are (1) to obtain a detailed understanding in Se speciation in Dutch agricultural soils and the key influencing factors; and (2) to obtain more insight of the relatively amount, chemical forms and pools of Se extracted in several commonly used single step extractions.

3.2. Materials and methods

3.2.1 Soil samples

Soil samples were collected in 2009 from 15 grassland fields and 26 arable land fields and in 2011 from 27 grassland fields and 15 arable land fields in the Netherlands. The samples were taken from top soils, i.e. 0–10 cm for grassland samples and 0–20 cm for arable land samples. The samples were oven dried at 40^oC under forced air ventilation for 24 to 48 h, ground, and sieved over a 2 mm sieve. The collected soil samples cover a wide range of different soil types, which are named following Dutch custom, i.e. sand, clay, loess, reclaimed peat, peat and clay-peat soils. The basic soil characteristics are presented in Table 1.

3.2.2 Soil extractions

Three single step extractions, i.e. ammonium oxalate extraction, 1 M NaOCl (pH 8) oxidation-extraction and aqua regia digestion (Table 2), were performed to determine Se speciation in the soils (see Section 3.3.1). Seven single step extractions, i.e. 0.01 M $CaCl_2$, 0.43 M HNO_3, hot water, ammonium oxalate – oxalic acid (pH 3), 0.1 M NaOH, 1 M NaOCl (pH 8) and aqua regia (Table 2), were conducted to study Se extractability in the soils (see Section 3.3.2). The 0.01 M $CaCl_2$, 0.43 M HNO_3, and ammonium oxalate extractions and aqua regia digestion were performed on the 83 soil samples collected (Table 1). Hot water extraction was performed on 21 selected grassland soils taken in 2011, whereas 0.1 M NaOH and 1 M NaOCl extractions were performed on 5 selected grassland soils and 5 selected arable land soils taken in 2011. In all the extractions, soil suspensions were shaken using a reciprocating horizontal shaker at constant temperature of 20 ± 1^oC, except for hot water extraction and aqua regia digestion. In the hot water extraction, initially the suspensions were shaken using a reciprocating horizontal shaker at 125 rpm for 30 minutes at 20 ± 1^oC and centrifuged at 3500 rpm for 20 minutes. The supernatant was discarded to remove readily water soluble C from soils, which may come from animal excreta and soluble plant residues (Ghani *et al.*, 2003). Afterwards, the same amount of ultrapure water was refilled to the soil residue in

Table 1. Basic soil characteristics (mean ± standard deviation).

Soil type	pH-$CaCl_2$	Organic matter (%)	Clay (%)	Total Se aqua regia ($mg\ kg^{-1}$)	Fe-oxalate ($g\ kg^{-1}$)[a]	Al-oxalate ($g\ kg^{-1}$)[a]	Fe-DCB ($g\ kg^{-1}$)[b]	Al-DCB ($g\ kg^{-1}$)[b]
Grassland								
Sand (n = 23)	5.3 ± 0.8	7.2 ± 3.7	2.8 ± 1.9	0.36 ± 0.26[c] (n = 20)[d]	2.6 ± 3.1	1.1 ± 0.5	3.2 ± 3.5	1.0 ± 0.4
Clay (n = 8)	6.7 ± 0.7	6.6 ± 3.5	24.7 ± 16.4	0.43 ± 0.30	4.9 ± 3.0	0.6 ± 0.4	10.2 ± 6.3	0.6 ± 0.3
Reclaimed peat (n = 3)	4.9 ± 0.2	13.9 ± 7.6	1.4 ± 0.6	0.65 ± 0.39	2.4 ± 1.4	1.2 ± 0.4	3.1 ± 2.1	1.2 ± 0.6
Peat (n = 2)	5.0 ± 0.2	22.2 ± 4.6	6.8 ± 0.7	1.44 ± 0.42	17.9 ± 0.6	2.0 ± 0.2	27.8 ± 4.7	2.3 ± 0.1
Clay-peat (n = 6)	5.2 ± 0.3	31.0 ± 17.3	44.8 ± 14.6	1.48 ± 0.36	14.0 ± 0.9	3.2 ± 1.5	14.5 ± 2.4	2.6 ± 1.6
Arable land								
Sand (n = 16)	5.2 ± 0.6	5.6 ± 3.3	3.0 ± 2.7	0.31 ± 0.05[c] (n = 13)[d]	1.7 ± 1.4	1.0 ± 0.3	2.3 ± 2.6	0.9 ± 0.3
Clay (n = 12)	6.8 ± 0.7	4.3 ± 1.9	28.8 ± 13.9	0.53 ± 0.24[c] (n = 8)[d]	4.8 ± 2.8	0.6 ± 0.3	10.1 ± 4.4	0.8 ± 0.1
Loess (n = 4)	6.7 ± 0.4	2.2 ± 0.5	16.5 ± 3.1	<LOQ[e]	2.4 ± 0.3	0.8 ± 0.02	8.4 ± 0.4	1.0 ± 0.1
Reclaimed peat (n = 2)	5.1 ± 0.2	10.5 ± 0.1	1.4 ± 1.2	0.35 ± 0.01	1.2 ± 0.1	1.3 ± 0.02	1.5 ± 0.2	1.2 ± 0.1
Peat (n = 1)	4.7	26.5	10.7	1.09	7.6	2.4	6.0	1.7
Clay-peat (n = 6)	5.2 ± 0.7	25.2 ± 12.5	26.2 ± 11.5	0.96 ± 0.29	8.5 ± 2.4	2.0 ± 0.8	10.0 ± 2.6	1.9 ± 0.9

[a] Measured in ammonium oxalate – oxalic acid extraction; [b] Measured in dithionite-citrate bicarbonate (DCB) extraction.

[c] Samples that have Se concentrations below the determination limits were excluded from the table.

[d] The actual number of soil samples included in the calculation after excluding the samples that have Se concentrations below the determination limit.

[e] Limit of quantification or determination limit.

Table 2. An overview of the single step soil extractions performed in this study and Se pool they represent as suggested in the references.

Extraction	Number of samples	Possible pool representing	Method adopted from	SSR[a]	Extraction/digestion (h)	Centrifugation (rpm and min)	Filter	Parameter measured
0.01M $CaCl_2$	83	Soluble (Houba *et al.*, 2000)	Houba *et al.* (2000)	1:10	2	3000; 10	0.45 µm	Se, DOC, pH
0.43 M HNO_3[b]	83	Reactively adsorbed	-	1:10	4	3000; 10	Paper	Se, DOC
Hot water	21	Labile organic matter associated (Ghani *et al.*, 2003)	Ghani *et al.* (2003)	1:10	16 (80^{o}C)	3500; 20	0.45 µm	Se, DOC, pH
Ammonium oxalate–oxalic acid (pH 3)	83[c]	Adsorbed to amorphous Fe and Al oxides (Keskinen *et al.*, 2009)	McKeague and Day (1966)	1:20	2 (in the dark)	3000; 10	No	Se, UV, Fe, Al, Mn
0.1 M NaOH	10[d]	Humic substances associated (Kang *et al.*, 1991b)	Van Zomeren and Comans (2007)	1:10	overnight	3500; 20	0.45 µm	Se, DOC
1M NaOCl (pH 8)	10	Organic Se (Tokunaga *et al.*, 1991)	Kaiser and Guggenberger (2003)	1:50	6; repeated 5 times	2930; 30	No	Se, Fe, Al, Mn
Aqua regia digestion	83	(Pseudo) total content (Keskinen *et al.*, 2009)	-	1:100	Overnight, plus 2 h in a microwave at 170^{o}C	No	Paper	Se

[a] Soil to solution ratio.
[b] Especially for soil samples that have pH ≥ 7, about 0.2 mL of 5 M HNO_3 was added to neutralise the carbonates in the soil samples.
[c] 24 out of 83 samples were fractionated on HPLC.
[d] All 10 samples of 0.1 M NaOH extraction were further fractionated with acidification and DAX-8 resin.

the same tubes and shaken by hand for a while to suspend the soil in the water. The capped tubes were placed in the oven at 80°C for 16 hours. In aqua regia digestion, around 1.00 g of soil was added to 2 mL of ultrapure water and subsequently added to 8 mL of a mixture of HCl and HNO_3 (volume ratio HCl : HNO_3 is 3:1). The suspensions were left to stand overnight under room temperature, followed by boiling in a microwave with a closed system at 170°C for 2 h. Afterwards, the suspensions were filtered with ash-free paper filters (Whatman 589/3 nr. 10300212) and filled up to 100 mL volume by adding ultrapure water. In 0.01 M $CaCl_2$, hot water and 0.1 M NaOH extractions, the supernatants were filtered with a 0.45 μm pore-size syringe filter (Whatman Aqua 30/0.45 CA), whereas in the 0.43 M HNO_3 extraction, as well as in aqua regia digestion, the supernatants were filtered with ash-free paper filters (Whatman 589/3 nr. 10300212). In the ammonium oxalate and NaOCl extractions the suspensions were only centrifuged and the supernatants were not filtered. All the solutions used in the soil extractions and analyses were prepared using ultrapure water with electrical conductivity resistance of 18.2 mΩ.

The filtrates or supernatants obtained from all the extractions were measured for Se concentrations. Dissolved organic carbon (DOC) concentrations were measured in the 0.01 M $CaCl_2$, 0.43 M HNO_3, hot water and 0.1 M NaOH extractions. Concentration of DOC in ammonium oxalate extraction was derived indirectly from UV-absorbance measured at 254 nm wavelength after diluting the extracts 50 times. Weishaar *et al.* (2003) suggested that UV absorbance at 254 nm wavelength was correlated to ferric ion (Fe^{3+}) concentration in the samples, therefore, a correction for Fe concentration in the ammonium oxalate soil extracts was made. The UV-absorbance of calibration solutions containing a mixture of humic acid material purified from a forest soil (Tongbersven) in the Netherlands and ferric ion (Fe^{3+}) in a 50 times diluted ammonium oxalate solution were measured as well. Concentrations of DOC in the ammonium oxalate soil extracts were estimated using a multiple linear regression equation derived from the calibration solutions, which relates the UV-absorbance to DOC and Fe^{3+} concentrations:

$$A_{254} = a*[DOC] + b*[Fe^{3+}]$$

where A_{254} is the total UV absorbance at 254 nm (cm^{-1}); *[DOC]* is DOC concentration (mg L^{-1}); $[Fe^{3+}]$ is soluble Fe^{3+} concentration (mg L^{-1}); a and b are coefficients of the regression. In the ammonium oxalate extraction, concentrations of Fe, Al and Mn were measured as well, which represent the amount of amorphous (hydr)oxides of the corresponding metals (Table 1). The amount of organic C oxidized in NaOCl oxidation-extraction (n = 10) was derived from the difference between total organic C in the original soil samples before NaOCl oxidation-extraction and residual organic C in the residue of soil samples after NaOCl oxidation-extraction. Before the analysis of organic C, the soil residues from NaOCl oxidation-extraction were washed with ultrapure water to remove chloride till the supernatants reach electrical conductivity < 40 µS cm^{-1}. Total soil organic C content in all the soil samples (n = 83) was calculated from the measured soil organic matter content assuming that soil organic matter contains 50% organic C. The percentage of organic C extracted in each extraction was relative to this calculated total organic C, except that the percentage of organic C in NaOCl oxidation-extraction was relative to the measured total organic C. The results showed that the total organic C measured in the 10 soil samples used in NaOCl oxidation-extraction is on average about 54% of the measured soil organic matter content of the corresponding soil samples, which is in line with the assumption used to estimate total organic C content in all soil samples (n = 83), i.e. soil organic matter contains 50% organic C.

Dithionite-citrate bicarbonate (DCB) extraction (soil to solution ratio 1:100) was also performed for the 83 soil samples to determine total Fe- and Al-(hydr)oxides in soils. The procedure of the extraction was described elsewhere (Hiemstra *et al.*, 2010). About 0.30 g soil sample was added to 30.0 mL extraction solution containing 0.66 M citrate, 0.11 M sodium bicarbonate, and 0.64 g sodium dithionite. The suspensions were shaken overnight (16 h) at constant temperature of 20 ± 1°C. Afterwards, the suspensions were centrifuged at 3000 rpm for 15 minutes. Concentrations of Fe and Al in the supernatants were measured.

3.2.3 Selenium speciation analyses

3.2.3.1 Inorganic Se in ammonium oxalate extraction measured using HPLC - ICP-MS

A High Performance Liquid Chromatography (HPLC) system with an anion exchange column coupled online with High Resolution ICP-MS was used to separate inorganic selenate, selenite and organic Se in ammonium oxalate extracts. Operational conditions modified from Guerin *et al.* (1997) were summarized in Table 3.

Table 3. Operational condition of HPLC coupled online with ICP-MS.

1	**HPLC system:**	
	a. Pump	Shimadzu LC 6A
	b. Pre-column	Hamilton Guard column PRP-X100; 50 mm x 4 mm
	c. Analytical/main column	Hamilton PRP-X100 anion exchange column; 10 µm; 250 mm x 4.1 mm
	d. Mobile phase	25 mM $(NH_4)_2HPO_4$ pH 8.5
	e. Flow rate	1 mL minute^{-1}
	f. Injected sample volume	100 µL
	g. Separation time in the column	15 minutes
2.	**ICP-MS system:**	Thermo Scientific, Element2; high resolution; isotope ^{78}Se; reading every 2.8 s.

Stock solutions of selenite and selenate (each 1000 mg L^{-1}) were prepared from sodium selenite pentahydrate ($Na_2SeO_3.5H_2O$) and sodium selenate decahydrate ($Na_2SeO_4.10H_2O$) (analytical reagents, Merck) with ultrapure water and stored at 4^{o}C. The stock solutions are stable for one month in storage (Guerin *et al.*, 1997). A 25 mM $(NH_4)_2HPO_4$ solution (analytical reagent, Merck) was adjusted to pH 8.5 by adding 25% ammonia solution (NH_4OH, Merck) and used as eluent. The eluent solution was filtered through a 0.20 µm cellulose membrane using a vacuum filter and subsequently degassed during 15 minutes before being pumped into the HPLC system. The filtration and degassing processes were conducted in order to remove particles and gas bubbles in the solutions that can clog the HPLC column. A 70% methanol solution (HPLC grade, Merck) was used for cleaning the HPLC system and the column each time after analysis. In addition, 100% methanol with 1% 6 M HNO_3 was used to clean the column before storage and to regenerate the column before use.

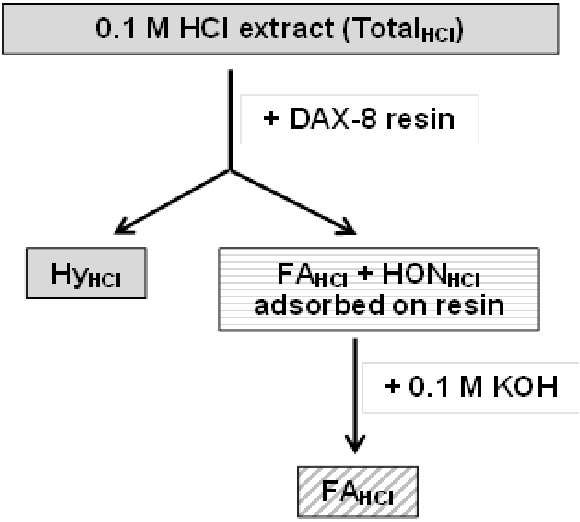
Standard solutions containing 0, 2, 5, and 10 µg L^{-1} Se (in the form of selenite or selenate) were prepared by diluting the stock solutions in the ammonium oxalate – oxalic acid (pH 3) solution on a daily basis. Before HPLC analysis, initial total selenite and selenate concentrations in the standard solutions were measured off line using High Resolution ICP-MS as a check. The recovery of selenite and selenate in the standard solutions analysed using HPLC was on average 100% and 97%, respectively, which were calculated from the peak area.

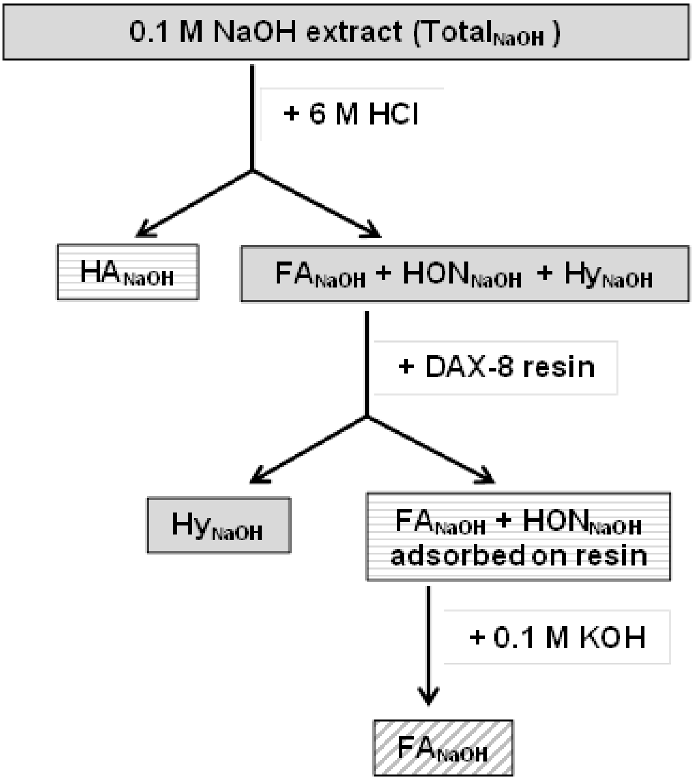
Nineteen soil samples from grassland and five soil samples from arable land taken in 2011 were selected from the sample set (Table 1). The soil samples were extracted using ammonium oxalate - oxalic acid solution as described in Section 3.2.2 (Table 2). The HPLC analysis was performed on the same day as the extraction. The extracts were stored in the fridge (4^oC) until the analysis in order to prevent any changes in Se speciation. A preliminary test showed that the Se species in the ammonium oxalate soil extracts were stable when the extracts were stored at cold temperature (4^oC) for a few hours after soil extraction, and all samples were analysed on the same day of the extraction. Concentrations of Se species in the soil extracts were calculated using an equation derived from the calibration curve of peak areas measured versus the known concentrations of selenite or selenate in the standard solutions with ammonium oxalate – oxalic acid (pH 3) matrix. Both the standard solutions and soil extracts were analysed on the HPLC-ICP-MS without dilution. Four soil samples analysed in duplicate showed good reproducibility with variation coefficients of 4% to 10% (as selenite) (on average 7%). Initial total Se concentration in the soil extracts was also measured before the HPLC analysis.

3.2.3.2 Fractionation of organic Se in 0.1 M NaOH extraction using acidification and DAX-8 resin

Fractionation of organic matter and Se in 0.1 M NaOH extraction was conducted in order to understand the distribution of Se in organic matter fractions. Five soil samples from grassland and five samples from arable land taken in 2011 were selected from the sample set (Table 1). The procedure of organic matter and Se fractionation was adopted from the rapid batch procedure of organic matter fractionation in solid materials (Van Zomeren and Comans, 2007). The soil samples were first extracted using 0.1 M HCl (soil to solution ratio 1:10) for 1 h at 180 rpm to remove carbonates, Fe-, Al-, and Mn-(hydr)oxides. After that, the soil suspension was centrifuged at 3500 rpm for 20 minutes and filtered with a 0.45 µm pore-size syringe filter (Whatman Aqua 30/0.45 CA). The supernatant of the 0.1 M HCl extraction was collected for further fractionation analysis. The soil residue was subsequently extracted with 0.1 M NaOH (soil to solution ratio 1:10 and pH ≥ 12) under N_2 atmosphere by shaking the soil suspension over night at 180 rpm. Afterwards, the soil suspension was centrifuged at 3500 rpm for 20 minutes and filtered with a 0.45 µm pore-size syringe filter (Whatman Aqua 30/0.45 CA). The supernatant of the 0.1 M NaOH extraction was collected for further fractionation analysis.

Organic matter in 0.1 M HCl extraction was fractionated into fulvic acids (FA), hydrophilic acids (Hy) and hydrophobic organic neutral (HON) using DAX-8 resin, whereas organic matter in 0.1 M NaOH extraction was fractionated into humic acids (HA), FA, Hy and HON using acid precipitation and DAX-8 resin (Fig. 1). Before fractionating organic matter in 0.1 M HCl extraction, subsamples were taken from the extracts for analysis ($\text{Total}_{\text{HCl}}$), representing the sum of FA, Hy, and HON fractions. To 35 mL of the remaining extract about 7.0 gram of DAX-8 resin was added to adsorb FA and HON ($(\text{FA+ HON})_{\text{HCl}}$), shaken at 220 rpm for 1 h, and filtered over 0.45 µm pore-size syringe filter (Whatman Aqua 30/0.45 CA). Subsamples were taken from the filtrate for analysis, representing the Hy fraction (Hy_{HCl}). About 25 mL 0.1 M KOH was added to the resin to desorb FA from the resin, shaken at 220 rpm for 1 h, and filtered over 0.45 µm pore-size syringe filter (Whatman Aqua 30/0.45 CA). Subsamples were taken from the filtrate for analysis, representing the FA fraction. The desorption of FA from the resin was repeated until the solution and resin are clear in colour, i.e. matching the blank solution and clean resin. Before fractionating organic matter in 0.1 M NaOH extraction, the soil extracts were diluted 24 times due to high organic C concentration in the soil extracts. Subsamples of the diluted extracts were taken for analysis, representing the sum of HA, FA, Hy, and HON fractions ($\text{Total}_{\text{NaOH}}$). About 40 mL of the diluted 0.1 M NaOH soil extract was adjusted to pH 1.0 to 1.5 with 6 M HCl and let stand overnight to precipitate HA fraction. Then the extract was centrifuged at 3500 rpm for 20 minutes to separate the HA pellet (HA_{NaOH}) and supernatant containing FA, Hy and HON fractions ($(\text{FA+Hy+HON})_{\text{NaOH}}$). The precipitated HA was re-dissolved in 0.1 M KOH for analysis, representing the HA fraction. About 35 mL supernatant containing FA, Hy and HON fractions was further fractionated using DAX-8 resin following similar procedure as fractionation in 0.1 M HCl extraction. From this fractionation, subsamples representing Hy (Hy_{NaOH}) and FA fractions were collected for analysis. Before used in the fractionation, the filters (Whatman Aqua 30/0.45 CA) were washed with ultrapure water to reduce DOC release.





■ : the measured Se or organic C concentration is used for that fraction

▤ : the Se or organic C concentration of that fraction is derived from mass balance

▨ : Se and organic C concentrations in this fraction were measured, but due to their oncentrations were too low, this fraction was not considered separately in the results.

Fig. 1. Procedure of organic matter and Se fractionation in 0.1 M NaOH (and 0.1 M HCl) soil extraction(s) using DAX-8 resin as adopted from Van Zomeren and Comans (2007).

Selenium and organic C concentrations were measured in all the subsamples collected in the fractionation analysis in 0.1 M HCl and 0.1 M NaOH extractions. The amounts of Se and organic C measured for the same fractions in the 0.1 M HCl and 0.1 M NaOH extractions were then summed (see equations below). However, fraction of Se and organic C measured in the summed FA fraction were only about 2% of total Se and 3% of total organic C in 0.1 M HCl plus 0.1 M NaOH extractions. In addition, the concentrations of Se and organic C measured in the FA fraction were mostly very low and have large uncertainties. Therefore, for a better accuracy, the data presented in this study did not distinguish between FA and HON fractions, which have in common that both remain soluble at low pH and bind to DAX-8 resin. Selenium concentrations measured in the HA fraction were also mainly below the determination limit and have a large uncertainty. On the other hand, organic C in the HA fraction was measurable. For a better accuracy and consistency, Se and organic C in the HA fraction were calculated from mass balance (see equations below). To summarize, Se and organic C in the Hy fraction were derived from the measured values (see equations below), whereas the concentrations of Se and organic C in the HA and summed FA and HON fractions were calculated from the mass balance (see equations below). For clarity, the derivations of different fractions for Se and organic C are represented in the expressions below:

HCl extraction:

$\text{Total}_{\text{HCl}}$ = measured

Hy_{HCl} = measured

$(\text{FA+HON})_{\text{HCl}}$ (calculated) = $\text{Total}_{\text{HCl}}$ (measured) – Hy_{HCl} (measured)

NaOH extraction:

$\text{Total}_{\text{NaOH}}$ = measured

Hy_{NaOH} = measured

HA_{NaOH} (calculated) = $\text{Total}_{\text{NaOH}}$ (measured) – $(\text{FA+Hy+HON})_{\text{NaOH}}$ (measured)

$(\text{FA+HON})_{\text{NaOH}}$ (calculated) = $(\text{FA+Hy+HON})_{\text{NaOH}}$ (measured) – Hy_{NaOH} (measured)

Sum of HCl and NaOH extraction:

$\text{Total}_{\text{sum}}$ = $\text{Total}_{\text{HCl}}$ (measured) + $\text{Total}_{\text{NaOH}}$ (measured)

Hy_{sum} = Hy_{HCl} (measured) + Hy_{NaOH} (measured)

$(FA+HON)_{sum}$ = $(FA+HON)_{HCl}$ (calculated) + $(FA+HON)_{NaOH}$ (calculated)

HA_{sum} = HA_{NaOH} (calculated)

Fraction calculation:

$\%Hy = Hy_{sum}/Total_{sum}*100$

$\%(FA+HON) = (FA+HON)_{sum}/Total_{sum}*100$

$\%HA = HA_{sum}/Total_{sum}*100$

3.2.4 Chemical analysis

3.2.4.1 Chemical analysis methods

Concentrations of Se in all extractions and concentrations of Fe, Al and Mn in 1 M NaOCl extraction were measured using High Resolution ICP-MS (Thermo Scientific, Element2). Concentrations of Fe, Al and Mn in ammonium oxalate extraction and concentrations of Fe and Al in DCB extraction were measured using ICP-AES (Thermo Scientific, Iris Advantage). For the ICP-MS and ICP-AES analysis, the soil extracts have to be diluted sometimes due to a too high salt level. The soil extracts were diluted using 0.14 M HNO_3 or ultrapure water depending on the matrix of the soil extracts and solubility and concentration of organic C extracted (to avoid organic C precipitation). The matrix effect on the measurement was corrected by preparing standard solutions in the same background as in the soil extracts. Concentrations of DOC in all extractions were measured using a segmented flow analyser (SFA) (3039 Skalar, the Netherlands), except that in 0.43 M HNO_3 extraction DOC concentration was measured using a TOC analyser (Shimadzu). The UV-absorbance of the ammonium oxalate extracts was determined using UV-spectrophotometer (Beckman Coulter or Genesys 10S Thermo Scientific). pH was measured using a pH meter (Radiometer Analytical PHM210) with a glass electrode (Beckman 511084). In the 10 selected soils used for NaOCl oxidation-extraction, total C in the original soil samples and their residues from NaOCl extraction was analysed using a CHN elementary analyser (Leco), whereas total inorganic C was measured only in the original soil samples using Scheibler method (Tatzber *et al.*, 2007). The total inorganic C in the original soil samples was very low, i.e. 0 to 3% of total C in soils (on average 0.4%, n = 10), therefore, the total inorganic C in the residues should be negligible. Total organic matter content in all the soil samples was analysed using loss on ignition (LOI) (Ball, 1964; NEN5754, 1992) or near infrared spectroscopy (NIR) (Reeves III, 2010; Soriano-Disla *et al.*, 2014).

3.2.4.2 Quality control for Se measurement

One challenge faced in measuring Se concentrations in the soil extractions is caused by the low amounts. To control the quality of the data, the detection and determination limits were calculated from the blanks of each batch of the extractions. The detection limits were calculated as 3 times the standard deviations of measurements of blanks, whereas the determination limits were calculated as 3.3 times the detection limits. Concentrations of Se in each extraction were compared to the detection and determination limits of the corresponding extraction. The results show that Se concentrations in the extractions are above the detection and determination limits (data not shown), except that in certain samples of the 0.43 M HNO_3 extraction (2 out of the 83 samples), ammonium oxalate extraction (24 out of 83 samples) and aqua regia (14 out of 83 samples) Se concentrations were found in between the detection and determination limits. These data contain relatively large uncertainties compared to the other data and should be handled with more care. To avoid clogging of the ICP-MS system, soil extracts had to be diluted in certain cases, depending on the matrix composition. The ammonium oxalate extraction has the largest dilution factor (i.e. 30 times) compared to that of other extractions, which resulted in a larger number of samples with a Se concentration below the determination limit. The variation of Se concentrations between duplicate (including data both above determination limit and between the detection and determination limits) is on average 2 – 15% (data not shown). The deviation between duplicate is relatively small for the 0.01 M $CaCl_2$, hot water, ammonium oxalate, 0.1 M NaOH and 1 M NaOCl extractions and aqua regia digestion (i.e. 2 – 9%) compared to that of 0.43 M HNO_3 extraction (15%). Tolu *et al.* (2011) also found a low reproducibility of Se extracted with 1 M HNO_3 (relative standard deviation 20%).

In the Results and discussion section below only the data that were above the determination limit were included. Taking into account the soil mass, extractant volume and dilution factor in each extraction, the ranges of determination limits for Se are 0.50–2.06 µg kg^{-1} in 0.01 M $CaCl_2$ extraction; 4.21–6.20 µg kg^{-1} in 0.43 M HNO_3 extraction; 2.56 µg kg^{-1} in hot water extraction; 17.82–40.33 µg kg^{-1} in ammonium oxalate extraction; 2.12-118.79 µg kg^{-1} in 0.1M NaOH extraction; 111.06–163.40 µg kg^{-1} in NaOCl extraction and 69.94–261.96 µg kg^{-1} in aqua regia digestion. The ranges of determination limits for Se in the organic matter fractionation analysis are 2.12–3.17 µg kg^{-1} in 0.1 M HCl extraction and 0–118.79 µg kg^{-1} in 0.1 M NaOH extraction. In the Se speciation analysis of ammonium oxalate soil extraction using HPLC - ICP-MS, the determination limit was derived from the concentration of selenite or selenate peak in the blank of ammonium oxalate solution in each batch of the analysis, i.e. 0–2.21 µg kg^{-1} for selenite and 0–7.59 µg kg^{-1} for selenate.

3.3 Results and discussion

3.3.1 Contribution of inorganic and organic Se to total Se content in soils

3.3.1.1 Total Se content in soils

Total Se contents measured in aqua regia are between 0.12 and 1.97 mg kg^{-1} (on average 0.62 mg kg^{-1}) for grassland soils and between 0.20 and 1.20 mg kg^{-1} (on average 0.53 mg kg^{-1}) for arable land soils (Table 6). The total Se content in the soils analysed in this study is within the range reported by Ure and Berrow (1982) *in* Yamada *et al*. (2009), i.e. 0.03 to 2.0 mg kg^{-1} with an average of 0.40 mg kg^{-1}, for 1623 soils throughout the world. Gupta and Gupta (2000) reported that in general, a total soil Se < 0.6 mg kg^{-1} is considered deficient. About 75% of the Dutch agricultural soils analysed in this study contain less than 0.6 mg Se kg^{-1}. The soils

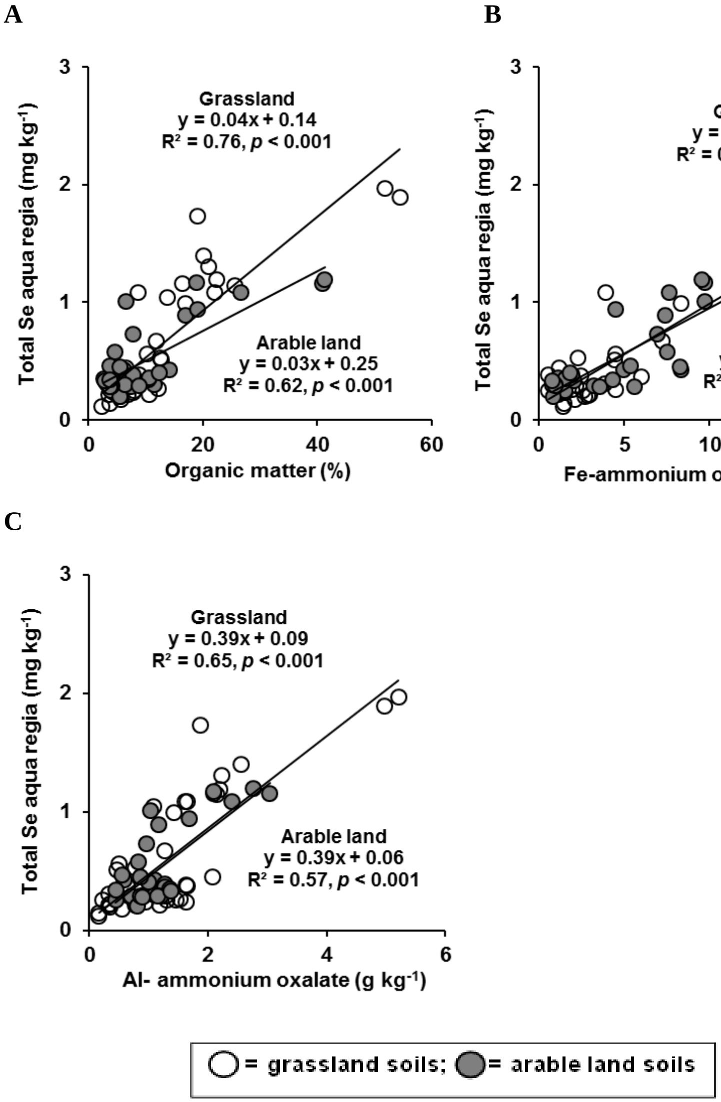


Fig. 2. Correlations between total Se content (aqua regia) and soil organic matter content (A), amorphous Fe-(hydr)oxide content (B) and amorphous Al-(hydr)oxide content as measured in ammonium oxalate extraction (C).

that have a total Se > 0.6 mg kg^{-1} consist of soils with a high organic matter content (7 to 54%) (Fig. 2A). Thus, based on the criteria proposed by Gupta and Gupta (2000), most of the agricultural soils in the Netherlands analysed in this study are to be considered Se deficient. Other areas of Europe, such as Belgium and Sweden also have low levels of Se contents in the agricultural soils, i.e. on average 0.30 mg kg^{-1} (Wang and Sippola, 1990; Johnsson, 1992; De Temmerman *et al.*, 2014). Soil organic matter and amorphous Fe- and Al-(hydr)oxides content show a strong correlation with total Se content (aqua regia) (Fig. 2A, B and C), whereas pH ($R^2 = 0.11$, $p < 0.05$ and $R^2 = 0.004$, $p > 0.05$ for grassland and arable land soils, respectively; data not shown) and clay content ($R^2 = 0.30$, $p < 0.01$ both for grassland and arable land soils; data not shown) show a weak correlation with total Se content (aqua regia).

3.3.1.2 Inorganic Se in soils derived from ammonium oxalate extraction

In ammonium oxalate extraction, amorphous metal (hydr)oxides can be dissolved due to the low pH (pH 3 ± 0.1) and metal-complexation of oxalate. Therefore, Se extracted in the ammonium oxalate has been considered as inorganic Se (selenate, selenite) adsorbed to amorphous metal (hydr)oxides (Keskinen *et al.*, 2009). However, in addition to Fe, Al and Mn, a large amount of organic matter was also extracted, i.e. on average 12% of total soil organic matter in grassland soils and 14% in arable land soils (Table 6). Bremmer and Lees (1949) suggested that oxalate has the same efficiency as pyrophosphate in extracting organic matter in soil due to removal of calcium in the form of insoluble calcium-oxalate precipitates. In addition, dissolution of metal (hydr)oxides during the extraction will lead to the release of organic matter that has been bound to the oxide surfaces. The relatively large amount of organic matter in the ammonium oxalate extraction suggests that a significant fraction of Se in this extraction may occur in an organic form.

To determine Se distribution among the inorganic selenate and selenite and organic Se in the ammonium oxalate extraction, nineteen soil samples from grassland and five soil samples from arable land were analysed using anion exchange HPLC coupled online with High Resolution ICP-MS. For all the 24 soil samples, a clear selenite peak was found in the chromatogram, but neither a selenate peak nor other obvious Se peaks were measured (Fig. A.1). The absence of selenate in the ammonium oxalate extraction can be caused by a very low concentration in combination with weak adsorption to oxide surfaces, therefore, the peak of selenate was hardly measurable using HPLC - ICP-MS (Fig A.1). Given that only inorganic selenite was recovered and identified, we assume that organic Se was not eluted from the column, which is probably due to a strong retention of the extracted organic matter in the (pre)column. Therefore, the organic Se in the extraction was calculated as the difference between total Se concentration and selenite concentration in the extraction (Table 4). On average 30% of Se in the ammonium oxalate extraction of both grassland soils and arable land soils is present as inorganic selenite and the rest on average 70% is in the form of organic Se (Table 4). These results indicate that the ammonium oxalate extraction is not a direct method to estimate the amount of inorganic selenate or selenite bound to metal oxides. The total selenite in both grassland and arable land soils determined in the ammonium oxalate extraction accounts for on average only 5% of total Se content (aqua regia) (Table 4).

The percentage of inorganic selenite over total Se in the ammonium oxalate extraction is in general decreasing in the order of clay soil > sandy soil > clay – peat soil > peat soil (Table 4). This trend corresponds to the amount of mineral surfaces that can bind selenite (clay, amorphous Fe-(hydr)oxide) relative to that of soil organic matter (Table 1). The larger the amount of clay and amorphous Fe-(hydr)oxide, the higher the amount of selenite extracted with ammonium oxalate, indicating that the inorganic selenite in soils is probably mainly associated with the soil mineral surfaces, i.e. clay and amorphous Fe-(hydr)oxide (Fig. 3A and B). For these soils, the amount of selenite in soils is somewhat better correlated with clay content ($R^2 = 0.52$, $p < 0.001$) than with amorphous Fe-(hydr)oxide content ($R^2 = 0.35$, $p < 0.01$). Other soil parameters, such as pH ($R^2 = 0.02$, $p > 0.05$), organic matter content ($R^2 = 0.07$, $p > 0.05$) and amorphous Al-(hydr)oxide content ($R^2 = 0.05$, $p > 0.05$) are hardly correlated with selenite amount in the soils (data not shown).

Table 4. Selenium speciation in ammonium oxalate extraction of selected samples from grassland and arable land (n = 24).

Soil type	Total Se ammonium oxalate (μg kg^{-1})		Inorganic selenite[a]/total Se ammonium oxalate (%)		Organic Se/total Se ammonium oxalate (%)		Total Se aqua regia (μg kg^{-1})	Inorganic selenite[a]/total Se aqua regia (%)
	Range	Average ± SD[b]	Range	Average ± SD[b]	Range	Average ± SD[b]	Average ± SD[b]	Average ± SD[b]
Sand (n = 7)	21– 71	40 ± 16	9 – 72	30 ± 20	28–91	70 ± 20	243 ± 81	5 ± 5
Clay (n = 8)	27– 198	64 ± 56	16 –67	45 ± 17	33– 84	55 ± 17	387 ± 194	8 ± 5
Clay-peat (n = 7)	108– 216	165 ± 36	7–31	21 ± 10	69– 93	79 ± 10	1288 ± 309	3 ± 1
Peat (n = 2)	164 – 201	183 ± 27	13	13 ± 0.7	87	87 ± 0.7	1114 ± 40	2 ± 0.1
Average ± SD[b]		**96 ± 69**		**31 ± 19**		**69 ± 19**	**668 ± 505**	**5 ± 4**

[a] Inorganic selenite as extracted with ammonium oxalate.

[b] Standard deviation.

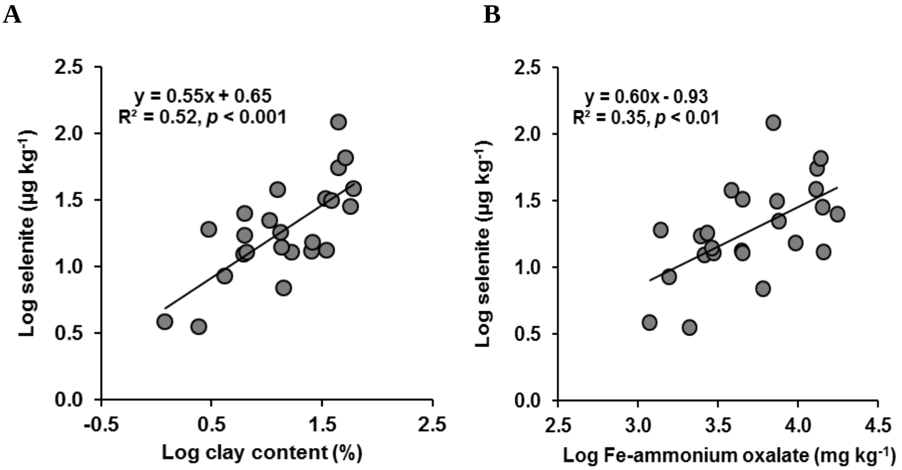


Fig. 3. Correlations between selenite extracted with ammonium oxalate and clay content (A) and amorphous Fe-(hydr)oxide content extracted with ammonium oxalate (B). The correlations include in total 24 samples, i.e. 19 samples from grassland and 5 samples from arable land (Table 4).

3.3.1.3 Organic Se in soils derived from NaOCl oxidation-extraction

The fraction of Se measured in the NaOCl oxidation-extraction over total Se content (aqua regia) corresponds to a large extent to the fraction of organic C oxidized in the NaOCl extraction (Table 5), indicating that Se in the NaOCl oxidation-extraction is mainly associated with organic matter. Tokunaga *et al.* (1991) suggest that Se measured in the NaOCl oxidation-extraction may include in addition to organic Se, also elemental Se (Se^0) that is an intermediate product of organic Se mineralization (Weres *et al.*, 1989) and adsorbed selenite (SeO_3^{2-}) that is protected by soil organic matter coating. We cannot be conclusive if and to which extent selenite adsorbed to soil minerals was desorbed during the NaOCl extraction. The Fe, Al and Mn concentrations extracted in the NaOCl extraction accounted for only 7%, 14% and 13% of the total Fe, Al and Mn measured in the ammonium oxalate extraction, respectively (data not shown), indicating that only a small fraction of amorphous metal (hydr)oxides was dissolved during the NaOCl extraction. Kaiser and Guggenberger (2003) and Siregar *et al.* (2005) suggest that oxidation with 1 M

NaOCl at moderate pH and temperature can remove effectively organic matter in soil, whereas oxides and clay minerals are largely unaffected. Because the total inorganic Se as determined in the HPLC analysis of the ammonium oxalate extracts were relatively small (5% of total Se-aqua regia; Table 4), the inorganic Se in the form of selenite (and selenate) contribution to Se extracted in NaOCl will in any case remain small. Therefore, in our calculation, we assumed that Se extracted in NaOCl is all organic Se. Elemental Se and selenides, if present, may have been oxidized into selenite and selenate during the NaOCl oxidation-extraction, therefore may contribute to the organic Se measured in this work.

3.3.1.4 Selenium speciation in soils

Mass balance calculations were carried out for the 10 selected soils for which both inorganic and organic Se measurement are available to quantify Se speciation in these soils (Table 5). With this analysis, the total Se (aqua regia) is fractionated into inorganic Se, organic Se and residual fraction. The HPLC analysis of ammonium oxalate extraction revealed that inorganic Se is mainly present in the selenite form, whereas the selenate content is hardly measurable (Table 4). Therefore, in the mass balance calculations, inorganic Se is set equal to the amount of selenite measured in the ammonium oxalate extraction using HPLC. Organic Se is derived from the total Se in the NaOCl oxidation-extraction. The residual fraction of Se was calculated as the difference between the total Se (aqua regia) and the sum of inorganic Se extracted in ammonium oxalate and organic Se extracted after NaOCl oxidation. The results show that (oxidizable) organic Se is the predominant Se species in soils from both grassland and arable land, which accounts for on average 87% and 78% of total Se (aqua regia), respectively (Table 5). Further analysis of Se associated with different fractions of soil organic matter was performed in 0.1 M NaOH soil extraction (Section 3.3.3), however, no effort was made to specify the molecular nature of organic Se compounds present in this extraction in the current study. The inorganic selenite accounts for on average 4% of total Se (aqua regia) in grassland soils and 5% in arable land soils (Table 5). The remaining is the residual or non-extractable fraction, which accounts for on average 10% of total Se (aqua regia) in

Table 5. Selenium speciation in the 10 selected soil samples. The percentage of Se species is relative to total Se (aqua regia) in soils.

Soil sample	pH	Organic matter (%)	Clay (%)	Fe-oxalate (g kg^{-1})	Total Se aqua regia (µg kg^{-1})	Inorganic Se (selenite) (%)	Organic Se (NaOCl extraction) (%)	Residual Se (%)	Organic C (NaOCl extraction) (%)[a]
Grassland									
Sand 1 (soil 6)	7.09	6.02	6.2	2.60	252.1	4.9	88	7.2	91
Sand 2 (soil 11)	5.20	7.76	1.2	1.18	236.1	1.6	90 ± 8.0[b]	8.3	93 ± 0.2[b]
Clay 1 (soil 5)	5.74	3.84	14.3	6.03	370.8	1.9 ± 0.2[b]	82	16.0	81 ± 0.2[b]
Clay 2 (soil 8)	6.07	10.20	34.3	4.48	558.0	5.8 ± 0.6[b]	91	3.3	92 ± 0.1[b]
Clay – peat (soil 9)	5.23	16.39	61.4	12.93	1155.9	3.3	83	13.4	88 ± 0.6[b]
Average ± SD[c]					**515 ± 381**	**4 ± 2**	**87 ± 4**	**10 ± 5**	**89 ± 5**
Arable land									
Sand (soil BK1)	5.07	7.42	4.2	1.55	284.1	3.0	82	15.5	93
Clay (soil K1)	6.04	7.81	44.5	6.95	726.1	16.7	68 ± 2.9[b]	15.6	91 ± 0.1[b]
Clay – peat 1 (soil BJ1)	4.81	41.32	26.1	9.57	1195.3	1.3	85 ± 0.6[b]	13.6	61 ± 2.2[b]
Clay – peat 2 (soil AI1)	6.54	16.92	38.5	7.41	886.7	3.5	71	25.4	93
Peat(soil BL1)	4.70	26.53	10.7	7.60	1085.5	2.0	82	15.7	84
Average ± SD[c]					**836 ± 357**	**5 ± 6**	**78 ± 8**	**17 ± 5**	**84 ± 13**
Range (all samples)					**236 – 1195**	**1.3 – 16.7**	**68 – 91**	**3 – 25**	**61 – 93**
Average ± SD[c] (all samples)					**675 ± 387**	**4.4 ± 4.6**	**82 ± 8**	**13 ± 6**	**87 ± 10**

[a] The percentage of organic C is relative to the measured total organic C in soils (see Section 3.2.2).

[b] The samples analysed in duplicate were presented as average ± standard deviation.

[c] Standard deviation.

grassland soils and 17% in arable land soils. The residual or non-extractable Se is probably Se in the structure of resistant organic matter, such as humin, which cannot be completely oxidized and extracted by NaOCl. The residual fraction of Se in most soils analysed is similar to the residual fraction of organic C (Table 5). The residual Se fraction may also include Se in the mineral structures.

3.3.1.5 Soil properties influencing Se speciation in soils

Organic Se is found to be the major Se pool in the soils both from grassland and arable land (Table 5). The importance of the organic Se pool in Dutch agricultural soils is supported by a positive correlation between the soil organic matter content and total Se content (aqua regia) that was observed for all soils included in this study (Fig. 2A). Such a correlation has also been found in the previous studies of Yamada *et al.* (2009), Johnsson (1992) and Tolu *et al.* (2014). A positive correlation was also found between amorphous Fe- or Al-(hydr)oxide content and total Se content (Fig. 2B and C), which is consistent with the correlation between the amorphous metal oxide content and soil organic matter content ($R^2 = 0.51$, $p < 0.001$ and $R^2 = 0.40$, $p < 0.001$ for the correlations between amorphous Fe-(hydr)oxide and organic matter content in the soils from grassland and arable land, respectively; and $R^2 = 0.79$, $p < 0.001$ and $R^2 = 0.78$, $p < 0.001$ for the correlations between amorphous Al-(hydr)oxide and organic matter content in the soils from grassland and arable land, respectively; data not shown).

The fraction of organic Se over total Se in the Dutch agricultural soils obtained in this study is higher (70 to 90% of total Se; Table 5) than that found in Japanese agricultural soils (52 to 79% of total Se) (Kang *et al.*, 1993; Yamada *et al.*, 2009). Correspondingly, the fraction of inorganic Se in the Dutch agricultural soils (1 to 17%, on average 5%, Table 4) is lower than that in the Japanese agricultural soils, i.e. on average 48% (Yamada *et al.*, 2009) and 9 to 16%, on average 13% (Kang *et al.*, 1993). Yamada *et al.* (2009) suggest that soils with a higher organic matter content have a higher organic Se fraction and vice versa. The Dutch agricultural soils have in general a higher organic matter content (2 to 54%, Table 1) than the

Japanese agricultural soils, i.e. 0.36 to 13% (Yamada *et al.*, 2009) and 3 to 30% (Kang *et al.*, 1993), whereas the total Se content is in the same range, i.e. 0.12 to 1.97 mg kg^{-1} in the Dutch soils (Table 6), 0.05 to 2.80 mg kg^{-1} in the Japanese soils from Yamada *et al.* (2009) and 0.17 to 1.23 mg kg^{-1} in the Japanese soils from Kang *et al.* (1993).

Besides the absolute amount of organic matter, the relative amount of soil organic matter to clay or Fe-(hydr)oxide also influences the relative importance of organic Se in soils. In our result, a positive relationship was found, especially between the ratio of soil organic matter to clay and ratio of organic Se to inorganic Se (R^2 = 0.42, $p < 0.05$; Fig. 4A), whereas the correlation between ratio of soil organic matter to amorphous Fe-(hydr)oxide and ratio of organic Se to inorganic Se was not significant (R^2 = 0.34, $p > 0.05$; Fig. 4B). Wang and Chen (2003) suggest that inorganic Se adsorbed to oxides is the major Se species in the soils with low organic matter but high metal oxide contents. The soils used in the study of Wang and Chen (2003) contain on average 16 and 1.5 times higher total Fe- and Al-(hydr)oxides extracted in DCB than the soils used in our study, whereas the organic matter content in the soils of our study is on average 5 times higher than that in the soils of study of Wang and Chen (2003). The total Se content in the soils from both studies is comparable (on average 624 µg kg^{-1} in grassland soils and 528 µg kg^{-1} in arable land soils in this study (Table 6) and on average 609 µg kg^{-1} from the study of Wang and Chen (2003)). Therefore, these differences in the properties of soils used in both studies explain why the fraction of organic Se in the soils from the study of Wang and Chen (2003) is lower (18 to 22% of total Se) than in the soils used in our study (70 to 90%, Table 5) and the inorganic Se is higher in the soils from Wang and Chen (2003) (45 to 57% of total Se) than in our soils (1 to 17%, Table 4).

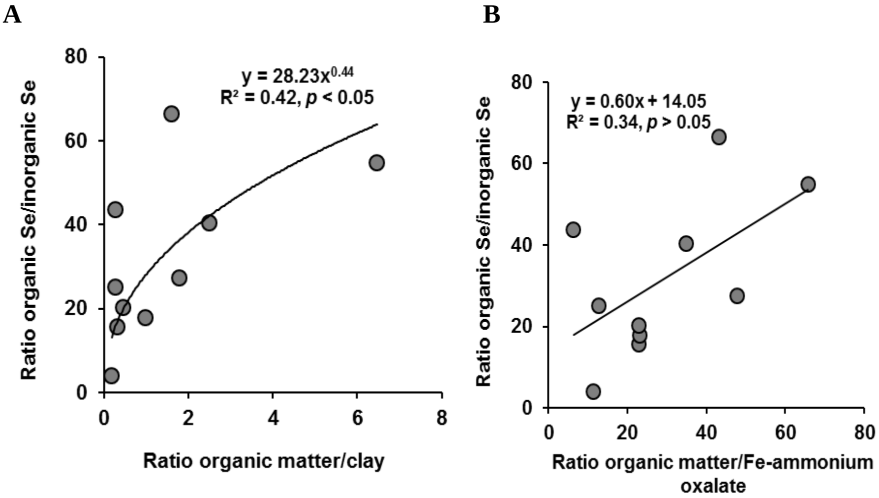


Fig. 4. Correlations between ratio of organic Se to inorganic Se and ratio of organic matter to clay (A) and ratio of organic matter to amorphous Fe-(hydr)oxide (B). The soil samples include five soil samples from grassland and five soil samples from arable land (Table 5).

3.3.2 Extractability of Se in single step extractions

3.3.2.1 Selenium extracted in single step extractions

Amounts of selenium measured in different extractions decrease in the order of aqua regia > NaOCl > NaOH > ammonium oxalate > hot water > HNO_3 > $CaCl_2$, both in the grassland and arable land soils (Table 6).

0.01 M $CaCl_2$. The 0.01 M $CaCl_2$ method extracted on average 1.5% of total Se (aqua regia) in grassland soils and 1.4% in arable land soils (Table 6), which is comparable to that obtained using 0.25 M KCl (Zhang and Moore, 1997; Wang and Chen, 2003) or water (Wang and Sippola, 1990; Kang *et al.*, 1991a; Séby *et al.*, 1997). The amount extracted with weak electrolytes or water is normally considered as an estimation of the soluble concentration in soil solution. Previous studies have shown that soluble Se measured in the 0.01 M $CaCl_2$ extraction of grassland soils in the Netherlands is mainly present as organic Se (Weng *et al.*, 2011; Supriatin *et al.*, 2015a). The predominance of organic Se in the 0.01 M $CaCl_2$ extraction indicates that the solubility of Se in Dutch agricultural soils is probably to a large extent determined by the solid-solution distribution of organic Se rather than the inorganic Se.

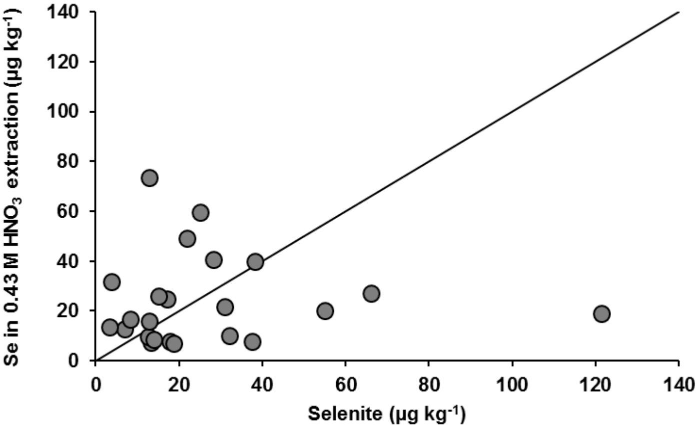


Fig. 5. Comparison between selenite content measured in ammonium oxalate extraction using HPLC - ICP-MS versus Se in 0.43 M HNO_3 extraction (n = 24). The line is 1:1 line.

0.43 M HNO_3. The fraction of Se extracted with 0.43 M HNO_3 (on average 5% of total Se (aqua regia) for both grassland and arable land soils, Table 6) is similar to that extracted with 0.1 M phosphate buffers at different pH values for soils with or without Se fertilization (Chao and Sanzolone, 1989; Wang and Chen, 2003; Hagarová *et al.*, 2005; Keskinen *et al.*, 2009). The latter is considered to extract the exchangeable Se, i.e. mainly inorganic Se adsorbed to soil mineral surfaces as a result of competition of phosphate for Se adsorption. Although adsorption of selenate and selenite to metal oxides increases with decreasing pH within normal soil pH range (Balistrieri and Chao, 1987, 1990), at pH < 1 in the 0.43 M HNO_3 extraction, adsorption of Se is expected to be weak due to protonation of selenate (at this pH as mainly $HSeO_4^-$) and selenite ions (as mainly $H_2SeO_3^0$), which leads to their desorption. Therefore, we expect that 0.43 M HNO_3 extracts mainly inorganic Se in soil, plus a small amount of organic Se as indicated by a small fraction of organic matter extracted (Table 6). In comparison with the total inorganic selenite (as measured using HPLC – ICP-MS), the 0.43 M HNO_3 extraction both overestimates and underestimates total inorganic selenite in soils (Fig. 5), indicating that in addition to ammonium oxalate extraction, 0.43 M HNO_3 extraction is not a direct method to estimate total inorganic selenite adsorbed in soils either.

Hot water. The fraction of Se extracted with hot water for the soil samples from grassland (soil samples from arable land were not analysed) is higher (4 to 14% of total Se (aqua regia), Table 6) than that in the study of Johnsson (1992) (0.7 to 2.2%) for non Se-fertilized soils, probably due to different methodologies used for the extraction. In our study, the soil suspensions were equilibrated at 80^{o}C for 16 hours, whereas in the study of Johnsson (1992), the soils were shaken in the hot water (100^{o}C) for 30 minutes. The hot water extraction in our study was aimed to determine (organic) Se associated with labile organic matter, whereas in the study of Johnsson (1992) it was aimed to measure soluble Se. In another study, Keskinen *et al.* (2009) boiled the soil suspensions in a boiling flask fitted to a water-cooled reflux condenser for hot water extraction of Se-fertilized soils, and found a similar extraction efficiency (5 to 10%) as in our study (4 to 14%). Since the soluble Se in 0.01 M $CaCl_2$ extraction was predominantly present in organic Se form and hardly inorganic selenate and selenite was measurable in this extraction (Weng *et al.*, 2011; Supriatin *et al.*, 2015a), we expect that Se extracted in the hot water performed in our study contains mainly organic Se as well. Hot water extracted a higher amount of dissolved organic C in soils (around 4% of total soil organic C) than 0.01 M $CaCl_2$ (around 0.6% of total soil organic C) (Table 6), due to a lower Ca^{2+} concentration and a higher temperature were employed in the hot water extraction than those in the 0.01 M $CaCl_2$ extraction.

Table 6. Amounts and percentage of Se and organic C extracted in the single step extractions (Table 2).

Extraction	Number of samples	Se extracted (µg kg^{-1})			Se extraction efficiency (% of total Se aqua regia)			Organic C extracted (g kg^{-1})			Organic C extraction efficiency (% of total organic C)			Ratio Se:C (µg g^{-1})
		Min.	Max.	Mean ± SD^{a}	Min.	Max.	Mean ± SD^{a}	Min.	Max.	Mean ± SD^{a}	Min.	Max.	Mean ± SD^{a}	Mean ± SD^{a}
Grassland														
0.01M $CaCl_2$	42	2.1	40.2	8.1 ± 8.0	0.5	3	1.5 ± 0.6	0.1	0.9	0.3 ± 0.2	0.2	1.0	0.6 ± 0.2	29 ± 13
0.43M HNO_3	42	5.2^{b}	73.3^{b}	22.4 ± 15.9^{b}	1.4^{b}	13^{b}	5 ± 3^{b}	0.6^{c}	8.6^{c}	2.4 ± 2.1^{c}	3^{c}	8^{c}	4 ± 1^{c}	10 ± 3^{bc}
Hot water	21	13.4	84.8	32.2 ± 19.9	4	14	8 ± 2	0.3	4.5	1.7 ± 1.2	2	5	4 ± 1	23 ± 13
Amm. ox.	42	18.3^{b}	267.2^{b}	84.3 ± 67.0^{b}	9^{b}	20^{b}	14 ± 3^{b}	1.0^{d}	28.6^{d}	6.8 ± 6.6^{d}	4^{d}	22^{d}	12 ± 5^{d}	14 ± 7^{b}
NaOH	5	206.7	640.6	345.5 ± 184.3	55	88	74 ± 12	8.5	26.8	16.7 ± 8.0	28	54	39 ± 10	22 ± 8
1M NaOCl	5	212.6	962.5	441.8 ± 314.3	82	91	87 ± 4	17.0^{e}	79.8^{e}	43.4 ± 24.3^{e}	81^{e}	93^{e}	89 ± 5^{e}	11 ± 5
Aqua regia	42	117.6^{b}	1968.0^{b}	623.8 ± 524.2^{b}	-	-	-	11.5^{f}	272.0^{f}	58.4 ± 56.1^{f}	-	-	-	11 ± 4^{b}
Arable land														
0.01M $CaCl_2$	41	1.7	11.5	5.1 ± 2.5	0.3	2.9	1.4 ± 0.6	0.1	0.5	0.2 ± 0.1	0.2	1.8	0.6 ± 0.3	33 ± 14
0.43M HNO_3	41	5.8^{b}	53.5^{b}	17.7 ± 12.3^{b}	1.6^{b}	13^{b}	5 ± 2^{b}	0.8^{c}	5.5^{c}	2.7 ± 1.6^{c}	2^{c}	7^{c}	4 ± 1^{c}	11 ± 4^{bc}
Amm. ox.	41	22.4^{b}	179.9^{b}	81.3 ± 42.6^{b}	8^{b}	27^{b}	16 ± 5^{b}	0.6^{d}	25.1^{d}	5.4 ± 5.5^{d}	5^{d}	31^{d}	14 ± 6^{d}	16 ± 8^{b}
NaOH	5	234.4	575.0	414.6 ± 124.9	37	83	55 ± 18	15.6	50.5	31.7 ± 16.8	24	57	37 ± 13	15 ± 8
1M NaOCl	5	231.7	1017.6	652.8 ± 314.2	68	85	78 ± 8	39.6^{e}	125.5^{e}	82.1 ± 40.9^{e}	61^{e}	93^{e}	84 ± 13^{e}	8 ± 3
Aqua regia	41	200.9^{b}	1195.3^{b}	527.8 ± 321.0^{b}	-	-	-	5.0^{f}	206.6^{f}	42.6 ± 46.7^{f}	-	-	-	13 ± 7^{b}
All samples														
0.01M $CaCl_2$	83	1.7	40.2	6.6 ± 6.1	0.3	3	1.5 ± 0.6	0.1	0.9	0.2 ± 0.2	0.2	1.8	0.6 ± 0.3	31 ± 14
0.43 M HNO_3	83	5.2^{b}	73.3^{b}	20.1± 14.3^{b}	1.4^{b}	13^{b}	5 ± 3^{b}	0.6^{c}	8.6^{c}	2.5 ± 1.9^{c}	2^{c}	8^{c}	4 ± 1^{c}	10 ± 3^{bc}
Hot water	21	13.4	84.8	32.2 ±19.9	4	14	8 ± 2	0.3	4.5	1.7 ± 1.2	2	5	4 ± 1	23 ± 13
Amm. ox.	83	18.3^{b}	267.2^{b}	83.1 ± 57.9^{b}	8^{b}	27^{b}	14 ± 4^{b}	0.6^{d}	28.6^{d}	6.1 ± 6.1^{d}	4^{d}	31^{d}	13 ± 5^{d}	14 ± 7^{b}
0.1 M NaOH	10	206.7	640.6	380.0 ± 152.8	37	88	65 ± 18	8.5	50.5	24.2 ± 14.7	24	57	38 ± 11	19 ± 8
1M NaOCl	10	212.6	1017.6	547.3 ± 316.5	68	91	82 ± 8	17.0^{e}	125.5^{e}	62.8 ± 37.7^{e}	61^{e}	93^{e}	87 ± 10^{e}	9 ± 4
Aqua regia	83	117.6^{b}	1968.0^{b}	582.1 ± 447.0^{b}	-	-	-	5.0^{f}	272.0^{f}	50.6 ± 51.9^{f}	-	-	-	12 ± 6^{b}

[a] Standard deviation.

[b] Samples that have Se concentrations below the determination limits were not included in the calculation.

[c] Analysed in 42 soil samples from grassland and arable land taken in 2011. The 41 soil samples taken in 2009 were not analysed.

[d] Determined using UV spectrophotometer.

[e] Derived from the difference between measured total C in the original soils and total inorganic C in the residues of soil samples used in NaOCl extraction.

[f] Estimated from the measured soil organic matter content assuming that soil organic matter contains 50% organic C.

Ammonium oxalate. Ammonium oxalate extraction is used to extract Se adsorbed to metal (hydr)oxides (Keskinen *et al.*, 2009). In our study, the ammonium oxalate extracted on average 14% of total Se (aqua regia) in grassland soils and 16% in arable land soils (Table 6), which is much lower than the values reported by Keskinen *et al.* (2009) (around 50%) for Finnish soils after > 11 years of Se fertilization. For Finnish soils before Se fertilization, 20 to 30% of total Se was extracted in ammonium oxalate (Yläranta (1983a) *in* (Keskinen *et al.*, 2009)). The increase of the ammonium extractable fraction has been attributed to accumulation of Se on oxide surfaces after Se fertilizer application (Keskinen *et al.*, 2009). Further analysis of Se in the ammonium oxalate extraction performed in our study shows that Se extracted includes both inorganic Se (on average 30%) and organic Se (on average 70%) (Table 4).

0.1 M NaOH. The 0.1 M NaOH extraction is aimed to determine Se associated with soil organic matter, especially the humic substances. The fraction of Se extracted in the 0.1 M NaOH is on average 74% of total Se (aqua regia) in grassland soils and 55% in arable land soils (Table 6), which is close to the Se fraction in 0.1 M NaOH extractions obtained in other studies for agricultural soils (Kang *et al.*, 1991b; Tolu *et al.*, 2011). The total Se extracted in the 0.1 M NaOH may also include part of the inorganic selenite adsorbed to metal oxides and clay minerals, which may be extracted in the initial step of the extraction using 0.1 M HCl (Section 3.2.3.2) and during 0.1 M NaOH extraction. However, the total inorganic selenite in the soils only accounts for on average 5% of total Se (aqua regia) (Table 4), which can thus only give a minor contribution to the Se concentrations in the NaOH extraction.

1 M NaOCl (pH 8). The 1 M NaOCl oxidation-extraction has been used to extract microelements (Se, Cu, Fe, Mn, Zn) associated with organic matter without disrupting oxides or clay fractions (Lavkulich and Wiens, 1970; Shuman, 1983; Tokunaga *et al.*, 1991; Kaiser and Guggenberger, 2003; Siregar *et al.*, 2005), which corresponds to our results presented in Section 3.3.1.3. The NaOCl extracted on average 87% and 78% of total Se (aqua regia) in grassland soils and arable land soils, respectively (Table 6), which is comparable to that found in the study of Tokunaga et al. (1991), i.e. around 85% of total Se (measured using X-ray fluorescence) was extracted in 1 M NaOCl (pH 8) from the seleniferous soil samples of Kesterson reservoir, California. The large fraction (87% and 78%) of Se extracted in NaOCl supports the conclusion that most of Se in the soils studied exists as organic Se (section 3.3.1.4).

3.3.2.2 Correlation between Se in different extractions

Selenium content in different extractions are positively correlated (Table 7). The correlations between Se-$CaCl_2$ and Se-NaOH, Se-$CaCl_2$ and Se-NaOCl, or Se-ammonium oxalate and Se-NaOCl were greatly increased if one soil sample (soil BJ1, clay-peat soil or soil K1, clay soil) was excluded. Generally, Se in hot water extraction was strongly ($R^2 > 0.83$) correlated with Se in all the other extractions, except in 0.43 M HNO_3. The Se in 0.43 M HNO_3 extraction shows a relatively weak correlation with other extractions (R^2 = 0.34 to 0.58), probably because that in the 0.43 M HNO_3 extraction Se is dominated by inorganic species, whereas in other extractions organic Se dominates (Section 3.3.2.1).

Table 7. R-squared (R^2) derived from the correlation between Se extracted in different extractions (μg kg^{-1}). Each correlation involves a pair of extractions from 83 soil samples from both grassland and arable land, except that for Se in hot water (n = 21), NaOH (n = 10), and NaOCl (n = 10) extractions, less samples were used.

	Se-HNO_3[a]	Se-hot water	Se-amm. ox.[a]	Se-NaOH	Se-NaOCl	Se-aqua regia[a]
Se-$CaCl_2$	0.52***	0.91***	0.69***	0.58* (0.85[b]***)	0.26 (0.92[b]***)	0.71***
Se-HNO_3[a]		0.38**	0.58***	0.36	0.34	0.58***
Se-hot water			0.83***	0.98[c]***	0.97[c]**	0.89***
Se-amm. ox.[a]				0.73**	0.51* (0.90[b]***)	0.88***
Se-NaOH					0.79***	0.80***
Se-NaOCl						0.97***

* Indicates $p < 0.05$; ** indicates $p < 0.01$; and *** indicates $p < 0.001$.

[a] The samples that have Se concentrations below the determination limits were not included in the correlation; [b] The R^2 after removing one soil sample from the correlation.

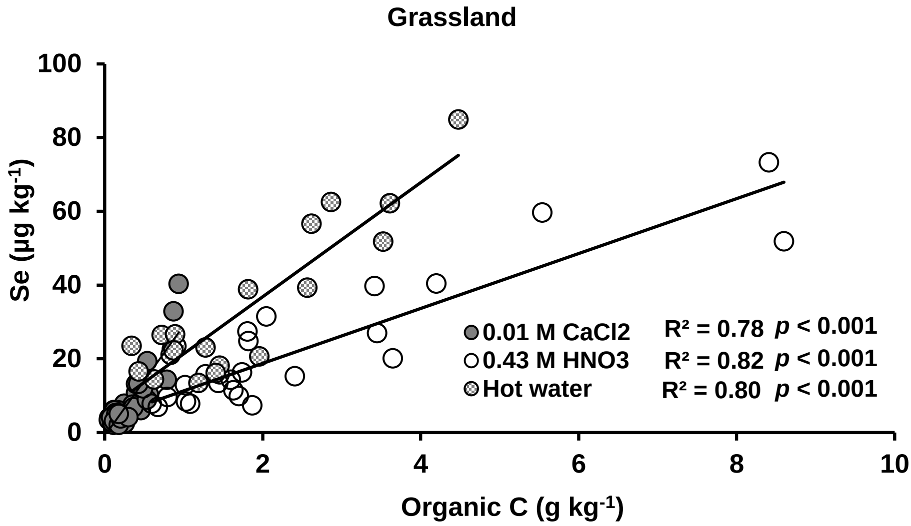
[c] Five soil samples were included in the correlation.

3.3.2.3 Correlation between Se and organic C extracted

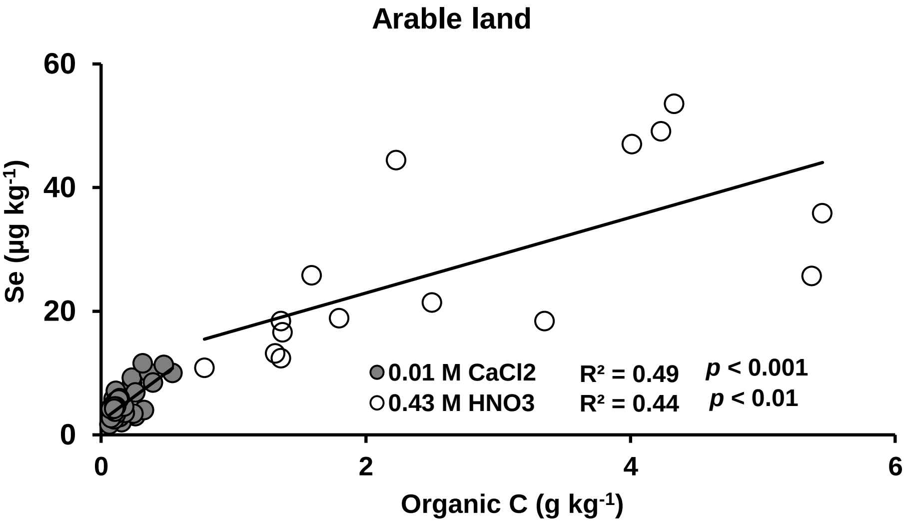
Positive correlations between Se and organic C extracted were found in all the extractions for both grassland soils and arable land soils (Fig. 6A, B, C and D). In general, the correlation of grassland soils is stronger than that of arable land soils, which may be due to the larger range of Se and organic C contents in the grassland soils compared to those in the arable land soils (Fig. 6A, B, C and D). The fraction of Se extracted in different extractions follows to a large extent the fraction of soil organic C extracted (Table 6).

Ratios of Se to organic C in 0.01 M $CaCl_2$, hot water, and 0.1 M NaOH extractions are higher compared to those in other extractions (0.43 M HNO_3, ammonium oxalate, 1 M NaOCl and aqua regia) (Table 6). In 0.01 M $CaCl_2$, hot water and 0.1 M NaOH extraction, fraction of Se extracted is about 2 times of fraction of organic C extracted (Table 6), whereas in 0.43 M HNO_3, ammonium oxalate and 1 M NaOCl extractions, fraction of Se extracted is similar to the fraction of organic C extracted. Our previous studies showed that soluble Se extracted in 0.01 M $CaCl_2$ is mainly organic Se (Weng *et al.*, 2011; Supriatin *et al.*, 2015a). Selenium in hot water extraction is expected to be Se associated with labile organic matter, such as microbial biomass, soluble carbohydrates and amines (Ghani *et al.*, 2003) and Se in 0.1 M NaOH is considered to consist mainly of Se associated with humic fractions of solid organic matter (Kang *et al.*, 1991b). The results indicate that soluble, labile organic matter and base-soluble humic substances are richer in Se compared to other soil organic matter. Further fractionation shows that the hydrophobic organic neutral (HON) and the hydrophilic (Hy) fractions of humic substances contain a relatively large amount of Se (Table 8 and Section 3.3.3). The relatively low Se to organic C ratios found in 0.43 M HNO_3, ammonium oxalate, and 1 M NaOCl extractions and aqua regia digestion indicate that the acid-soluble, amorphous metal oxide associated organic matter as well as the majority of the soil organic matter is mainly poor in Se. No profound differences were found between grassland and arable land samples with regard to Se to organic C ratios in various extractions (Table 6).

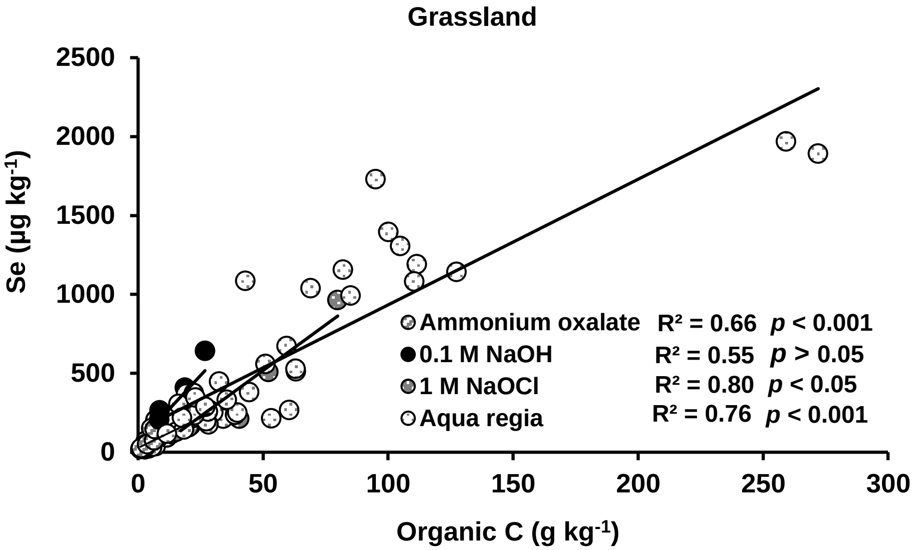
A


Grassland
Se (µg kg-1)
100
80
60
40
20
0
0
2
4
6
8
10
Organic C (g kg-1)
0.01 M CaCl2 R² = 0.78 p < 0.001
0.43 M HNO3 R² = 0.82 p < 0.001
Hot water R² = 0.80 p < 0.001

B


Arable land
Se (µg kg-1)
60
40
20
0
0
2
4
6
Organic C (g kg-1)
0.01 M CaCl2 R² = 0.49 p < 0.001
0.43 M HNO3 R² = 0.44 p < 0.01

C



D

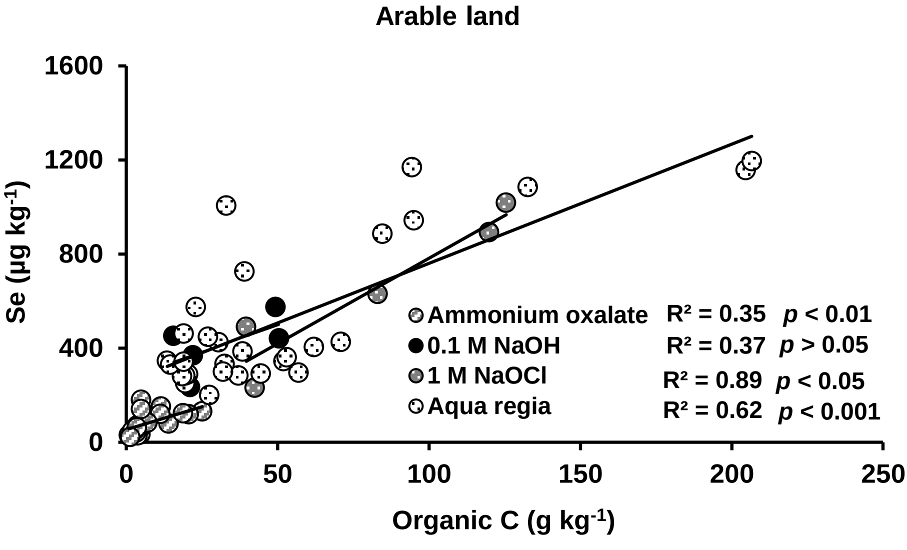


Fig. 6. Correlations between Se and organic C in 0.01 M $CaCl_2$, 0.43 M HNO_3 and hot water extractions of soil samples from grassland (A) and arable land (B);

correlations between Se and organic C in ammonium oxalate and 0.1 M NaOH extractions or organic C oxidized in 1 M NaOCl pH 8 extraction, and correlation between Se in aqua regia and total soil organic C of soil samples from grassland (C) and arable land (D).

3.3.3 Selenium in organic matter fractions extracted in 0.1 M NaOH

The distribution of Se (in terms of Se amount not Se to organic C ratio) among the organic matter fractions extracted in 0.1 M NaOH of grassland soils and arable land soils follows the order of HA > FA + HON > Hy, whereas the distribution of organic C follows the order of HA > FA + HON ≈ Hy (Table 8). In the FA + HON fraction, Se and organic C are mainly present in HON (on average 23% for Se and 12% for organic C, respectively, relative to total Se and organic C in the 0.1 M NaOH extraction) and to a lesser extent in the FA (2% for Se and 3% for organic C, respectively, relative to total Se and organic C in the 0.1 M NaOH extraction) (data not shown). The comparable distribution of Se and organic C among the fractions of organic matter in the NaOH extraction supports the idea that Se in this extraction is mainly associated with organic matter. Some other studies have also measured Se distribution in 0.1 M NaOH extractions (Kang *et al.*, 1991b; Kang *et al.*, 1993; Qin *et al.*, 2012), in which only the HA fraction was separated from the rest of the fractions (FA + HON + Hy). Their conclusions that more Se amount was found in the FA + HON + Hy fraction (though it was called in these references FA fraction) than in the HA fraction is in contrast with our findings (Table 8). In our results the Se in the HA fraction is slightly higher than that in the FA + HON + Hy fraction (Table 8).

The ratio of Se to organic C in each fraction of organic matter in the NaOH extraction decreases in the order of FA + HON > Hy > HA fraction (Table 8). The results indicate that the FA + HON (mainly HON) fraction of solid organic matter is richer in Se content per unit C than the Hy and HA fractions, which is comparable with the results obtained by Gustafsson and Johnsson (1992). Gustafsson and Johnsson (1994) suggested that Se is mainly incorporated into the hydrophobic

Table 8. Percentage of Se and organic C and Se:organic C ratio in organic matter fractions extracted in 0.1 M NaOH.

Soil type	pH	Organic matter (%)	Clay (%)	Fe-oxalate (g kg^{-1})	Total Se (µg kg^{-1})	Se in organic matter fractions (%)			Total organic C (g kg^{-1})	Organic matter fractions (%)			Ratio Se:C (µg g^{-1})		
						HA^a	FA + HON^a	Hy^b		HA^a	FA + HON^a	Hy^b	HA^a	FA + HON^a	Hy^b
Grassland															
Sand 1 (soil 6)	7.09	6.02	6.2	2.60	206.7	55	25	20	8.5	65	18	18	21	35	27
Sand 2 (soil 11)	5.20	7.76	1.2	1.18	207.9	51	33	17	20.8	68	16	16	7	21	11
Clay 1 (soil 5)	5.74	3.84	14.3	6.03	263.8	53	31	15	8.7	61	18	21	27	52	22
Clay 2 (soil 8)	6.07	10.20	34.3	4.48	408.4	61	22	18	18.8	70	15	16	19	33	25
Clay – peat (soil 9)	5.23	16.39	61.4	12.93	640.6	64 ± 0.4^c	19 ± 1.0^c	17 ± 0.6^c	26.8 ± 0.7^c	70 ± 0.1^c	15 ± 0.2^c	15 ± 0.1^c	22 ± 0.6^c	31 ± 2^c	27 ± 0.1^c
Average ± SD^d						**57 ± 5**	**26 ± 6**	**18 ± 2**		**67± 4**	**16 ± 2**	**17 ± 2**	**19 ± 7**	**34 ± 11**	**23 ± 7**
Arable land															
Sand (soil BK1)	5.07	7.42	4.2	1.55	234.4	60	27	14	21.1	76	14	10	9	21	16
Clay (soil K1)	6.04	7.81	44.5	6.95	452.0	42	27	32	15.6	65	16	19	19	50	48

Table 8. Continued.

Soil type	pH	Organic matter (%)	Clay (%)	Fe-oxalate (g kg^{-1})	Total Se (µg kg^{-1})	Se in organic matter fractions (%)			Total organic C (g kg^{-1})	Organic matter fractions (%)			Ratio Se:C (µg g^{-1})		
						HA[a]	FA + HON[a]	Hy[b]		HA[a]	FA + HON[a]	Hy[b]	HA[a]	FA + HON[a]	Hy[b]
Clay – peat 1 (soil BJ1)	4.81	41.32	26.1	9.57	442.0	72	19	10	50.5	78	14	8	8	12	11
Clay – peat 2 (soil AI1)	6.54	16.92	38.5	7.41	369.4	57	24	20	22.0	68	15	17	14	27	19
Peat (soil BL1)	4.70	26.53	10.7	7.60	575.0	70	19	12	49.3	79	14	8	10	16	18
Average ± SD[d]						**60 ± 12**	**23 ± 4**	**17 ± 9**		**73 ± 6**	**15 ± 1**	**12 ± 5**	**12 ± 4**	**25 ± 15**	**22 ± 15**
Average ± SD[d] (all samples)						**58 ± 9**	**25 ± 5**	**18 ± 6**		**70± 6**	**15 ± 2**	**15 ± 5**	**16 ± 7**	**30 ± 14**	**22± 11**

[a] Calculated values from mass balance (see Section 3.2.3.2).

[b] Measured values (see Section 3.2.3.2).

[c] The sample was analysed in duplicate. The values are the mean ± standard deviation of the duplicate.

[d] Standard deviation.

fulvate fraction instead of the humate fraction of organic matter through microbial processes. It is interesting to point out that in clay soils the Se to organic C ratio in the FA + HON fraction is in general higher than in the other soil types (Table 8). It should be noted that inorganic Se (mainly selenite) probably has contributed to the Se measured in the Hy fraction of NaOH extraction, and it may increase the Se to organic C ratio in the Hy fraction.

Comparison was made between the Se to organic C ratio in different single step extractions (Table 6) with the Se to organic C ratio in different fractions of NaOH extraction (Table 8). The ranking of Se to organic C ratio follows the order of $CaCl_2$ (on average for grassland and arable land soils 31 ± 14 $\mu g\ g^{-1}$) ≈ <u>HON-NaOH</u> (30 ± 14 $\mu g\ g^{-1}$) > hot water (23 ± 13 $\mu g\ g^{-1}$) ≈ <u>Hy-NaOH</u> (22 ± 11 $\mu g\ g^{-1}$) > NaOH (19 ± 8 $\mu g\ g^{-1}$) > <u>HA-NaOH</u> (16 ± 7 $\mu g\ g^{-1}$) ≈ ammonium oxalate (14 ± 7 $\mu g\ g^{-1}$) ≈ aqua regia (12 ± 6 $\mu g\ g^{-1}$) ≈ HNO_3 (10 ± 3 $\mu g\ g^{-1}$) ≈ NaOCl (9 ± 4 $\mu g\ g^{-1}$). The results show that the Se to organic C ratio in the $CaCl_2$ extraction is comparable to that of the HON (+ FA) fraction in the NaOH extraction, although it has been shown that Se (and organic C) in $CaCl_2$ extraction is mainly present in the Hy fraction, and to a less extent in the FA (+ HON) fraction (Supriatin *et al.*, 2015a). In the hot water extraction, the relatively high Se to organic C ratio indicates that the extraction contains to a large extent Se-rich organic matter. For the NaOH extraction, its average Se to organic C ratio is resulted from the mixture of Se-rich (HON, Hy) and Se-poor (HA) fractions (Table 8). In the rest of the single step extractions (0.43 M HNO_3, ammonium oxalate, 1 M NaOCl and aqua regia), the average Se to organic C ratio is close to that found for the HA fraction in the NaOH extraction. Based on the results in Table 8, we can deduce that the 0.1 M NaOH non-extractable organic C fraction, i.e. the so-called humin fraction, probably has a Se to organic C ratio that is similar or somewhat lower than that of HA. Summarizing, these findings show that the humin, HA and probably also the FA fractions in the solid organic matter have a relatively low Se to organic C ratio compared to the HON and Hy fractions in the solid organic matter and the soluble organic matter in hot water or in weak electrolyte.

3.4 Conclusions

The total Se content measured in Dutch agricultural soils ranges from 0.12 to 1.97 mg kg^{-1} in grassland soils and 0.20 to 1.20 mg kg^{-1} in arable land soils, which is positively correlated with soil organic matter, amorphous Fe- and Al-(hydr)oxide content in soils. Most of Se (on average 82% of total Se) in Dutch agricultural soils is present in organic forms. Inorganic selenium is mainly in the form of selenite, whereas selenate is hardly measurable. Selenite accounts for on average 5% of total Se, and soil selenite content is positively correlated with clay and amorphous Fe-(hydr)oxide content in soils. The relative importance of inorganic Se to total Se in soils increases in general with the increase of ratio between clay to soil organic matter content.

Fractions of Se extracted in the single step extractions follows the order of aqua regia (as 100%) > 1 M NaOCl pH 8 (82 ± 8%) > 0.1 M NaOH (65 ± 18%) > ammonium oxalate pH 3 (14 ± 4%) > hot water (8 ± 2%) > 0.43 M HNO_3 (5 ± 3%) > 0.01 M $CaCl_2$ (1.5 ± 0.6%). No noteworthy differences have been found in the Se extraction efficiency between grassland and arable land soils. The Se extraction efficiency follows to a large extent the extraction efficiency of organic C, but the fractions of Se extracted in 0.01 M $CaCl_2$, hot water and 0.1 M NaOH is about twice of that of organic C, whereas in 0.43 M HNO_3, ammonium oxalate, 1 M NaOCl and aqua regia the extracted fractions of Se and organic C are similar. All the extractions are expected to contain mainly organic Se, except the 0.43 M HNO_3 and ammonium oxalate extractions. Amounts of Se extracted in different extractions are correlated, especially in those in which organic Se predominates.

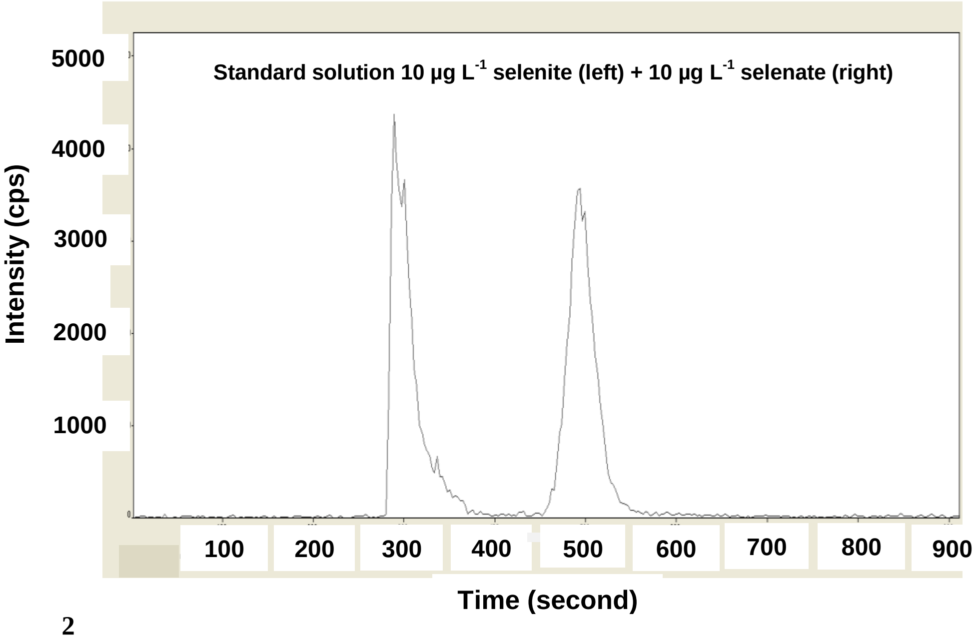
The HON fraction of soil organic matter in NaOH extraction is richer in Se, followed by the Hy fraction. The humin, HA, and probably also the FA fraction in the soil organic matter are relatively poor in Se compared to the HON and Hy fractions. The ratio of Se to organic C follows the order of $CaCl_2$ ≈ HON-NaOH > hot water ≈ Hy-NaOH > NaOH > HA-NaOH ≈ ammonium oxalate ≈ aqua regia ≈

HNO_3 ≈ NaOCl. Organic matter in the $CaCl_2$, hot water, and NaOH extractions is more enriched with Se compared to that in the other extractions.

These results have shown that organic Se is the major Se pool both in soil solution and solid phase of Dutch agricultural soils, and provided first insights in specific Se-rich fractions of soil organic matter. The findings of this study suggest that more attention should be paid to organic Se behaviour in soils when assessing Se availability in agricultural soils of the Netherlands. Further research focusing on the speciation, distribution and turn-over of the organic Se pool is recommended to increase the reliability in predicting the bioavailability of native soil Se and the effectiveness of Se fertilization.

Appendix

1



2

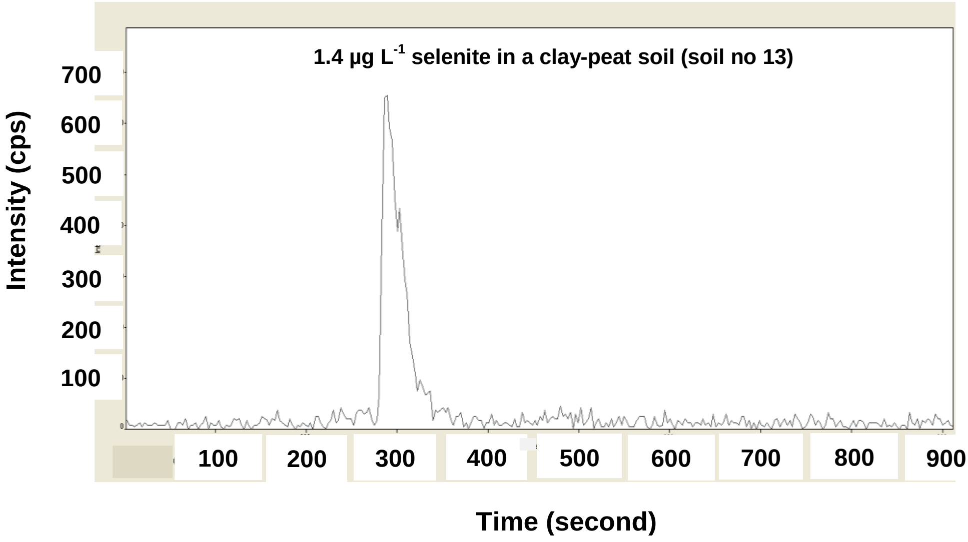


Fig. A.1. Chromatograms of (1) selenite and selenate peaks in a standard solution and (2) one soil sample as examples.

Chapter 4

Selenium-rich Dissolved Organic Matter Determines Selenium Uptake in Wheat Grown on Low-selenium Arable Land Soils

Supriatin Supriatin, Liping Weng, Rob N. J. Comans

Published as 'Selenium-rich dissolved organic matter determines selenium uptake in wheat grown on low-selenium arable land soils' in Plant and Soil, 2016, DOI: 10.1007/s11104-016-2900-7

Abstract

The study aimed to find soil parameters that are best related to Se plant uptake for low Se soils with predominantly organic Se, and to explore the mechanisms that control Se bioavailability in the soils under study. A pot experiment using nineteen soil samples taken from different fields of arable land (potato fields) in the Netherlands was conducted on summer wheat (*Triticum aestivum* L.). Selenium in wheat shoots and soil parameters, including basic soil properties, C:N ratio, inorganic selenite content, and Se and organic C in different soil extractions (0.01 M $CaCl_2$, 0.43 M HNO_3, hot water, ammonium oxalate, aqua regia) were analysed. Regression analysis was performed to identify soil parameters that determine Se content in wheat shoots. The regression model shows that Se:DOC ratio in 0.01 M $CaCl_2$ soil extraction explained about 88% of the variability of Se content in wheat shoots. Selenium plant uptake increased with the increase of Se:DOC ratio in $CaCl_2$ extraction, which can be interpreted as a measure of the content of soluble Se-rich organic molecules. Selenium:DOC ratio in $CaCl_2$ extraction and Se plant uptake increased towards higher soil pH and lower soil C:N ratio. The soil C:N ratio is also negatively correlated to Se:organic C ratio in other extractions (0.43 M HNO_3, hot water, ammonium oxalate, aqua regia), indicating that at low soil C:N ratio soil organic matter is richer in Se. Contrarily, the soil pH is positively and strongly correlated to Se:organic C ratio in $CaCl_2$ and hot water extractions, but only weakly correlated to Se:organic C ratio in other extractions. Selenium-rich dissolved organic matter is the source of bioavailable Se in low Se soils with predominantly organic Se. The soil pH and quality of soil organic matter (i.e. soil C:N ratio) are the main soil properties determining Se bioavailability in these soil types.

4.1 Introduction

Selenium (Se) is an essential micronutrient for cattle and humans, which plays a role in the production of anti-oxidant in cell systems. Generally, Se enters cattle and humans through grasses or crops in the food chain. An adequate level of Se in grasses and crops is determined by the level of available Se in soil. In agricultural soils with low Se availability, Se fertilizer can be applied to increase the Se content in plants. Understanding soil parameters and mechanisms affecting Se uptake by plants may help us to accurately predict the Se status in soils, design sustainable soil management and Se fertilization recommendations, avoid problems of Se deficiency, toxicity and environmental pollution.

In soil, Se can be present as inorganic and organic species. There are several oxidation states of inorganic Se, ranging from +6 (selenate), +4 (selenite), 0 (elemental Se), to -2 (selenides) (Hartikainen, 2005). The inorganic selenate (SeO_4^{2-}) and selenite (SeO_3^{2-}) can be found as soluble forms or as adsorbed (mainly selenite) to soil mineral surfaces, such as clay and metal (hydr)oxides, whereas elemental Se (Se^0) and selenides (Se^{2-}) are present as insoluble forms (Hartikainen, 2005). The chemical nature of organic Se in soils is not very well known. Previous studies have reported different forms of organic Se present in soil, such as selenomethionine ($C_5H_{11}NO_2Se$), methane seleninic acids (CH_3SeOOH) and trimethyl selenium ion ($(CH_3)_3Se^+$) (Abrams and Burau, 1989; Yamada and Hattori, 1989; Abrams *et al.*, 1990a; Stroud *et al.*, 2012; Tolu *et al.*, 2014). It has also been suggested that organic Se in soil is present as seleno-amino acids incorporated into protein or peptides in humic and fulvic acid fractions of soil organic matter (Kang *et al.*, 1991b). Spectroscopic evidence shows that organic Se in soils is present in a structure of C-Se-C, similar to that in methyl-selenocysteine and selenomethionine (Mehdawi *et al.*, 2015). Our previous studies showed that Se in low Se Dutch agricultural soils is predominantly in organic form both in soil solution and solid phase, and only a small fraction (on average 5%) is present as inorganic Se (mainly selenite) (Supriatin *et al.*, 2015a; Supriatin *et al.*, 2015b).

Mechanisms and soil parameters controlling Se availability and plant uptake are still not well understood. Among different Se forms present in soil, soluble Se is the main source available for plant uptake. However, in the soil solution, Se can be present as both inorganic selenate, selenite and as organic Se (Weng *et al.*, 2011). Probably not all soluble Se species can be taken up by plants directly. Inorganic selenate can be taken up directly by roots and easily translocated to the shoots, whereas selenite tends to be accumulated in root tissue (Zayed *et al.*, 1998; Hopper and Parker, 1999; Li *et al.*, 2008; Kikkert and Berkelaar, 2013). Previous studies have shown that some small organic Se molecules, such as selenomethionine and selenocystine, can also be taken up by plants (Abrams *et al.*, 1990b; Williams and Mayland, 1992; Kikkert and Berkelaar, 2013), and selenomethionine is more readily taken up by roots and quickly transported to the shoots than selenocystine (Kikkert and Berkelaar, 2013). However, Supriatin *et al.* (2015a) showed that most of soluble Se in Dutch agricultural soils consists of colloidal-sized organic Se, whereas inorganic selenite or selenate and small organic Se molecules were hardly measurable. The colloidal-sized organic Se, due to its relatively large molecular size, is probably not directly available for plants. In addition to the concentration of soluble, directly available Se species, the release rate of Se into soil solution from different forms of Se in soil solid phase determines the buffer capacity of soil in case of fast uptake.

Both pot and field experiments have been performed to derive soil parameters that control Se uptake by plants. Most of these pot experiments were aimed to understand the soil parameters influencing the efficiency of Se uptake from Se fertilizers added (Hurd-Karrer, 1938; Gissel-Nielsen, 1971; Johnsson, 1991; Eich-Greatorex *et al.*, 2007). It was found that soil organic matter content, pH, clay content and sulphate concentration influence Se plant uptake under inorganic Se fertilization treatments (Hurd-Karrer, 1938; Gissel-Nielsen, 1971; Johnsson, 1991; Eich-Greatorex *et al.*, 2007). Studies on the relationship between soil parameters and Se content in plant without Se fertilization were conducted mainly in field experiments, in which organic matter content, pH, total Se content, potassium dihydrogen phosphate extractable S, and water, potassium dihydrogen phosphate or ammonium acetate-EDTA extractable Se were found of relevance (Wang and Sippola, 1990; Zhao *et al.*, 2005; Stroud *et al.*, 2010a; De Temmerman *et al.*, 2014). In general, the above mentioned soil parameters were weakly correlated with Se content in crops, explaining 48% of the variation at maximum (Wang and Sippola, 1990; De Temmerman *et al.*, 2014). However, Stroud *et al.* (2010a) showed that the variability of Se content in wheat grains can be predicted accurately ($R^2 = 0.86$, $p < 0.001$) by taking into account both total Se and potassium dihydrogen phosphate extractable Se and S in soil. Their experiment was performed in soils with low levels of total Se (i.e. 245 to 590 µg kg^{-1}) and up to 70% of extractable soil Se is inorganic selenite. Similarly, Zhao *et al.* (2005) showed that potassium dihydrogen phosphate extractable Se was strongly correlated with Se content of tea plant ($R^2 = 0.95$, $p < 0.01$) grown on selenium rich soils. Up to now, there is no comprehensive research determining the important soil factors controlling Se uptake in crops grown on non-fertilized soils under controlled conditions, especially in low Se soils with predominantly organic Se, such as in Dutch agricultural soils, and there is a lack of understanding of the underlying controlling factors and mechanisms.

The objectives of this study are (1) to find soil parameters that are best related to plant uptake of Se under controlled conditions for Dutch agricultural soils with a relatively low Se content and predominantly organic Se, and (2) to explore the mechanisms that control the bioavailability of Se in the soils under study.

4.2 Materials and methods

4.2.1 Soil samples

Nineteen soil samples were taken from arable fields (potato fields, top soil 0 to 20 cm) in the Netherlands in May 2013. The soil samples consist of four soil types, i.e. sand, clay, loess, and reclaimed peat soils (Table 1). Most of them (n = 16) have been used in the previous study on the effects of drying on soluble Se and Cu concentrations in soils (Supriatin *et al.*, 2015a). Before sampling, mineral nitrogen

Table 1. Characteristics of soil samples, total Se content (aqua regia), and soluble Se, dissolved organic C (DOC) and Se to DOC ratio in 0.01 M $CaCl_2$ extraction of soil samples used for the pot experiment. The data are presented as mean ± standard deviation per soil type. Values in brackets are ranges of the parameters per soil type. The clay soils were further divided into two groups due to the differences in Se uptake.

Soil type	pH[a]	Organic matter (%)	Clay (%)	C:N ratio	Fe-oxalate[b] (g kg^{-1})	Al-oxalate[b] (g kg^{-1})	Se-aqua regia (mg kg^{-1})	Se-$CaCl_2$ (µg kg^{-1})	DOC-$CaCl_2$ (mg kg^{-1})	Se:DOC ratio (µg g^{-1})
Sand (n = 5)	5.5 ± 1.0	6.2 ± 4.9	2.8 ± 1.5	14.1 ± 3.1	3.3 ± 3.6	1.2 ± 0.5	0.44 ± 0.18[c]	2.6 ± 0.6	70.9 ± 19.5	37.8 ± 6.9
							(0.27 – 0.69)[c]	(2.0 – 3.5)	(46 – 90)	(26 – 44)
Clay ≥ 20%, pH > 6 (n = 3)	7.3 ± 0.1	3.1 ± 1.9	26.7 ± 4.0	9.0 ± 0.7	2.2 ± 0.9	0.8 ± 0.4	0.26 ± 0.04	4.4 ± 1.4	45.6 ± 6.8	95.7 ± 17.1
							(0.22 – 0.31)	(3.3 – 6.0)	(39 – 52)	(84 – 115)
Other clay (n = 6)	6.4 ± 0.9	3.1 ± 1.5	17.5 ± 6.6	12.6 ± 2.8	4.2 ± 2.3	0.8 ± 0.4	0.39 ± 0.19	4.0 ± 1.1	78.6 ± 55.0	67.2 ± 40.6
							(0.23 – 0.73)	(2.5 – 5.0)	(35 – 184)	(27 – 140)
Loess (n = 1)	6.4	5.3	33.0	10.2	10.4	1.4	0.73	4.2	94.4	44.1
Reclaimed peat (n = 4)	5.0 ± 0.4	10.8 ± 4.1	1.8 ± 1.5	21.8 ± 1.9	2.6 ± 3.0	1.1 ± 0.1	0.40 ± 0.10	1.6 ± 0.3	84.2 ± 12.3	18.9 ± 4.1
							(0.26 – 0.50)	(1.2 – 1.9)	(66 – 93)	(13 – 22)
Average ± SD[d]							**0.40 ± 0.17**	**3.2 ± 1.4**	**73 ± 34**	**53 ± 35**

[a] Measured in 0.01 M $CaCl_2$ extraction at soil to solution ratio 1:10 (Table 2).

[b] Measured in ammonium oxalate-oxalic acid (pH 3) extraction at soil to solution ratio 1:20 (Table 2).

[c] One sample that has a total Se content (aqua regia) below the determination limit was excluded from the table.

[d] Standard deviation.

(N), phosphorus (P), and potassium (K) fertilizers have been applied in the fields for the coming season following the scheme of individual farmers. No Se fertilizer was applied to the soils. After sampling, the soils were oven dried at 20^{o}C under forced air ventilation for 48 h. Afterwards, the roots and gravels were removed from the soils and the soil samples were ground, passed through a 2 mm sieve, and stored at room temperature (20 ± 1^{o}C) for further use in the pot experiment. Sub soil samples were oven-dried at 40^{o}C under forced air ventilation for 24 to 48 h, ground, and passed through a 2 mm sieve for further soil analysis.

4.2.2 Pot experiment

A pot experiment under controlled greenhouse conditions was designed to derive relations between soil parameters and Se content in shoots of summer wheat (*Triticum aestivum* L.). The pot experiment was conducted between June and August, 2013. Each pot was filled with 1.5 kg of dried soil, in duplicates for each soil sample. Before filling the pots, each soil was adjusted to 60% of its field water holding capacity by adding deionized water. The moistened soils in the pots were allowed to equilibrate for 1 week at room temperature (20 ± 1^{o}C) before sowing. The pots were covered with a plastic sheet during the equilibration time to prevent evaporation. Afterwards, the pots were moved to the greenhouse. Six seeds of summer wheat were sown in each pot. The pots were covered with a plastic sheet for one more week until the seeds were germinated. After that, the plastic sheet was removed and the plants were thinned to three or four plants per pot. The water contents of the soils were maintained at 60% of field water holding capacity during the growth of the plants by daily watering. The experiments were conducted in the greenhouse with a photoperiod of 16 h (06:00 to 21:00 h), light intensity of 400 W m^{-2} (16 SON-T agro lamps), day/night temperature of 21/19°C, and relative humidity of 60%. The location of the pots on the greenhouse table was reshuffled randomly every 3 days. The weeds, pests and insects were suppressed during the growth of the plants.

4.2.3 Plant samples

The plants were harvested 53 days after sowing. At this stage, the spikes and flowers have already emerged. The green shoots were harvested, which include the second to fifth leaves, stems, spikes, and green tillers. The yellow shoots including the first leaf and yellow coloured tillers were also harvested and separated from the green shoots. Afterwards, the green and yellow shoots were washed with deionized water to remove soil particles and oven dried at 70^{o}C for 72 h. The total dry matter of shoots per pot was weighted (dry weight of green plus yellow shoots). Only the dried green shoots were milled for further Se analysis.

4.2.4 Plant digestion

Selenium content in plant samples was determined after digestion with concentrated HNO_3 and H_2O_2 in a microwave with a closed system. A preliminary test on this method was performed using standard reference sample 1573a (tomato leaves) obtained from NIST and IPE 100 (grass, *Poaceae*, GR 94) obtained from Wepal, the Netherlands, three grass samples in which one was spiked with selenite, two maize shoot samples, and two potato tuber samples. Recovery of Se was 98 ± 2% and 96 ± 4% in the standard reference sample 1573a and IPE 100, respectively and 92 ± 2% in the spiked grass sample. The variations among three replicates of Se content in the two grass samples, two maize shoot samples, and two potato tuber samples were on average 5%, 2% and 6%, respectively.

In the digestion, 0.4 g of plant sample was put into a Teflon destruction vessel and 5 mL of concentrated HNO_3 was added to the sample and mixed. Afterwards, the destruction vessel was closed and allowed to stand overnight at room temperature (20 ± 1^{o}C). After that, the vessel was put into a microwave to allow the first digestion step to take place. After the first digestion step was completed, the sample was taken out and cooled down. About 1 mL of H_2O_2 was added to the sample and the second digestion step in the microwave took place. At the end, the sample was transferred into a 50 mL grainer tube and the vessel was washed twice with ultrapure water. The volume of the sample was made up to 50 mL by adding ultrapure water. Selenium concentration in the sample was measured.

4.2.5 Soil extractions

A series of single-step extractions was performed on the soil samples, including 0.01 M $CaCl_2$, 0.43 M HNO_3, hot water, and ammonium oxalate extractions and aqua regia digestion. An overview of the extraction methods is presented in Table 2, and more details can be found in Supriatin *et al.* (2015b). Selenium, dissolved organic C (DOC), pH, major anions (i.e. S = total extracted sulphur, P = total extracted phosphorus, $P\text{-}PO_4$ = total extracted ortho-phosphate), soluble inorganic N ($N\text{-}NH_4$, $N\text{-}NO_3$ + $N\text{-}NO_2$) and total soluble N were measured in the corresponding soil extractions as presented in Table 2. Concentration of dissolved organic N (DON) in 0.01 M $CaCl_2$ extraction was derived from the difference between total soluble N and soluble inorganic N. Concentrations of Fe and Al in the ammonium oxalate extraction were measured as well, which represent the amount of amorphous (hydr)oxides of the corresponding metals (Table 1 and 2). Concentration of DOC in ammonium oxalate extraction was derived indirectly from UV-absorbance measured at 254 nm wavelength after diluting the extracts 50 times and correcting for Fe concentration in the extracts as described in detail in Supriatin *et al.* (2015b).

4.2.6 Inorganic selenite in ammonium oxalate extraction measured using HPLC - ICP-MS

A High Performance Liquid Chromatography (HPLC) system with an anion exchange column coupled online with High Resolution ICP-MS was used to measure inorganic selenite, selenate and organic Se in the ammonium oxalate extraction of the soil samples. The details of the HPLC and ICP-MS conditions, solutions and procedure used in the HPLC analysis were described elsewhere (Supriatin *et al.*, 2015b). The soil samples were extracted using ammonium oxalate - oxalic acid (pH 3) solution (Table 2). Initial total Se concentrations in the soil extracts were measured before the HPLC analysis. Concentrations of selenite and

Table 2. An overview of soil extractions performed on the soil samples used in this study.

Extraction	Possible pool representing	Method adopted from	SSR[b]	Extraction/digestion (h)	Centrifugation (rpm and min)	Filter	Parameter measured
0.01 M $CaCl_2$	Soluble (Houba *et al.*, 2000)	Houba *et al.* (2000)	1:10	2	3000; 10	0.45 µm[d]	Se, DOC, pH, S, P, PO_4, NH_4, NO_3 + NO_2, total soluble N
0.43 M HNO_3[a]	Reactively adsorbed	-	1:10	4	3000; 10	Paper[e]	Se, DOC
Hot water	Labile organic matter associated (Ghani *et al.*, 2003)	Ghani *et al.* (2003)	1:10	16 (80°C)	3500; 20	0.45 µm[d]	Se, DOC, pH, S, P, PO_4
Ammonium oxalate – oxalic acid (pH 3)[c]	Adsorbed to amorphous Fe and Al oxides (Keskinen *et al.*, 2009)	McKeague and Day (1966)	1:20	2 (in the dark)	3000; 10	No	Se, UV, S, P, Fe, Al
Aqua regia digestion	(Pseudo) total content (Keskinen *et al.*, 2009)	-	1:100	Overnight, plus 2 h in a microwave at 170°C	No	Paper[e]	Se

[a] Especially for soil samples that have pH ≥ 7, about 0.2 mL of 5 M HNO_3 was added to neutralise the carbonates in the soil samples.

[b] Soil to solution ratio.

[c] The soil extracts were further fractionated on HPLC - ICP-MS.

[d] Syringe filter (Whatman Aqua 30/0.45 CA).

[e] Ash-free paper filter (Whatman 589/3 nr. 10300212).

selenate in the soil extracts were determined from the peak areas of the chromatograms. Concentrations of organic Se were calculated from the difference between total Se concentrations and inorganic Se (selenite plus selenate) concentrations in the corresponding soil extracts. There was no interference for Se in the soil extracts was observed during the ICP-MS analysis because we used a High Resolution ICP-MS in high resolution mode to measure isotope ^{78}Se for Se measurement in the samples (see Table 3 in Chapter 3). This high resolution mode of ICP-MS can separate isotope ^{78}Se from all the possible interferences, except that for Kr that cannot be separated from ^{78}Se, however, we solve that by measuring ^{83}Kr and compensate the ^{78}Se signal for that (Peter Nobels, *Personal communication*). Similar ICP-MS conditions were also applied for Se analysis in other matrices of soil extractions (Table 2).

4.2.7 Chemical analysis

Concentrations of Se in plant digestion samples and all soil extractions, and concentrations of P in 0.01 M $CaCl_2$ and hot water extractions were measured using High Resolution ICP-MS (Thermo Scientific, Element2). Concentrations of S in 0.01 M $CaCl_2$ and hot water extractions and S, P, Fe and Al in ammonium oxalate extraction were measured using ICP-AES (Thermo Scientific, Iris Advantage). Concentrations of DOC in 0.01 M $CaCl_2$, 0.43 M HNO_3 and hot water extractions, soluble inorganic N and total soluble N in 0.01 M $CaCl_2$ extraction, and ortho-phosphate P (P-PO_4) in 0.01 M $CaCl_2$ and hot water extractions were measured using a segmented flow analyser (SFA) (3039 Skalar, the Netherlands). The UV-absorbance of the ammonium oxalate extracts was determined using UV-spectrophotometer (Genesys 10S Thermo Scientific). pH was measured using a pH meter (Radiometer Analytical PHM210) with a glass electrode (Beckman 511084). Total C and N in the soil samples were analysed using a CHN elementary analyser (Leco). Total organic matter content in the soil samples was analysed using near infrared spectroscopy (NIR) (Reeves III, 2010; Soriano-Disla *et al.*, 2014). Total organic C content in the soil samples was calculated from the measured soil organic matter content assuming that soil organic matter contains 50% organic C.

4.2.8 Linear regression analysis

Single and multiple linear regression analyses were performed to correlate Se content in the wheat shoots and soil parameters measured in order to identify soil parameters that determine Se content in wheat shoots. Since the Se contents in the wheat shoots were not normally distributed, the data were transformed into Log(10) before used in the regression analyses.

4.3 Results

4.3.1 Total and extractable Se content in soils

Total Se content (aqua regia) in the arable land soils ranges from 0.22 up to 0.73 mg kg^{-1}, which is mostly less than 0.6 mg Se kg^{-1} (Table 1). Gupta and Gupta (2000) suggested a threshold value of 0.6 mg kg^{-1} for Se deficient soils. Our previous study has also shown that about 75% of 83 soil samples taken from grasslands and other arable lands in the Netherlands contain less than 0.6 mg Se kg^{-1} (Supriatin *et al.*, 2015b). The findings suggest that most of agricultural soils in the Netherlands are Se deficient. Other parts of Europe, such as Belgium, Sweden, Germany, Czech Republic, Spain (Mediterranean area), also have low total Se content in their soils i.e. mainly < 0.50 mg kg^{-1} (Hartfiel and Bahners, 1988; Johnsson, 1992; Roca-Perez *et al.*, 2010; De Temmerman *et al.*, 2014; Száková *et al.*, 2015). Total Se (aqua regia) in soils used in this study is hardly correlated to amorphous Al-(hydr)oxide content ($R^2 = 0.16$, $p > 0.05$) and weakly correlated to soil organic matter content ($R^2 = 0.26$, $p < 0.05$) and to amorphous Fe-(hydr)oxide content ($R^2 = 0.51$, $p < 0.001$). In contrast, our previous study using 83 soil samples taken from grasslands and other arable lands in the Netherlands showed that the total Se (aqua regia) is strongly correlated with soil organic matter content and amorphous Fe- and Al-(hydr)oxide content (Supriatin *et al.*, 2015b). This difference is probably due to a narrow range of soil organic matter content, amorphous Fe- and Al-(hydr)oxide content, and total Se (aqua regia) content in the soils used in this study (Table 1). The fraction of inorganic Se (mainly selenite) in the soils is on average 3 ± 3% of total Se (aqua regia) (Table A.2; n = 15), indicating that most of the Se is present in organic form, despite that only a weak correlation was found between total Se content and soil organic matter content.

The amount of Se measured in different extractions followed the order of aqua regia (224 to 734 µg kg^{-1}) > ammonium oxalate (38 to 146 µg kg^{-1}) > hot water (6 to 29 µg kg^{-1}) ≥ 0.43 M HNO_3 (7 to 32 µg kg^{-1}) > 0.01 M $CaCl_2$ (1.2 to 6.0 µg kg^{-1}) (Table 1 and A.1). Soluble Se concentration in 0.01 M $CaCl_2$ extraction accounts for on average 1.0 ± 0.7% of total Se (aqua regia) (Table 1), slightly lower than that observed in the previous study for grassland soils (1.5 ± 0.6%) and other arable land soils (1.4 ± 0.6%) (Supriatin *et al.*, 2015b). The fraction of Se in other extractions is on average 4 ± 2% in 0.43 M HNO_3; 5 ± 2% in hot water; and 21 ± 7% in ammonium oxalate (Table A.1), comparable to that measured in previous study (Supriatin *et al.*, 2015b). On average 85% of Se in ammonium oxalate extraction is organic Se and 15% is inorganic Se (mainly selenite) (Table A.2; n = 16). Previous studies suggested that Se in 0.01 M $CaCl_2$ and hot water extraction is mainly in organic Se form, whereas 0.43 M HNO_3 extracts mainly inorganic Se plus a small amount of organic Se (Supriatin *et al.*, 2015a; Supriatin *et al.*, 2015b).

4.3.2 Selenium content in wheat shoots

Selenium content in the wheat shoots is between 7 and 101 µg kg^{-1} (plant dry weight), with a mean of 28 µg kg^{-1} (n = 19) (Fig. A.1.1). In general, Se content in the wheat shoots grown on clay soils with clay content ≥ 20% and pH > 6 is higher than that in the wheat shoots grown on other soil types (Fig. 1A). The variability of Se content in the wheat shoots grown on “other clay” soils is high due to that one wheat shoot sample (grown on a clay soil with clay content 18% and pH 7.30) contains 101 µg kg^{-1} Se whereas the other five samples (grown on clay soils with clay content 12 to 30% and pH 5.20 to 7.30) contain about 12 to 40 µg kg^{-1} Se. Since the wheat shoots were harvested at the flowering stage, we do not have data of Se content in wheat grain. It has been shown that Se content in wheat grain is correlated to Se content in wheat stems or leaves, and that the grain Se content is largely similar to that in the wheat stems but much lower than the Se content in wheat leaves (Jiang *et al.*, 2015). Therefore, we expect that Se content in wheat grain in our current study would be less than that measured in the shoots (i.e. 7 to 101 µg kg^{-1} Se).

A Se content in plants of 50 to 100 µg Se kg^{-1} dry weight has been proposed as the minimum amount required for animal and human intake (Gardiner and Gorman, 1963; Gissel-Nielsen *et al.*, 1984). Previous studies showed that Se content in wheat grain grown on 41 Danish soils range between 4 and 67 µg kg^{-1}, with an average of 20 µg kg^{-1} (Gissel-Nielsen, 1975) and in the wheat grain grown on UK soils is on average between 25 to 33 µg kg^{-1} (n = 85 to 187) over three years of sampling (Adams *et al.*, 2002). Total Se contents in Danish soils and UK soils are between 0.14 to 0.52 mg kg^{-1} (Hamdy and Gissel-Nielsen (1976) *in* Gupta and Gupta (2000)) and on average 0.50 mg kg^{-1} (Adams *et al.*, 2002), respectively, which are in a similar range as in the soils in our current study (Table 1). Based on these findings, we expect that without Se fertilizer application, Se content in wheat grain grown on the soils in our study would be mostly below the minimum amount of Se for animal and human intake, especially in the soils other than clay soil with a combination of clay content ≥ 20% and pH > 6 (Fig. 1A), indicating a generally low Se bioavailability in the arable soils in the Netherlands.

The yield of wheat shoots ranged from 2 to 9 g dry weight pot^{-1} (Fig. A.1.1). Soil type had no effect on crop yield (Fig. 1B). Hardly any correlation between yield and Se content in the wheat shoots (µg Se kg^{-1} dry biomass) was observed ($R^2 = 0.001$, $p > 0.05$; Fig. A.1.1), indicating that Se is not a limiting factor for plant growth. A lack of correlation between yield and Se content in Italian rye grass (*Lolium multiflorum*) was also observed in the study of Yläranta (1983). The variation in yield of wheat shoots among the different soils (around 4 times; Fig. A.1.1) is smaller than that in Se content (15 times; Fig. A.1.1) or total Se uptake (17 times; Fig. A.1.2) in wheat shoots, and the absolute Se content in the wheat shoots (in µg Se kg^{-1} dry biomass) correlates well with the total Se uptake per pot (in µg Se pot^{-1}) ($R^2 = 0.93$, $p < 0.001$; Fig. A.1.2). Therefore, for the purpose of comparison among soils, there is not much difference if the absolute Se content or total Se uptake is used.

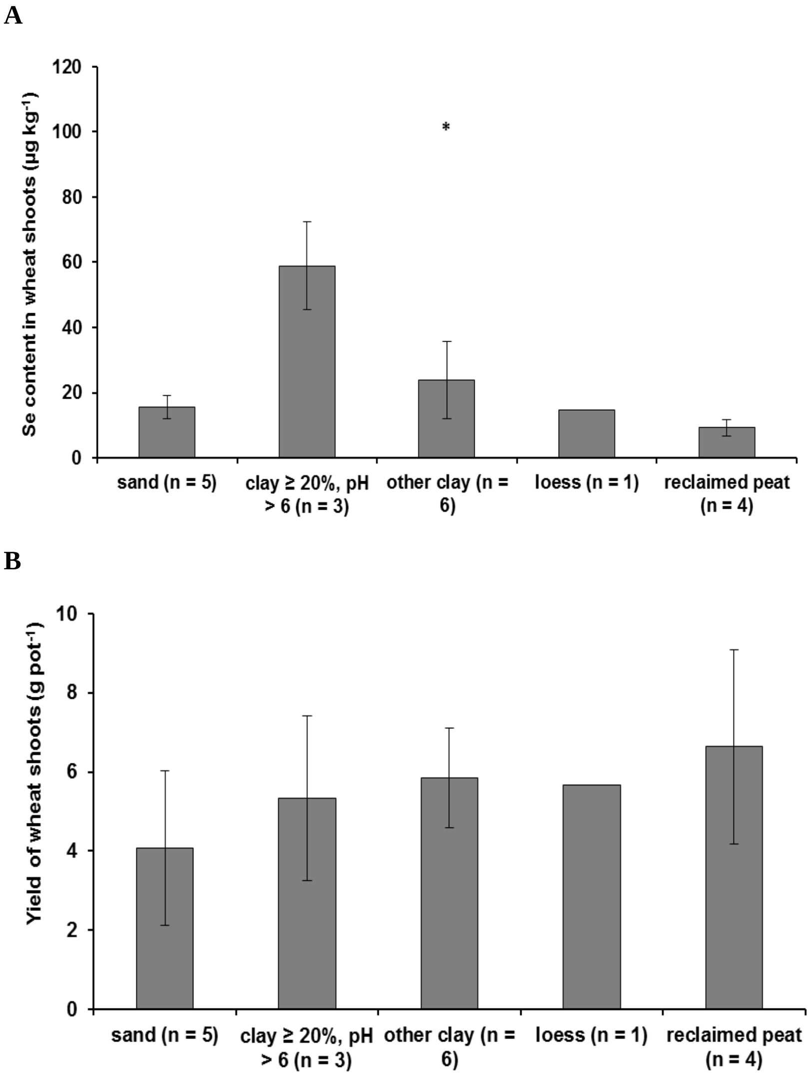


Fig. 1. Selenium content (A) and yield of wheat shoots (B) as affected by soil type. The bars are means and the error bars are the standard deviations per soil type. * is an outlier.

4.3.3 Regression model for Se uptake

To identify which soil parameters are important in determining Se content in the wheat shoots, firstly single factor regression analysis between Se content in the wheat shoots and each individual soil parameter was performed (Table 3). Among the soil property parameters, pH measured in 0.01 M $CaCl_2$ extraction (pH-$CaCl_2$), pH in hot water extraction (pH-hot water) and clay content are positively correlated to Se content in the wheat shoots, whereas organic matter content and soil C:N ratio are negatively correlated, and amorphous Fe- and Al-(hydr)oxide content are not correlated to Se content in the wheat shoots. Among Se concentrations measured in different extractions, Se in 0.01 M $CaCl_2$ extraction (Se-$CaCl_2$) and Se in hot water extraction (Se-hot water) are positively correlated to Se content in the wheat shoots, whereas total Se content (aqua regia) and total inorganic selenite content are negatively correlated, and Se in ammonium oxalate and 0.43 M HNO_3 extractions are not correlated to Se content in the wheat shoots. Besides, DOC and DON in 0.01 M $CaCl_2$ extraction (DOC-$CaCl_2$ and DON-$CaCl_2$, respectively) and DOC in hot water extraction (DOC-hot water) are negatively correlated to Se content in wheat shoots, whereas other soil parameters considered, such as S, P and PO_4 in 0.01 M $CaCl_2$ and hot water extractions, and S and P in ammonium oxalate extraction are

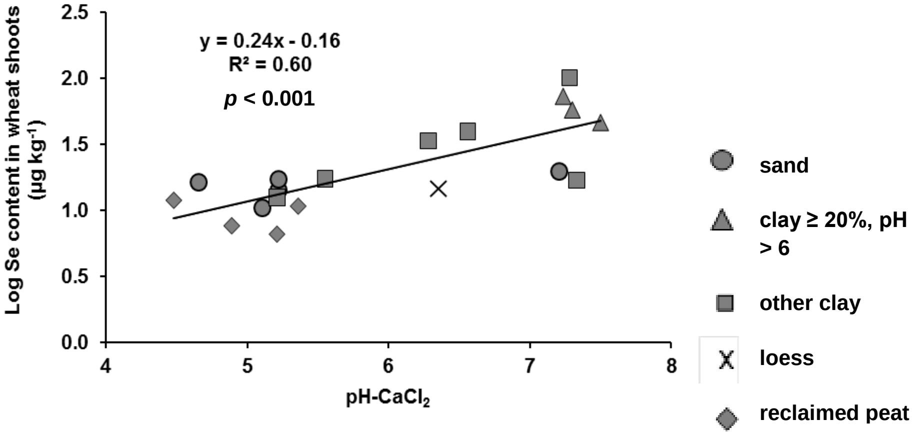


Fig. 2. Correlation between Se content in wheat shoots and soil pH measured in 0.01 M $CaCl_2$ extraction (n = 19).

not correlated to Se content in the wheat shoots. Among the single soil parameters, soil pH explains most (about 60%) of the variation of Se content in wheat shoots (Table 3 and Fig. 2), showing that Se content in wheat shoots increases with the increase of soil pH (Fig. 2).

Following the single parameter regression, multiple linear regression analysis was performed by combining two or more soil parameters. We included in this multiple regression the soil factors of pH-$CaCl_2$, pH-hot water, clay content, soil organic matter content, soil C:N ratio, Se-$CaCl_2$, Se-hot water, total Se content (aqua regia), inorganic selenite content, DOC-$CaCl_2$, DON-$CaCl_2$ and DOC-hot water, all of which have been found correlated with Se content in the wheat shoots (Table 3). For all combinations of two factors regression, the best regression model obtained includes Se-$CaCl_2$ and DOC-$CaCl_2$, which explains 85% (adjusted $R^2 = 0.85$, $p < 0.001$) of the variability of Se content in the wheat shoots (Eq. 1 and Model 25 in Table A.3). We have shown above that in general the Se content in the wheat shoots grown on clay soils with a combination of clay content ≥ 20% and pH > 6 is higher than that in the wheat shoots grown on other soil types (Fig. 1A). The two factors regression including clay content and pH-$CaCl_2$ leads to an adjusted R^2 of 0.58, $p < 0.001$ (Eq. 2 and Model 8 in Table A.3). Extending the regression to three or four factors can only slightly raise the maximum adjusted R^2 from 0.85 to 0.87 (Table A.3), and since the benefit of including additional factors is relatively small we focus our further evaluation on the one factor and two factors regressions.

$$\text{Log(Se-wheat shoots)} = 3.09 + 1.01\ \text{Log(Se-CaCl}_2) - 1.23\ \text{Log(DOC-CaCl}_2)$$
$$\text{Adj. } R^2 = 0.85,\ p < 0.001 \quad (1)$$

$$\text{Log(Se-wheat shoots)} = 0.07 + 0.19\ (\text{pH-CaCl}_2) + 0.15\ \text{Log(clay)}$$
$$\text{Adj. } R^2 = 0.58,\ p < 0.001 \quad (2)$$

$$\text{Se-wheat shoots} = 0.69\ (\text{Se:DOC-CaCl}_2) - 8.39$$
$$R^2 = 0.88 \text{ or Adj. } R^2 = 0.87,\ p < 0.001 \quad (3)$$

Unit: Se-wheat shoots (μg kg^{-1}); Se-CaCl$_2$ (mg kg^{-1}); DOC-CaCl$_2$ (mg kg^{-1}); clay (%); Se:DOC-CaCl$_2$ (μg g^{-1}).

Table 3. Regression parameters of the equation: Log$_{10}$(Se content in wheat shoots, μg kg^{-1}) = a + b*Log$_{10}$(soil parameter) (n = 19).

Soil parameter	Intercept (a)	Coefficient (b)	R^2	*p* value
pH-CaCl$_2$[a]	-0.16	0.24	**0.60**	< 0.001
pH-hot water[a]	-0.14	0.22	**0.58**	< 0.001
Clay (%)	0.98	0.39	**0.46**	< 0.01
Organic matter (%)	1.78	-0.74	**0.50**	< 0.001
Soil C:N ratio	2.99	-1.49	**0.41**	< 0.01
Amorphous Fe-(hydr)oxide (mg kg^{-1})	1.66	-0.10	0.01	> 0.05
Amorphous Al-(hydr)oxide (mg kg^{-1})	3.71	-0.81	0.21	0.05
Se-CaCl$_2$ (μg kg^{-1})	0.79	1.12	**0.45**	< 0.01
Se-HNO$_3$ (μg kg^{-1})	2.14	-0.75	0.21	0.05
Se-hot water (μg kg^{-1})	-0.04	1.09	**0.22**	< 0.05
Se-ammonium oxalate[b] (μg kg^{-1})	1.54	-0.18	0.02	> 0.05
Total Se (aqua regia)[b] (μg kg^{-1})	4.15	-1.10	**0.30**	< 0.05
Selenite[b] (μg kg^{-1})	2.13	-0.67	**0.42**	< 0.05
DOC-CaCl$_2$ (mg kg^{-1})	3.77	-1.35	**0.51**	< 0.001
DOC-hot water (mg kg^{-1})	1.13	-0.97	**0.53**	< 0.001
DON-CaCl$_2$ (mg kg^{-1})	1.88	-0.60	**0.34**	< 0.01
S-CaCl$_2$ (mg kg^{-1})	1.60	-0.18	0.12	> 0.05
S-hot water (mg kg^{-1})	1.80	-0.34	0.14	> 0.05
S-ammonium oxalate (mg kg^{-1})	2.23	-0.47	0.19	> 0.05
P-CaCl$_2$ (mg kg^{-1})	1.27	0.09	0.009	> 0.05
P-hot water (mg kg^{-1})	1.74	-0.33	0.08	> 0.05
P-ammonium oxalate (mg kg^{-1})	2.44	-0.42	0.03	> 0.05
PO$_4$-CaCl$_2$ (mg kg^{-1})	1.28	0.10	0.02	> 0.05
PO$_4$-hot water (mg kg^{-1})	1.43	-0.12	0.02	> 0.05

[a] pH is not converted into Log$_{10}$.

[b] The samples that have Se concentrations below the determination limit were not included in the regression analysis.

4.4 Discussion

4.4.1 Selenium to DOC ratio in 0.01 M $CaCl_2$ extraction determines Se uptake in wheat shoots

Soluble Se and DOC in 0.01 M $CaCl_2$ extraction explain the majority (around 85%) of Se variability in wheat shoots grown on low Se soils under controlled conditions (Eq. 1 and Model 25 in Table A.3). This model can predict Se content in the wheat shoots quite well, however, when the measured Se content in the wheat shoots is above 60 µg kg^{-1}, the model underestimates the measured values (Fig. 3). This model, which is based on the two major explanatory variables, can be further improved and reduced to a single factor regression model by replacing soluble Se and DOC in 0.01 M $CaCl_2$ extraction with the Se to DOC ratio in 0.01 M $CaCl_2$ extraction ($R^2 = 0.88$, $p < 0.001$) (Eq. 3 and Fig. 7A), indicating that the richness of Se in dissolved organic matter (DOM) is a good predictor of bioavailable Se in low Se soils with predominantly organic Se.

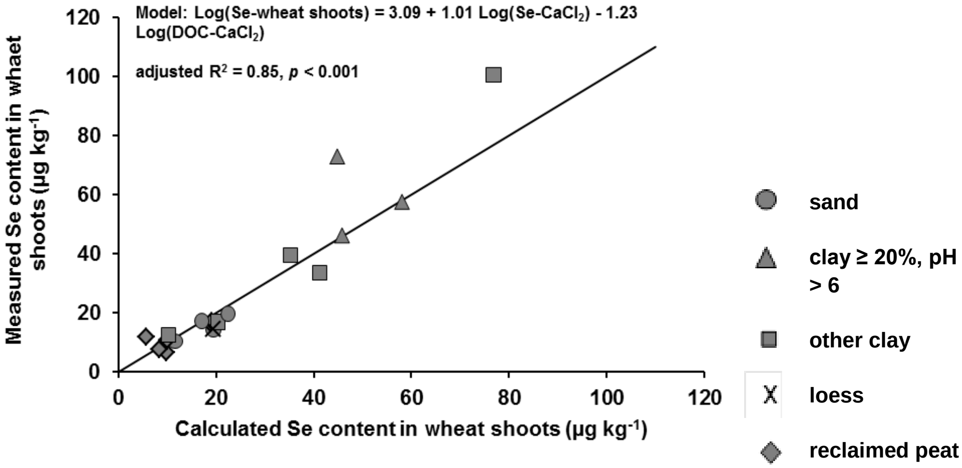


Fig. 3. Comparison between the measured and calculated Se content in the wheat shoots grown in the pot experiment. The model used to calculate Se content in the wheat shoots includes soluble Se-$CaCl_2$ and DOC-$CaCl_2$ as soil parameters (Eq. 1

and Model 25 in Table A.3). The line is 1:1 line at which the measured and calculated Se content in wheat shoots are equal.

Our observation that in general more Se was taken up in the wheat shoots grown on clay soils with clay content ≥ 20% and pH > 6 (Fig. 1A), is not directly reflected in the model in Equation 1 (two factors model including Se and DOC in $CaCl_2$ extraction) or in the single factor model with Se to DOC ratio in $CaCl_2$ extraction (Eq. 3). The model in the Equation 2, which includes clay content and pH-$CaCl_2$, has a lower predictive power ($R^2 = 0.58$, $p < 0.001$) than the model in Equation 1 ($R^2 = 0.85$, $p < 0.001$). A closer examination shows that the higher Se uptake in the wheat shoots grown on clay soils with clay content ≥ 20% and pH > 6 is primarily related to the high Se to DOC ratio in 0.01 M $CaCl_2$ extraction of these soils (Table 1). These findings indicate that bioavailability of Se in low Se soils dominated by organic Se is related to the solubility and quality of Se containing DOM in soil solution.

The Se and DOC concentrations in 0.01 M $CaCl_2$ extraction in the moist soil samples taken freshly from the fields have also been measured (Supriatin *et al.*, 2015a). Consistent with the dried soils, Se to DOC ratio in 0.01 M $CaCl_2$ extraction of the moist soils is also positively correlated to Se content in the wheat shoots ($R^2 = 0.70$, $p < 0.001$; n = 19), although the correlation for the moist soils is less strong than for dried soils ($R^2 = 0.88$, $p < 0.001$; Eq. 3 and Fig. 7A). Previous study showed that an increase of both DOC (on average 2.5 to 2.7 fold) and Se (on average 2 to 4 fold) occurred in 0.01 M $CaCl_2$ extraction upon soil drying, but the Se to DOC ratio remained quite stable (Supriatin *et al.*, 2015a). However, the very low concentrations of soluble Se measured in 0.01 M $CaCl_2$ extraction of moist soils can be a challenge to derive an indicator of Se bioavailability using the moist soil samples (Supriatin *et al.*, 2015a).

In our preliminary study, selenite adsorption in several selected Dutch agricultural soil samples other than the soils used in the current study was measured by adding selenite (data not shown). By extrapolating the results of this adsorption experiment to the amount of selenite initially present in the soils used in the current study (< 40 µg kg^{-1}) (Table A.2), the K_d values of selenite (K_d = ratio adsorbed selenite in µg kg^{-1}:soluble selenite in µg L^{-1}) and amount of soluble selenite in 0.01 M $CaCl_2$ extraction were estimated for the soils under current study. These calculations suggest that the K_d values for selenite in these soils are between 1500 and 4600 kg L^{-1} and that the soluble selenite concentration in 0.01 M $CaCl_2$ are mainly < 10 ng L^{-1} (< 0.1 µg kg^{-1}). This very low concentration explains why the soluble selenite was hardly measurable in the 0.01 M $CaCl_2$ soil extraction (Supriatin *et al.*, 2015a). The estimated concentration of soluble (inorganic) selenite accounts for mostly < 1% of total soluble Se measured in 0.01 M $CaCl_2$ extraction (1.2 to 6.0 µg kg^{-1}; Table 1), which supports the above conclusion that the content of soluble Se-rich organic molecules determines the Se plant uptake.

Different forms of soluble organic Se, such as selenomethionine ($C_5H_{11}NO_2Se$), methane seleninic acids (CH_3SeOOH) and trimethyl selenium ion ($(CH_3)_3Se^+$) have been measured in soils (Abrams and Burau, 1989; Yamada and Hattori, 1989; Abrams *et al.*, 1990a; Stroud *et al.*, 2012; Tolu *et al.*, 2014). Kikkert and Berkelaar (2013) have shown that selenium amino acids, such as selenomethionine, is more readily taken up by plants than selenocystine. Selenium content in selenium amino acids, such as selenocystine ($C_6H_{12}N_2O_4Se_2$), selenocysteine ($C_3H_7NO_2Se$) and selenomethionine ($C_5H_{11}NO_2Se$), is high with a Se to C mass ratio of 2.2, 2.2 and 1.3 g g^{-1}, respectively, which is in contrast to the much lower ratio of Se to DOC measured in the $CaCl_2$ extraction (13 to 140 µg g^{-1}; Table 1). If we take a Se to C ratio of 1.3 g g^{-1} based on selenomethionine as an assumed representative of Se-rich molecule in the $CaCl_2$ extractions of the soils studied, and assume that the rest of DOM has a Se to C ratio of 10 µg g^{-1} (i.e. the overall Se to organic C ratio in bulk soil organic matter; Supriatin *et al.* 2015b), we can calculate the concentration of Se-rich molecule using the measured Se to DOC ratio in the $CaCl_2$ extraction. This calculation leads to 0.28 to 5.51 µg Se kg^{-1} soil of selenomethionine-like Se-rich organic molecule in the $CaCl_2$ extraction of the soils studied, which would account for 24% to 93% of the measured total soluble Se in the $CaCl_2$ extractions, but account for only 0.00024% to 0.01% of the measured total DOC. The fraction of Se-rich molecule calculated in this study is in similar range of selenomethionine fractions measured in water (on average 20% of soluble Se) or 0.016 M KH_2PO_4 (52 to 83% of soluble Se) soil extractions from previous studies (Stroud *et al.*, 2012; Tolu *et al.*, 2014). A positive correlation between the amount of calculated Se-methionine in the $CaCl_2$ extraction and Se content in the wheat shoots was found ($R^2 = 0.63$, $p < 0.001$) (Fig. 4), which supports that the Se to DOC ratio in $CaCl_2$ extraction can be considered as a measure of the amount of Se-rich organic molecule in the extraction.

Previous study of Supriatin *et al.* (2015a), however, found hardly any Se-rich small organic molecules (< 1 nm) in the $CaCl_2$ soil extraction, suggesting that the Se-rich organic molecules are probably associated with larger organic molecules, forming colloidal sized structures (1 nm to 0.45 µm). In addition, the Se to DOC ratios in the hydrophilic acid (Hy) and fulvic acid (FA) fractions of DOM in $CaCl_2$ extraction are comparable, and the ratio of Se to DOC for both the Hy and FA fractions increases with pH, especially in the soil samples from potato fields (Supriatin *et al.*, 2015a), which supports the idea that most of Se-rich molecules are probably associated with colloidal sized Hy and FA molecules. A correlation analysis using Se to organic C ratio of Hy and FA fractions of DOM in 0.01 M $CaCl_2$ extraction of soil samples taken from the same fields as used in this study (n = 8) (Supriatin *et al.*, 2015a) showed that Se content in the wheat shoots is strongly positively correlated with Se to organic C ratio of the Hy fraction ($R^2 = 0.93$, $p < 0.001$) and less strongly correlated to Se to organic C ratio of the FA fraction ($R^2 = 0.74$, $p < 0.05$). These findings indicate that the Se plant uptake is probably more influenced by Se-rich compounds in the more rapidly degradable Hy fraction than the less easily degradable FA fraction.

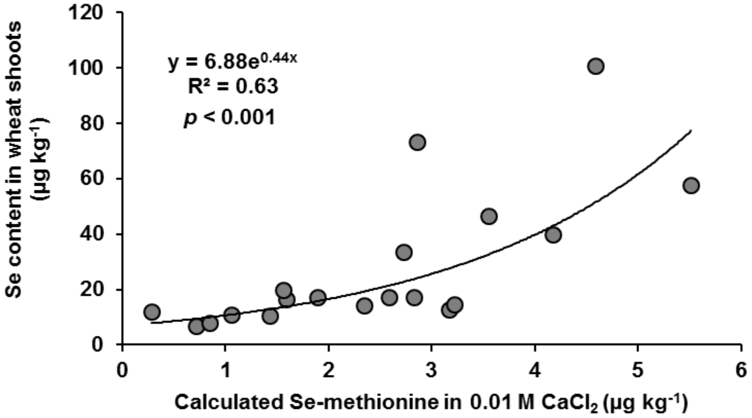


Fig. 4. Relationship between Se content in wheat shoots and calculated selenomethionine-like Se-rich organic molecule in the $CaCl_2$ extraction estimated from the measured Se to DOC ratio in $CaCl_2$ extraction.

4.4.2 Soil properties determining Se to DOC ratio in 0.01 M $CaCl_2$ extraction and Se in wheat shoot

The above results showed that soluble Se and DOC in 0.01 M $CaCl_2$ extraction, which are reflected by the Se to DOC ratio in $CaCl_2$ extraction, are the main soil parameters determining Se content in the wheat shoots (Eq. 1 and 3), suggesting that Se uptake in the wheat shoots is not only related to solution Se concentration but also to the Se speciation in the solution. To further explore the basic soil properties that can potentially determine the concentration and speciation of soluble Se, their correlation to soluble Se and Se to DOC ratio in 0.01 M $CaCl_2$ extractions is investigated and discussed below. Both pH-$CaCl_2$ and clay content are positively correlated to soluble Se and Se to DOC ratio, whereas organic matter content and soil C:N ratio are negatively correlated (Table 4). Among these soil parameters, soil pH correlates most strongly with Se to DOC ratio (R^2 = 0.66, $p < 0.001$), but correlates the least with soluble Se (R^2 = 0.26, $p < 0.05$) (Table 4), indicating that the soil pH is the primary factor that controls the solubility of Se-rich DOM. These findings correspond to our observations that pH, clay content, soil organic matter content and soil C:N ratio are also significantly correlated with Se content in the

Table 4. Single factor linear regression between Se concentration or Se to DOC ratio in 0.01 M $CaCl_2$ extraction and basic soil properties (n = 19).

Soil parameter	Se-$CaCl_2$ ($\mu g\ kg^{-1}$) = a + b*soil parameter				Log_{10}(ratio Se:DOC-$CaCl_2$, $\mu g\ g^{-1}$) = a + b*Log_{10}(soil parameter)			
	Intercept (a)	Coefficient (b)	R^2	*p* value	Intercept (a)	Coefficient (b)	R^2	*p* value
pH-$CaCl_2$	-0.78	0.66	**0.26**	< 0.05	0.35	0.21	**0.66**	< 0.001
Clay (%)	2.11	0.09	**0.53**	< 0.001	1.37	0.32	**0.44**	< 0.01
Organic matter (%)	4.18	-0.17	**0.29**	< 0.05	2.08	-0.69	**0.62**	< 0.001
Soil C:N ratio	6.05	-0.20	**0.52**	< 0.001	3.20	-1.38	**0.51**	< 0.001
Amorphous Fe-(hydr)oxide ($mg\ kg^{-1}$)	3.19	$4.28*10^{-6}$	$9*10^{-5}$	> 0.05	2.06	-0.12	0.02	> 0.05

wheat shoots, and that soil pH is the single factor that correlates most strongly with Se content in wheat shoots ($R^2 = 0.58$ to 0.60, $p < 0.001$) (Table 3).

The amount of Se-rich DOM and Se content in the wheat shoots increase with the increase of pH (Table 3 and 4 and Fig. 2 and A.2.1), consistent with previous studies that have shown the solubility of Se-containing organic matter increases with the increase of pH (Gustafsson and Johnsson, 1994; Yanai *et al.*, 2015). In addition to controlling the solubility of Se-containing organic matter, we expect that pH also plays a role in the mineralization of soluble organic Se (Fig. 5). The soluble organic Se, which may not be directly available for plant uptake when in colloidal form, is likely to be mineralized into soluble inorganic Se (selenate or selenite) or soluble small organic Se molecules that are available for plant uptake (Fig. 5). Curtin *et al.* (1998) suggested that mineralization of soil organic matter increased with an increase of soil pH from 5.7 to 7.3. Further, the soluble Se and Se to DOC ratio in 0.01 M $CaCl_2$ extraction, as well as Se content in the wheat shoots, are increased at a low soil C:N ratio (Table 3 and 4), suggesting that the quality of solid soil organic matter strongly influences the amount of Se-rich DOM (Fig. 5). Our previous studies indicated that Se in both the soil solid phase and soil solution is mainly present as organic Se (Supriatin *et al.*, 2015a; Supriatin *et al.*, 2015b), and we expect, therefore, that mineralization of the organic Se controls the solubility and bioavailability of Se in these soils (Fig. 5). A low soil C:N ratio is related to both a relatively high Se content in soil organic matter (Fig. 6E) and to some extent a likely faster mineralization of soil organic matter (Post *et al.*, 1985; Zech *et al.*, 1997). In addition, soluble Se and Se to DOC ratio in 0.01 M $CaCl_2$ extraction and Se content in the wheat shoots have shown a positive correlation with clay content and a negative correlation with soil organic matter content (Table 3 and 4). These correlations may be caused by the fact that clay and soil organic matter content are correlated with soil C:N ratio ($R^2 = 0.62$, $p < 0.001$ and $R^2 = 0.42$, $p < 0.01$ for the correlations between soil C:N ratio and clay content or soil C:N ratio and organic matter content, respectively) and pH ($R^2 = 0.39$, $p < 0.01$ and $R^2 = 0.40$, $p < 0.01$ for the correlations between pH and clay content or pH and organic matter content, respectively) of these soils. Homann *et al.* (2007) indicated that the association of soil N with clay is stronger than that of soil organic C with clay, which results in a decrease of soil C:N ratio with an increase of clay content as found in this study (data not shown). On the other hand, a positive correlation between soil organic matter content and soil C:N ratio was found in this study (data not shown), reflecting a decline of the quality of soil organic matter (i.e. increase of soil C:N ratio) in high organic matter soils.

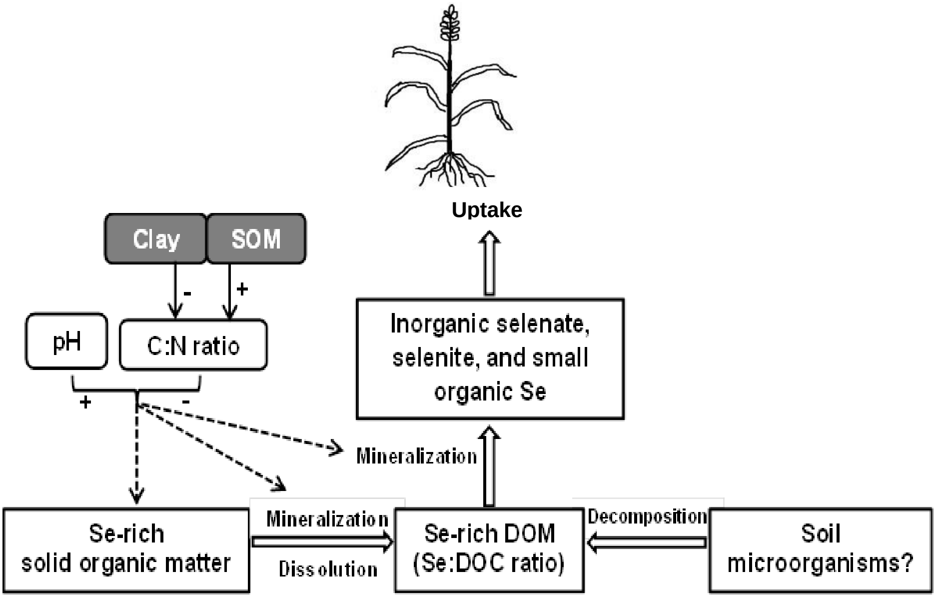


Fig. 5. Schematic pathways between Se in the soil solid phase, soil solution, and plants, and the basic soil properties influence these processes. In soils that contain low total Se with predominantly organic Se, Se-rich organic matter in the soil solid phase is released into the soil solution upon mineralization/dissolution. The decay of soil microorganisms may also contribute to Se-rich DOM. The Se-rich DOM can either be directly taken up by plants (in case of Se-amino acids), or after being mineralized into chemical forms that are directly available (selenate, selenite, small organic Se). The amount of Se-rich DOM can be measured as the Se:DOC ratio in soil solution. pH has a positive, and soil C:N ratio has a negative effect on the

amount of Se-rich solid organic matter, the mineralization/dissolution of solid organic Se into soluble organic Se, and the mineralization of soluble organic Se. Clay content has a positive correlation, and soil organic matter content has a negative correlation with Se bioavailability, probably through its correlation with soil C:N ratio. Soil C:N ratio is negatively related to clay content and positively related to soil organic matter content.

4.4.3 Mass balance estimation

Total Se uptake in the wheat shoots is in the range of 0.03 to 0.59 µg Se pot^{-1} (Fig. A.1.2). If we assume that the dissolved Se concentration in the soil pore water during the pot experiment is the same as that measured in the 0.01 M $CaCl_2$ extraction (0.12 to 0.60 µg L^{-1}; Table 1), taking into account the soil water content of the pot experiment (230 to 504 mL pot^{-1}), the dissolved Se in the soils used during the pot experiment is 0.04 to 0.24 µg Se pot^{-1}. When calculated in this way, the total amount of Se taken up by wheat shoots accounts for 37% to 439% or about 0.4 to 4 times the dissolved Se in the pore water for the whole 53 days of wheat growth. However, it has been shown that the soluble Se extracted in 0.01 M $CaCl_2$ of moist potato field soils as used in this study is 2 to 4 times lower than that measured in the same extraction using the dried soils (Supriatin *et al.*, 2015a). Therefore, we expect that the dissolved Se concentration in the pore water during the pot experiment is less than that estimated above based on soluble Se measured in the $CaCl_2$ extraction of dried soils. When assuming that the dissolved Se concentration in the pore water is the same as that measured in the $CaCl_2$ extraction of the moist soils (0.04 to 0.48 µg L^{-1}; Supriatin *et al.* 2015a), the total amount of Se taken up in the wheat shoots is 0.8 to14 times the dissolved Se in the pore water. These values can be higher if Se taken up by the wheat roots and lost due to possible phyto-volatilization are also included (Zayed *et al.*, 1998).

The above calculation shows that to supply the amount of Se taken up by wheat during the pot experiment the dissolved Se in the pore water needs to be replenished by about 0.8 to 14 times within the 53 days period, corresponding to a total turnover time of about 4 to 67 days. It has been shown that the turnover of free amino acids in soil through microbial mineralization is very fast (within a few hours) (Jones and Kielland, 2002). The mineralization of the colloidal sized organic Se might be slower than the free amino acids. Nevertheless, up to 40% of DOM in soil solution can be mineralized within a period of days to a few months (Kalbitz *et al.*, 2000). The amount of Se taken up in the wheat shoots is only 0.008% to 0.38% of total Se (aqua regia) in soils. On the other hand, the amount of labile organic Se as extracted in hot water accounts for on average 5% of total Se (aqua regia) (Table A.1), which is much higher than the Se amount taken up by wheat shoots. The labile organic matter extracted in hot water is relatively rich in Se (Supriatin *et al.*, 2015b), which may represent the Se-rich solid organic matter in soils (Fig. 5). The above simplified calculations show that the amount of Se generated during the mineralization of Se-rich solid and dissolved organic matter is potentially sufficient to explain the amount of Se taken up by wheat shoots (Fig. 5).

4.4.4 Selenium to organic C ratio in different extractions

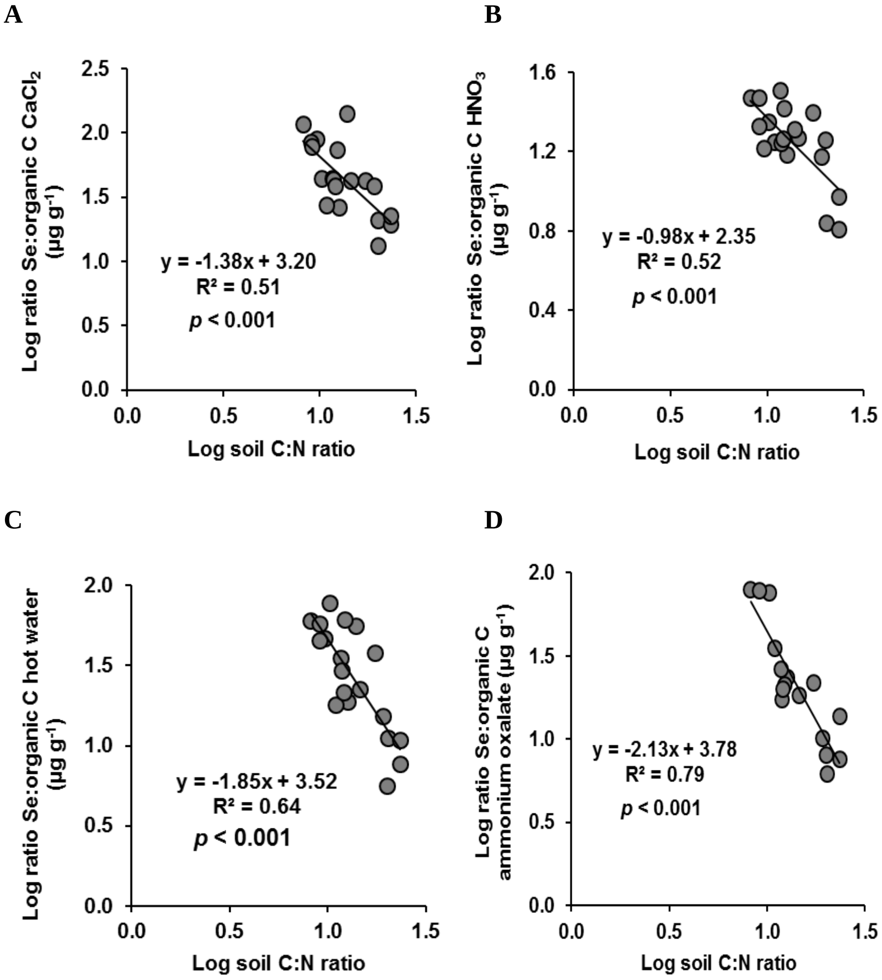
The results above showed that among other soil properties, soil pH correlates most strongly with both Se to organic C ratio in 0.01 M $CaCl_2$ extraction and Se content in the wheat shoots (Table 3 and 4 and Fig. 2 and A.2.1). In addition to soil pH, soil C:N ratio is also related to both Se to organic C ratio in 0.01 M $CaCl_2$ extraction and Se content in the wheat shoots (Table 3 and 4 and Fig. 6A). Below, a further analysis of the correlation between soil pH or soil organic matter quality (i.e. soil C:N ratio) and Se to organic C ratio in different extractions was performed. Moreover, correlation analysis between the Se to organic C ratio in different extractions and Se content in the wheat shoots was carried out.

These correlation analyses show that the soil pH is strongly positively correlated to the Se to organic C ratio in 0.01 M $CaCl_2$ and hot water extractions ($R^2 = 0.66$, $p < 0.001$ and $R^2 = 0.69$, $p < 0.001$, respectively), but weakly correlated to the Se to organic C ratio in 0.43 M HNO_3 and ammonium oxalate extractions and aqua regia digestion ($R^2 = 0.26$ to 0.39) (Fig. A.2), indicating that pH influences the amount of

Se-rich organic matter mainly in the soluble and labile fractions. On the other hand, in general, soil C:N ratio is strongly negatively correlated with the Se to organic C ratio in all the soil extractions ($R^2 = 0.51$ to 0.79, $p < 0.001$) (Fig. 6), suggesting that at a low soil C:N ratio soil organic matter is richer in Se than at a high soil C:N ratio, regardless of the different pools of soil organic matter (soluble, acid extractable, labile, oxide associated or bulk of organic matter). These findings suggest that both the soil pH and soil organic matter quality (i.e. soil C:N ratio) control the content of Se-rich organic matter in the soluble (0.01 M $CaCl_2$ extraction) and labile (hot water extraction) organic matter pools, whereas in the rest of the organic matter pools (HNO_3 and ammonium oxalate extractions and aqua regia digestion), the soil organic matter quality (i.e. soil C:N ratio) has a larger influence on the Se to organic C ratio than the soil pH (Fig. 6 and A.2).

As a comparison with DOC, the amount of DON in the $CaCl_2$ extraction was also measured, which range from 2 to 33 mg N kg^{-1} soil. The ratio of Se to DON in $CaCl_2$ extraction is between 42 and 1578 µg g^{-1}, with an average of 488 µg g^{-1}, which is substantially higher than the average ratio of total Se (aqua regia) to total N in these soils, i.e. 189 µg g^{-1}. Despite the observed correlation between the soil C:N ratio and Se to DOC ratio in the $CaCl_2$ extraction (and also in other extractions) (Fig. 6), only a weak correlation occurs between soil C:N ratio and DON concentration in $CaCl_2$ extraction ($R^2 = 0.22$, $p < 0.05$), and hardly any correlations between soil C:N ratio and DOC to DON ratio in $CaCl_2$ extraction (DOC:DON ratio = 2 to 28) ($R^2 = 0.09$, $p > 0.05$), between DON and soluble Se concentration in $CaCl_2$ extraction ($R^2 = 0.10$, $p > 0.05$), and between DOC to DON ratio and Se to DOC ratio in $CaCl_2$ extraction ($R^2 = 0.01$, $p > 0.05$). These results indicate that although the overall soil organic matter quality (i.e. soil C:N ratio) is an important factor determining the amount of soluble Se-rich organic matter (i.e. ratio of soluble Se to DOC in $CaCl_2$ extraction) (Fig. 6A), the amount of soluble Se-rich organic molecules is not directly related to the solubility of N-containing organic molecules in general.

Among the Se to organic C ratios in all the soil extractions, Se to organic C ratio in 0.01 M $CaCl_2$ extraction correlates most strongly with Se content in the wheat shoots ($R^2 = 0.88$, $p < 0.001$; Fig. 7). On the other hand, Se to organic C ratio in other extractions (0.43 M HNO_3, hot water and ammonium oxalate extractions and aqua regia digestion) are weakly correlated with Se content in the wheat shoots ($R^2 = 0.15$ to 0.40; Fig. 7). These findings confirm that the amount of Se-rich DOM in soil solution is the main limiting factor for Se plant uptake.



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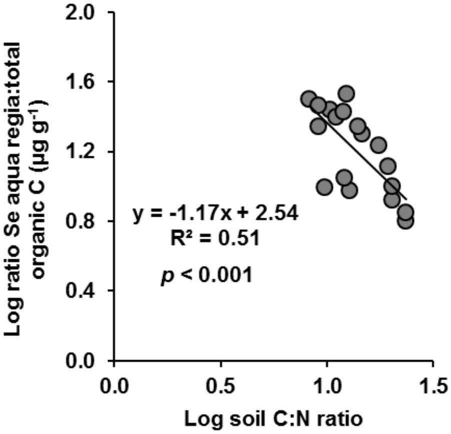

Fig. 6. Correlations between soil C:N ratio and Se to organic C ratio in 0.01 M $CaCl_2$ (A), 0.43 M HNO_3 (B), hot water (C), ammonium oxalate (three samples with Se concentrations in the extraction below the determination limit were not included in the correlation) (D) and Se (aqua regia) to total organic C ratio (one sample with Se concentration in aqua regia below the determination limit was not included in the correlation) (E).

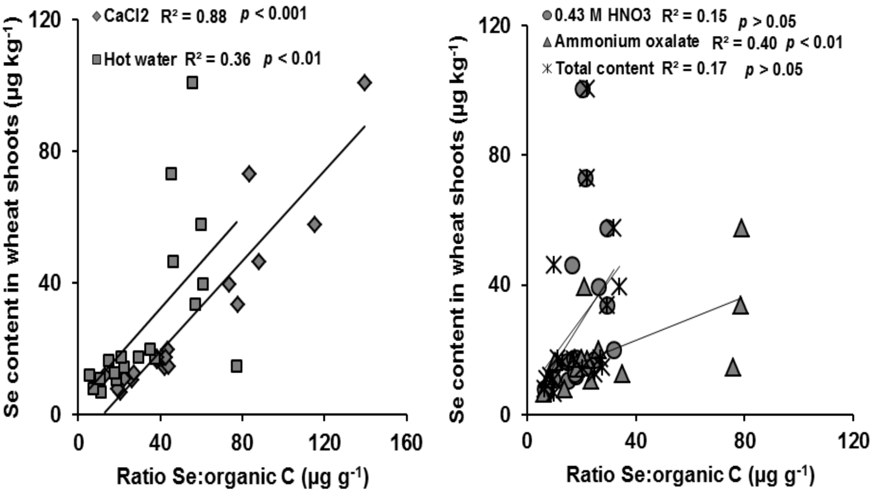

Fig. 7. Correlation between Se content in wheat shoots and Se to organic C ratio in 0.01 M $CaCl_2$ and hot water extractions (A), and correlation between Se content in wheat shoots and Se to organic C ratio in 0.43 M HNO_3, ammonium oxalate and total content (Se aqua regia:total organic C) (three samples in ammonium oxalate extraction and one sample in aqua regia digestion with Se concentrations in the extractions below the determination limit were not included in the correlation) (B).

4.4.5 Implication of the regression uptake model for prediction of Se bioavailability in other soil samples

The results of current study apply to soil samples that have low level of Se (total 0.22 to 0.73 mg kg^{-1} and soluble Se 0.12 to 0.60 µg L^{-1}) with predominantly organic Se, and with a low amount of DOC (3.5 to 18.4 mg L^{-1}) (Table 1). In a previous study we have found that the Se to DOC ratio in 0.01 M $CaCl_2$ extraction of the majority of (dried) potato field soils used in the current study (on average 64 ± 33 µg g^{-1}; n = 16) is higher than that in similar extraction of (dried) soils from grassland fields (34 ± 13 µg g^{-1}; n = 27) and maize fields (30 ± 16 µg g^{-1}; n = 15), and in general the grassland soils (0.21 to 4.02 µg L^{-1} for Se and 5.5 to 93.9 mg L^{-1} for DOC) and maize field soils (0.26 to 1.13 µg L^{-1} for Se and 11.2 to 53.9 mg L^{-1} for DOC) have a higher level of soluble Se and DOC in 0.01 M $CaCl_2$ extraction than those in potato field soils (Supriatin *et al.*, 2015a). By including three additional soil samples, the average Se to DOC ratio in 0.01 M $CaCl_2$ extraction of (dried) potato field soil samples used in the current study is 53 ± 35 µg g^{-1} (n = 19; Table 1). These results indicate that the dissolved organic matter in potato field soils is richer in Se than that in grassland soils and maize field soils, and the dissolved organic matter in grassland soils contain similar amount of Se as that in maize field soils. Further research is needed to evaluate the validity of the Se uptake model derived in this study in other soils (i.e. grassland soils and maize field soils) with different quality of Se-containing DOM (i.e. lower Se to DOC ratio).

4.5 Conclusions

Total Se content (aqua regia) in the potato field soils is in the range of 0.22 to 0.73 mg kg^{-1}, of which a small fraction (on average 3 ± 3 % of total Se) is present as inorganic Se (mainly selenite) and the majority is in organic form. Soluble Se in 0.01 M $CaCl_2$ extraction is between 1.2 to 6.0 µg kg^{-1} and accounts for on average 1.0 ± 0.7% of total Se. The soluble Se is mainly present as colloidal organic Se, whereas inorganic selenate, selenite, and small organic Se were hardly measurable. The Se content in the wheat shoots grown on these low total and soluble Se soils was found to be between 7 and 101 µg kg^{-1} (plant dry weight), with an average of 28 µg kg^{-1}. In general, Se content in the wheat shoots grown on clay soils with a combination of clay content ≥ 20% and pH > 6 is higher compared to that in the wheat shoots grown on other soil types (i.e. sand, “other clay”, loess and reclaimed peat soils).

A regression uptake model developed from these data shows that around 88% of variability of Se content in the wheat shoots grown under controlled conditions can be explained by the Se to DOC ratio in 0.01 M $CaCl_2$ extraction, indicating that Se-rich DOM is the main source of bioavailable Se in these low Se soils with predominantly organic Se. The Se content in the wheat shoots is particularly strongly and positively correlated with Se to organic C ratio of the hydrophilic fraction of DOM in 0.01 M $CaCl_2$ extraction ($R^2 = 0.93$, $p < 0.001$), suggesting that Se-rich compounds present in the easily degradable DOM fraction control Se bioavailability. Soil pH, C:N ratio, clay content and organic matter content have been identified as the major soil properties determining the Se to DOC ratio in 0.01 M $CaCl_2$ extraction. The Se to DOC ratio in 0.01 M $CaCl_2$ extraction increased towards high pH, high clay content, low soil C:N ratio and low soil organic matter content. Among these soil properties, soil pH correlates most strongly with Se to DOC ratio in 0.01 M $CaCl_2$ extraction ($R^2 = 0.66$, $p < 0.001$) and Se content in wheat shoots ($R^2 = 0.60$, $p < 0.001$). In addition to soil pH, our findings suggest that soil organic matter quality (i.e. soil C:N ratio) controls to a large extent the amount and release of Se-rich organic molecules from the soil solid phase to soil solution and subsequent uptake.

The results of this study suggest that Se bioavailability in low Se soils with predominantly organic Se is determined by the solubility of Se-containing DOM in soil solution (i.e. soluble organic Se). Further research is recommended to validate the regression uptake model for prediction of Se bioavailability in soil samples from other land uses with different quality of soil organic matter (i.e. lower soluble Se to DOC ratio).

Appendices

Table A.1. Amount and fraction of Se extracted in 0.43 M HNO_3, hot water and ammonium oxalate. The data are presented as mean ± standard deviation per soil type. Values in brackets are ranges of the Se amount and fraction in each extraction per soil type.

Soil type	Se extracted (µg kg^{-1})			Se extraction efficiency (% of total Se-aqua regia)		
	HNO_3	hot water	ammonium oxalate	HNO_3	hot water	ammonium oxalate
Sand (n = 5)	23.3 ± 7.9	17.7 ± 2.4	80.0 ± 37.7	7 ± 3[a]	5 ± 2[a]	21 ± 3[a]
	(12.0 – 32.0)	(13.8 – 19.7)	(38 – 140)	(3 – 11)[a]	(3 – 7)[a]	(18 – 25)[a]
Clay ≥ 20%, pH > 6 (n = 3)	8.3 ± 1.2	20.5 ± 0.5	86.0[b]	3 ± 0.7	8 ± 1	38[b]
	(7.0 – 9.0)	(19.9 – 20.9)		(3 – 4)	(6 – 9)	
Other clay (n = 6)	12.2 ± 5.4	19.8 ± 5.2	77.6 ± 40.2[b]	3 ± 0.4	6 ± 2	19 ± 9[b]
	(7.0 – 22.0)	(14.1 – 29.5)	(50 – 146)[b]	(3 – 4)	(2 – 9)	(12 – 34)[b]
Loess (n = 1)	18.0	25.2	146.0	2	3	20
Reclaimed peat (n = 4)	11.8 ± 3.9	12.2 ± 4.7	66.5 ± 15.8	3 ± 1	3 ± 1	17 ± 4
	(8.0 – 17.0)	(6.4 – 16.9)	(50 – 80)	(2 – 4)	(2 – 4)	(11 – 19)
Average ± SD[c]	**14.7 ± 7.5**	**18.1 ± 5.0**	**80.4 ± 34.7**	**4 ± 2**	**5 ± 2**	**21 ± 7**

[a] One sample that has a total Se content (aqua regia) below the determination limit was excluded from the table.

[b] Soil samples that have Se concentrations below the determination limit were excluded from the table.

[c] Standard deviation.

Table A.2. Selenium speciation in ammonium oxalate extraction using HPLC - ICP-MS.

Soil type	Total Se ammonium oxalate ($\mu g\ kg^{-1}$)		Inorganic selenite[a]/total Se ammonium oxalate (%)		Organic Se[b]/total Se ammonium oxalate (%)		Total Se aqua regia ($\mu g\ kg^{-1}$)	Inorganic selenite[a]/total Se aqua regia (%)
	Range	Average ± SD[c]	Range	Average ± SD[c]	Range	Average ± SD[c]	Average ± SD[c]	Average ± SD[c]
Sand (n = 5)	38 - 140	80 ± 38	0 – 28	15 ± 12	72 – 100	85 ± 12	437 ± 179[d]	3 ± 2[d]
Clay ≥ 20%, pH > 6 (n = 3)	n.d.[ef]	86[e]	n.d.[ef]	19[e]	n.d.[ef]	81[e]	262 ± 43	7[e]
Other clay (n = 6)	50 -146[e]	78 ± 40[e]	10 – 45[e]	28 ± 13[e]	55 – 90[e]	72 ± 13[e]	392 ± 187	5 ± 2[e]
Loess (n = 1)	n.d.[f]	146	n.d.[f]	7	n.d.[f]	93	734	1.4
Reclaimed peat (n = 4)	50 - 80	67 ± 16	n.d.[f]	0	n.d.[f]	100	404 ± 103	0
Average ± SD[c]		**80 ± 35**		**15 ± 14**		**85 ± 14**	**734 ± 168**	**3 ± 3**

[a] Inorganic selenite as extracted with ammonium oxalate.

[b] Organic Se in ammonium oxalate extraction was derived from the difference between total Se concentration and inorganic selenite in the ammonium oxalate extraction.

[c] Standard deviation.

[d] One sample that has a total Se content (aqua regia) below the determination limit was excluded from the table.

[e] In ammonium oxalate extraction, soil samples that have Se concentrations below the determination limit were excluded from the table.

[f] Not determined.

Table A.3. Results of multiple (two to four factors) linear regressions between Log_{10}(Se in wheat shoots, µg kg^{-1}) as a dependent parameter (Y) and Log_{10}(soil parameters) as independent parameters (X_1, X_2, ...) (n = 19), with an adjusted R^2 > 0.50.

Model	Intercept (a)	Coefficient (independent parameter)				Adjusted R^2	*p* value
		b_1 (X_1)	b_2 (X_2)	b_3 (X_3)	b_4 (X_4)		
Model 1	0.90	-0.65 (soil C:N ratio)	0.19 (pH-$CaCl_2$)[a]			0.60	< 0.001
Model 2	0.96	-0.68 (soil C:N ratio)	0.17 (pH hot water)[a]			0.59	< 0.001
Model 3	4.59	-1.12 (soil C:N ratio)	-1.10 (DOC-$CaCl_2$)			0.70	< 0.001
Model 4[b]	5.18	-1.33 (soil C:N ratio)	-0.92 (total Se-aqua regia)			0.57	< 0.001
Model 5	0.48	0.17 (pH-$CaCl_2$)[a]	-0.33 (organic matter)			0.60	< 0.001
Model 6	1.53	0.17 (pH-$CaCl_2$)[a]	-0.67 (DOC-$CaCl_2$)			0.62	< 0.001
Model 7	0.43	0.21 (pH-$CaCl_2$)[a]	-0.38 (DON-$CaCl_2$)			0.68	< 0.001
Model 8	**0.07**	**0.19 (pH-$CaCl_2$)[a]**	**0.15 (clay)**			**0.58**	**< 0.001**
Model 9	-0.07	0.18 (pH-$CaCl_2$)[a]	0.60 (Se-$CaCl_2$)			0.65	< 0.001
Model 10[b]	0.70	0.18 (pH-$CaCl_2$)[a]	-0.40 (selenite)			0.58	< 0.01

Table A.3. Continued.

Model	Intercept (a)	Coefficient (independent parameter)				Adjusted R^2	*p* value
		b_1 (X_1)	b_2 (X_2)	b_3 (X_3)	b_4 (X_4)		
Model 11[b]	0.94	0.23 (pH-$CaCl_2$)[a]	-0.39 (total Se-aqua regia)			0.64	< 0.001
Model 12	0.53	0.15 (pH-hot water)[a]	-0.35 (organic matter			0.59	< 0.001
Model 13	0.24	0.15 (pH-hot water)[a]	-0.36 (DOC-hot water)			0.55	< 0.001
Model 14	0.10	0.16 (pH-hot water)[a]	0.16 (clay)			0.57	< 0.001
Model 15	-0.52	0.20 (pH-hot water)[a]	0.42 (Se-hot water)			0.56	< 0.001
Model 16[b]	0.73	0.16 (pH-hot water)[a]	-0.42 (selenite)			0.57	< 0.01
Model 17[b]	1.09	0.20 (pH-hot water)[a]	-0.44 (total Se-aqua regia)			0.62	< 0.001
Model 18	2.10	-0.61 (organic matter)	-0.42 (DON-$CaCl_2$)			0.60	< 0.001
Model 19	1.43	-0.50 (organic matter)	0.24 (clay)			0.56	< 0.001
Model 20	1.32	-0.51 (organic matter)	0.68 (Se-$CaCl_2$)			0.56	< 0.001
Model 21	0.95	-0.65 (organic matter)	0.63 (Se-hot water)			0.51	< 0.01

Table A.3. Continued.

Model	Intercept (a)	Coefficient (independent parameter)				Adjusted R^2	*p* value
		b_1 (X_1)	b_2 (X_2)	b_3 (X_3)	b_4 (X_4)		
Model 22[b]	2.51	-0.74 (organic matter)	-0.28 (total Se-aqua regia)			0.52	< 0.01
Model 23	3.66	-1.09 (DOC-$CaCl_2$)	-0.37 (DON-$CaCl_2$)			0.58	< 0.001
Model 24	3.12	-1.14 (DOC-$CaCl_2$)	0.32 (clay)			0.78	< 0.001
Model 25	**3.09**	**-1.23 (DOC-$CaCl_2$)**	**1.01 (Se-$CaCl_2$)**			**0.85**	**< 0.001**
Model 26[b]	3.47	-1.01 (DOC-$CaCl_2$)	-0.22 (selenite)			0.56	< 0.01
Model 27	1.28	-0.41 (DON-$CaCl_2$)	0.89 (Se-$CaCl_2$)			0.54	< 0.001
Model 28	0.54	-0.86 (DOC-hot water)	0.49 (Se-hot water)			0.51	< 0.01
Model 29[b]	2.65	-0.85 (DOC-hot water)	-0.58 (total Se-aqua regia)			0.58	< 0.001
Model 30[b]	1.74	0.31 (clay)	-0.62 (selenite)			0.51	< 0.01
Model 31[b]	3.63	0.38 (clay)	-1.02 (total Se-aqua regia)			0.68	< 0.001
Model 32[b]	3.32	1.08 (Se-$CaCl_2$)	-0.98 (total Se-aqua regia)			0.66	< 0.001

Table A.3. Continued.

Model	Intercept (a)	Coefficient (independent parameter)				Adjusted R^2	*p* value
		b_1 (X_1)	b_2 (X_2)	b_3 (X_3)	b_4 (X_4)		
Model 33[b]	2.84	1.23 (Se-hot water)	-1.19 (total Se-aqua regia)			0.51	< 0.01
Model 34	0.79	-0.53 (soil C:N ratio)	0.18 (pH-$CaCl_2$)[a]	0.05 (clay)		0.58	< 0.01
Model 35	3.14	-0.05 (soil C:N ratio)	-1.22 (DOC-$CaCl_2$)	0.98 (Se-$CaCl_2$)		0.84	< 0.001
Model 36	0.17	-0.16 (soil C:N ratio)	0.18 (pH-$CaCl_2$)	0.53 (Se-$CaCl_2$)		0.63	< 0.001
Model 37	3.42	-0.27 (soil C:N ratio)	-1.11 (DOC-$CaCl_2$)	0.27 (clay)		0.77	< 0.001
Model 38	3.35	-0.86 (soil C:N ratio)	0.08 (pH-$CaCl_2$)	-0.84 (DOC-$CaCl_2$)		0.70	< 0.001
Model 39	0.20	-0.20 (soil C:N ratio)	0.18 (pH-$CaCl_2$)[a]	-0.03 (clay)	0.55 (Se-$CaCl_2$)	0.60	< 0.01
Model 40	3.81	-0.25 (soil C:N ratio)	-0.04 (pH-$CaCl_2$)[a]	-1.24 (DOC-$CaCl_2$)	0.32 (clay)	0.76	< 0.001
Model 41	3.05	-0.04 (soil C:N ratio)	0.007 (pH-$CaCl_2$)[a]	-1.20 (DOC-$CaCl_2$)	0.97 (Se-$CaCl_2$)	0.83	< 0.001
Model 42	2.71	0.26 (soil C:N ratio)	-1.21 (DOC-$CaCl_2$)	0.16 (clay)	0.81 (Se-$CaCl_2$)	0.86	< 0.001
Model 43	2.99	0.009 (pH-$CaCl_2$)[a]	-1.20 (DOC-$CaCl_2$)	0.99 (Se-$CaCl_2$)		0.84	< 0.001

Table A.3. Continued.

Model	Intercept (a)	Coefficient (independent parameter)				Adjusted R^2	*p* value
		b_1 (X_1)	b_2 (X_2)	b_3 (X_3)	b_4 (X_4)		
Model 44	3.02	-1.18 (DOC-$CaCl_2$)	0.13 (clay)	0.74 (Se-$CaCl_2$)		0.87	< 0.001
Model 45	3.71	-0.06 (pH-$CaCl_2$)[a]	-1.39 (DOC-$CaCl_2$)	0.19 (clay)	0.76 (Se-$CaCl_2$)	0.87	< 0.001
Model 46	3.58	-0.04 (pH-$CaCl_2$)[a]	-1.27 (DOC-$CaCl_2$)	0.37 (clay)		0.77	< 0.001
Model 47	0.42	0.16 (pH-$CaCl_2$)[a]	-0.32 (DON-$CaCl_2$)	0.48 (Se-$CaCl_2$)		0.73	< 0.001
Model 48	0.94	0.15 (pH-$CaCl_2$)[a]	-0.28 (organic matter)	-0.36 (DON-$CaCl_2$)		0.70	< 0.001
Model 49	0.97	-0.38 (soil C:N ratio)	0.18 (pH-$CaCl_2$)[a]	-0.33 (DON-$CaCl_2$)		0.68	< 0.001
Model 50	0.46	0.19 (pH-$CaCl_2$)[a]	-0.35 (DON-$CaCl_2$)	0.04 (clay)		0.66	< 0.001

[a] pH is not converted into Log_{10}.

[b] The samples that have Se concentrations below the determination limit were not included in the regression analysis.

Unit of independent parameters: DOC-$CaCl_2$, DON-$CaCl_2$; DOC-hot water (mg kg^{-1}); clay content (%); organic matter content (%); Se-$CaCl_2$; Se-hot water, selenite, total Se-aqua regia (µg kg^{-1}).

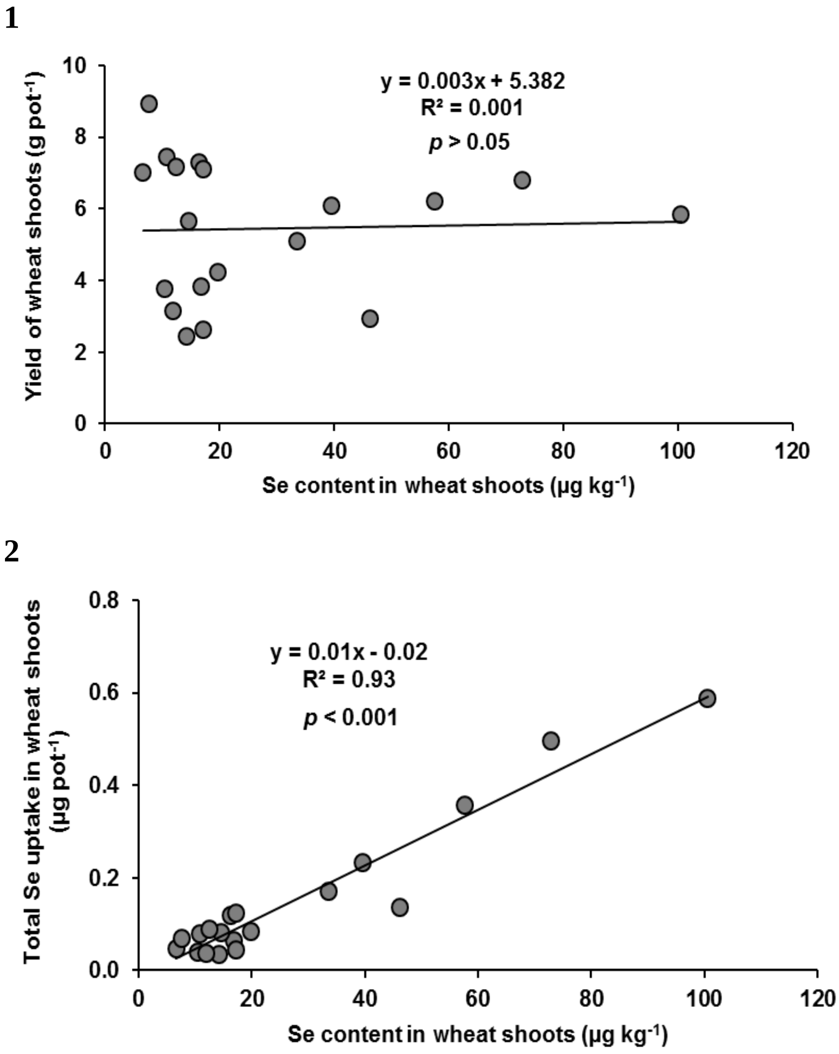

Fig. A.1. Correlation between (1) yield and Se content of wheat shoots, and (2) correlation between total Se uptake and Se content of wheat shoots.

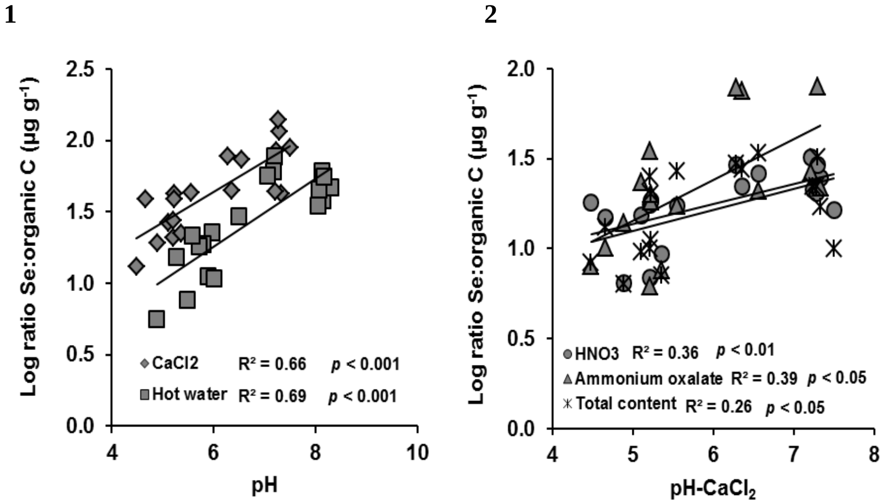

Fig. A.2. Correlation between (1) soil pH (pH measured in 0.01 M $CaCl_2$ and hot water extractions) and Se to organic C ratio in 0.01 M $CaCl_2$ and hot water extractions, respectively, and (2) correlation between soil pH measured in 0.01 M $CaCl_2$ extraction and Se to organic C ratio in 0.43 M HNO_3, ammonium oxalate extractions and total content (Se aqua regia:total organic C).

Chapter 5

Soil Parameters Influencing Selenium Uptake in Grass Grown Under Field Conditions

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Under preparation

Abstract

This study was aimed to identify the major soil factors controlling Se bioavailability under field conditions for low Se grassland soils with predominantly organic Se, and to evaluate the effect of NPK fertilization on Se plant uptake in these soils. Field experiments without and with NPK applications were conducted in the Netherlands on 21 and 15 grasslands, respectively. Soil parameters, including basic soil properties, C:N ratio, inorganic selenite content, total Se, S and P in different soil extractions (0.01 M $CaCl_2$, 0.43 M HNO_3, hot water, ammonium oxalate, aqua regia) and DOC in hot water and 0.01 M $CaCl_2$ soil extraction, were analysed on soil samples taken from the experimental field without NPK application in early Spring before the growing season. Selenium content in grass was analysed after harvest. Regression analysis was performed to identify soil parameters that determine Se content in grass. Among all the soil parameters measured in this study, Se in hot water extraction correlates most strongly with Se content in grass (without NPK application), which explains about 41% of the Se variability in grass grown under field conditions. This finding indicates that > 50% of the Se variability in grass grown under field conditions cannot be described by the soil parameters considered in the current study. Selenium in hot water extraction is mostly in organic form, representing the labile organic Se in soils. The content of amorphous Fe-(hydr)oxide and clay in soils has a positive effect on both the amount of Se in hot water extraction and Se in grass, suggesting that adsorption and desorption processes on soil mineral surfaces control the bioavailability of labile organic Se in the soils. Generally, application of NPK fertilizers decreased Se content in grass, especially on organic rich soils. This effect becomes stronger with increasing soil organic matter content, suggesting that interaction between NPK fertilizer and soil organic matter results in a synergetic effect in reducing Se bioavailability in soils. A possible explanation is that NPK application increases soil microbial activity, which reduces Se into a lower valence state that is subsequently immobilized into soil organic matter.

5.1 Introduction

Selenium (Se) is an essential micronutrient for livestock and human, which is important for functioning of the immune system. Previous studies have shown that lack of Se intake in livestock results in several health disorders, such as white muscle disease in sheep and cattle, liver necrosis in pigs or other subclinical Se deficiency related problems, which further cause impaired immune system, slow growth, infertility and production losses (Tinggi, 2003). For humans, the lack of Se intake may cause several possible health problems, such as impaired immune function, viral infection, infertility (especially for male), adverse mood status, and cancer risk (Rayman, 2000). In China, Keshan disease, an endemic cardiomyopathy, and Keshin-Beck disease, a deforming arthritis, were identified in humans living in areas with low soil Se content, i.e. < 0.125 mg kg^{-1} (Tan *et al.*, 2002). On the other hand, elevated Se intake in livestock and humans can cause Se toxicity (i.e. selenosis), which has occurred in several countries such as in some areas of China with an excessive soil Se content, i.e. on average 2.31 mg kg^{-1} (Tan *et al.*, 2002). In general, Se intake by humans in most parts of Europe is low, i.e. mainly < 40 µg day^{-1}, due to low Se content in the soils, crops and agricultural products (Rayman, 2000). For the benefit of human health, the recommended Se daily intake proposed by the Panel on Dietary Antioxidants and Related Compounds, USA, is 55 µg day^{-1} for both adult men and women (Monsen, 2000). Selenium fertilization on agricultural soils can be an option to increase Se level in food crops or fodder crops and consequently to increase Se intake by cattle and humans. In order to enable a sufficient Se intake by livestock and humans, a minimum level of 50 to 100 µg Se kg^{-1} dry weight (Gardiner and Gorman, 1963; Gissel-Nielsen *et al.*, 1984) or an optimum level of 150 to 200 µg Se kg^{-1} dry weight (Gierus *et al.*, 2002) in crops is recommended. Knowledge about the major factors controlling Se plant uptake in fields is important to design sustainable soil management strategies for Se availability and uptake in crops, including recommendations for effective Se fertilization.

In soil, Se is present as inorganic and organic compounds. The inorganic Se in soil ranges from selenate (SeO_4^{2-}) and selenite (SeO_3^{2-}) in oxidized soils to elemental Se (Se^0) and selenides (Se^{2-}) in reduced soils. The exact nature of organic Se in soil is not known yet. Research has shown the presence of selenomethionine ($C_5H_{11}NO_2Se$), methane seleninic acids (CH_3SeOOH), and trimethyl selenium ions ($(CH_3)_3Se^+$), and suggests that organic Se in soil is probably present as seleno-amino acid incorporated into protein or peptides in humic and fulvic acid fractions of soil organic matter (Abrams and Burau, 1989; Yamada and Hattori, 1989; Abrams *et al.*, 1990a; Kang *et al.*, 1991b; Stroud *et al.*, 2012; Tolu *et al.*, 2014). Among these inorganic and organic Se forms, the soluble inorganic selenate and selenite and soluble organic Se, such as selenomethionine and selenocystine, can be taken up directly by plants (Abrams *et al.*, 1990b; Williams and Mayland, 1992; Zayed *et al.*, 1998; Hopper and Parker, 1999; Li *et al.*, 2008; Kikkert and Berkelaar, 2013). Our previous study has shown that agricultural soils in the Netherlands contain mostly low amounts of Se (0.12 to 1.97 mg kg^{-1}, with an average of 0.58 mg kg^{-1}), with most of Se in organic form and only a small fraction of inorganic selenite (Supriatin *et al.*, 2015b). Generally, agricultural soils in other European countries, such as Belgium, Germany, France, Sweden, Finland, United Kingdom, Czech Republic, Spain (Yläranta, 1983; Hartfiel and Bahners, 1988; Wang and Sippola, 1990; Johnsson, 1992; Adams *et al.*, 2002; Roca-Perez *et al.*, 2010; De Temmerman *et al.*, 2014; Tolu *et al.*, 2014; Száková *et al.*, 2015) and Denmark (Hamdy and Gissel-Nielsen (1976) *in* Gupta and Gupta, (2000)), also have a low total Se content, i.e. mostly < 0.70 mg kg^{-1}. Some studies have shown that the major Se form in soils from Finland, France and Spain is also associated with soil organic matter (Keskinen *et al.*, 2009; Roca-Perez *et al.*, 2010; Tolu *et al.*, 2014).

Correlation studies between Se content in plants grown under field conditions and soil Se content (total and/or extractable Se) have been performed in previous studies for both Se low and rich soils (Wang and Sippola, 1990; Zhao *et al.*, 2005; Stroud *et al.*, 2010a; De Temmerman *et al.*, 2014). The predictive power of derived relationships describing the variability of Se content in plants from these studies ranges from 9% to 95% (R^2 = 0.09 to 0.95). A strong correlation (R^2 = 0.86 to 0.95) was found between Se content in plant and total soil Se and potassium dihydrogen phosphate soluble Se and S, with up to 70% of soluble Se is in inorganic selenite, for the low Se soils (0.25 to 0.59 mg kg^{-1}) in UK, and between plant uptake and potassium dihydrogen phosphate extractable Se for the Se rich soils (1.01 to 2.60 mg kg^{-1}) in China (Zhao *et al.*, 2005; Stroud *et al.*, 2010a). On the other hand, a weak correlation (R^2 of 0.09 to 0.48) was found between Se content in plant and total soil Se or water- or acid ammonium acetate-EDTA-extractable Se for low Se soils (0.06 to 0.82 mg kg^{-1} in soil samples collected from 13 European countries) (Wang and Sippola, 1990; De Temmerman *et al.*, 2014), probably dominated by (soluble) organic Se. These studies indicate that the Se plant uptake is more related to the amount and forms of Se in soil solution, than to the total amount of Se in soils per se.

Our previous study has shown that Se content in wheat shoots grown under controlled conditions (pot experiment) on arable land (potato fields) soils from the Netherlands with low Se content (predominantly organic Se for both the total Se and soluble Se) is determined by the amount of Se-rich dissolved organic matter (Supriatin *et al.*, 2016). In those soils, the amount of Se-rich dissolved organic matter increases in general with increasing soil pH and with decreasing soil C:N ratio (Supriatin *et al.*, 2016). This study has shown that high soil pH and low soil C:N ratio favour the release of Se-rich dissolved organic matter, which is directly or after mineralization available for plant uptake.

In addition to the amount and speciation of Se in soils, agricultural management practices, such as mineral NPK fertilization, may also influence Se plant uptake. Generally, farmers apply mineral NPK fertilizers on their fields to increase the yields of crops. Previous studies suggested a decrease of Se plant uptake upon N fertilization due to the dilution effect from the increase of crop yield (Gissel-Nielsen (1974) *in* Gissel-Nielsen *et al.* (1984)) and (Gissel-Nielsen, 1975). However, the effect of NPK fertilization on Se uptake in plants is still unknown. No comprehensive study about the effect of NPK fertilization on the uptake of native soil Se or added Se fertilizer by plants has so far been performed.

In this study, field experiments on a number of grassland fields in the Netherlands on low Se soils with predominantly organic Se, without NPK and Se fertilizer applications, were conducted to identify the major soil factors controlling Se availability and uptake in grass grown under field conditions. These grassland soils contain lower amount of Se-rich dissolved organic matter (i.e. lower Se to DOC ratio in 0.01 M $CaCl_2$ soil extraction) compared to the potato field soils used in our previous research in Chapter 4 (Supriatin *et al.*, 2015a; Supriatin *et al.*, 2016). Moreover, field experiments on the same grassland fields with NPK application were performed to study the effect of NPK fertilization on Se uptake in grass, and to identify soil properties that potentially influence Se uptake in grass grown on soils applied with NPK.

5.2 Materials and methods

5.2.1 Grassland field experiments

5.2.1.1 Field experiment without NPK application

In 2011, field experiments on 21 grasslands in the Netherlands on low Se soils with predominantly organic Se were conducted to identify the soil parameters that determine Se availability and uptake in grass grown under field conditions. The grassland fields consist of four soil types, i.e. sand, clay, clay-peat, and peat soils (Table 1). The size of each plot was 8 m x 1.5 m. The plots did not receive any mineral NPK and Se fertilizer applications during that season, except for 3 plots that received 78.3 kg N ha^{-1} (two plots, sandy and clay soils) and 94.5 kg N ha^{-1} (one plot, sandy soil) from mineral N fertilizer, and neither manure has been applied. We included the three plots with N application because we found no effect of the N application on Se uptake in grass grown on these three plots (data not shown). Soil samples (top soil 0 to 10 cm) were taken in March 2011. These soil samples have also been used in the study of drying effects on soluble Se and Cu in 0.01 M $CaCl_2$

Table 1. Characteristics of soil samples, total Se content (aqua regia), soluble Se and Se extraction efficiency in 0.01 M $CaCl_2$ extraction of soil samples from the 2011 field experiment. The data are presented as mean ± standard deviation per soil type. Values in brackets are ranges of the parameters per soil type.

2011 field experiment									
Soil type	pH[a]	Organic matter (%)	Clay (%)	C:N ratio	Fe-oxalate[b] (g kg^{-1})	Al-oxalate[b] (g kg^{-1})	Se-aqua regia (mg kg^{-1})	Se-$CaCl_2$ (µg kg^{-1})	Extraction efficiency Se-$CaCl_2$ (% of total Se-aqua regia)
Sand (n =10)	5.7 ± 0.9	6.2 ± 2.7	3.5 ± 2.2	13.3 ± 2.5	1.9 ± 1.1	0.9 ± 0.6	0.23 ± 0.07 (0.12 – 0.37)	3.9 ± 1.1 (2.8 – 5.9)	1.8 ± 0.6 (1.1 – 2.9)
Clay (n = 6)	6.6 ± 0.8	6.8 ± 3.8	20.5 ± 10.9	10.2 ± 1.6	4.1 ± 1.2	0.4 ± 0.1	0.36 ± 0.15 (0.20 – 0.56)	6.1 ± 1.4 (3.9 – 7.7)	1.9 ± 0.7 (1.3 – 2.8)
Clay-peat (n = 4)	5.4 ± 0.2	19.9 ± 2.5	53.5 ± 7.3	12.8 ± 1.0	13.5 ± 0.6	2.3 ± 0.2	1.26 ± 0.11 (1.16 – 1.40)	16.4 ± 5.5 (10.7 – 22.6)	1.3 ± 0.5 (0.9 – 1.9)
Peat (n = 1)	5.1	25.5	6.3	15.0	17.5	2.2	1.14	13.7	1.2
All samples (n = 21)							**0.51 ± 0.42**	**7.4 ± 5.4**	**1.7 ± 0.6**

[a] Measured in 0.01 M $CaCl_2$ extraction at soil to solution ratio 1:10.

[b] Measured in ammonium oxalate-oxalic acid (pH 3) extraction at soil to solution ratio 1:20.

Table 2. Characteristics of soil samples taken from the same grassland fields in 2011 and 2012 (n = 15). The soil samples from both 2011 and 2012 field experiments were taken in Spring and in the case of 2012 before NPK application.

Field name	Soil type	2011 field experiment				2012 field experiment			
		pH	Clay (%)	Organic matter (%)	CEC (mmol(+) kg^{-1})	pH	Clay (%)	Organic matter (%)	CEC (mmol(+) kg^{-1})
Roelofs Hall	Sand	5.57	7	5.6	85	5.56	5	4.5	76
Winkel Branden	Sand	5.35	6	4.5	67	5.11	1	4.8	49
Winkel BBL	Sand	4.85	2	5.6	53	5.13	2	5.2	49
De Jong 13	Sand	5.23	4	12.1	115	4.99	8	10.6	157
De Jong 11.1	Sand	4.80	2	6.8	63	4.89	3	6.3	80
V. Rein Links	Sand	5.00	1	7.9	72	5.16	2	5.9	84
Harm Draai	Sand	5.20	1	7.8	66	n.d.[a]	n.d.[a]	7.8	88
Bakker Achter	Clay	6.07	34	10.2	341	5.81	36	14.8	386
Bakker Voor	Clay	5.98	35	12.7	325	6.12	33	16	375
Roelofs Kanaal	Clay	5.74	14	3.8	111	5.91	10	4	106
Klever v Veen A.	Clay-peat	5.23	61	16.4	453	5.22	49	18.4	440
Klever Ringkade	Clay-peat	5.23	57	22.3	524	5.28	40	19.2	416
Roayen 14	Clay-peat	5.45	51	20.0	399	5.32	41	17.4	348
Roayen 8	Clay-peat	5.64	44	21.0	453	5.83	37	21.8	426
Draai Linde	Peat	5.11	6	25.5	247	n.d.[a]	n.d.[a]	25.7	275

[a] Not determined.

soil extraction (Supriatin *et al.*, 2015a) and the study of Se speciation and extractability in different soil extractions (Supriatin *et al.*, 2015b). Grass samples were harvested from the first cut in May 2011.

5.2.1.2 Field experiment with NPK application

In 2012, field experiments with NPK application were performed on 15 grasslands out of the 21 fields used in the 2011 experiment (Table 2) to study the effect of NPK fertilization on Se uptake in grass. Each plot received mineral N (120 kg N ha^{-1}), P (31 kg P_2O_5 ha^{-1}), and K (60 kg K_2O ha^{-1}) fertilizer applications in March or April 2012. No Se fertilizer was applied to the soils. Soil samples (top soil 0 to 10 cm) were taken in March 2012 before mineral NPK fertilizer applications. In general, the properties of soil samples taken from these 15 grassland fields in 2012 field experiment are well correlated and comparable to those of soil samples taken from the same fields in 2011 field experiment (Table 2; the properties of soil samples from 15 grassland fields in 2011 field experiment overlap with data in Table 1). However, for some data points the differences are large because the locations of the plots are not exactly at the same spots of the corresponding fields. Grass samples were taken from the first cut in May 2012. The effect of NPK application on Se content in grass was evaluated by comparing Se content in grass grown on soils without NPK application (2011 field experiment as control plots) with that in grass grown on the same fields with NPK application (2012 field experiment as NPK plots) (n = 15; Table 2).

5.2.2 Pre-treatment of soil and grass samples

After removing the plant residues and gravels, the soil samples were oven-dried at 40^{o}C under forced air ventilation for 24 to 48 h, ground, and sieved with a 2 mm sieve for further analysis. The grass samples were oven dried at 70^{o}C for 72 h and milled for further Se content analysis. The yield of grass (kg dry matter ha^{-1}) was also determined. The grass samples taken from the fields were dominated by *Lolium perenne*.

5.2.3 Soil extractions

A series of single-step extractions, including 0.01 M $CaCl_2$, 0.43 M HNO_3, hot water, and ammonium oxalate extractions, and aqua regia digestion, was performed on the soil samples from the 2011 field experiment. An overview of the extraction methods is presented in detail in Supriatin *et al.* (2015b). Selenium, dissolved organic C (DOC), pH, major anions (i.e. S = total extractable sulphur, P = total extractable phosphorus, $P\text{-}PO_4$ = total extractable ortho-phosphate), Fe and Al were measured in the corresponding extractions (Table 3).

5.2.4 Inorganic selenite in ammonium oxalate extraction measured using HPLC - ICP-MS

A High Performance Liquid Chromatography (HPLC) system with an anion exchange column coupled online with High Resolution ICP-MS was used to measure inorganic selenite, selenate and organic Se extracted with ammonium oxalate from the soil samples from 2011 field experiment (Table 3). The details of the HPLC - ICP-MS system, solutions and procedure were described elsewhere (Supriatin *et al.*, 2015b). The soil samples were extracted using ammonium oxalate - oxalic acid (pH 3) solution. Initial total Se concentrations in the soil extracts were measured before the HPLC analysis. Concentrations of selenite and selenate were determined from the peak areas of the chromatograms. Concentrations of organic Se in the soil extracts were calculated from the difference between total Se concentrations and inorganic Se (selenite plus selenate) concentrations in the corresponding soil extracts.

5.2.5 Grass digestion

To determine Se content in the grass samples, the samples were digested using concentrated HNO_3 and H_2O_2 in a microwave with a closed system. The digestion method was described in detail in Supriatin *et al.* (2016).

5.2.6 Chemical analysis

Concentrations of Se in all soil extractions and grass digestion samples, and concentrations of P in 0.01 M $CaCl_2$ and hot water extractions were measured using High Resolution ICP-MS (Thermo Scientific, Element2). Concentrations of S in 0.01 M $CaCl_2$ and hot water extractions and S, P, Fe and Al in ammonium oxalate extraction were measured using ICP-AES (Thermo Scientific, Iris Advantage). Concentrations of DOC in 0.01 M $CaCl_2$ and hot water extractions and concentrations of ortho-phosphate P ($P\text{-}PO_4$) in hot water extraction were measured using a segmented flow analyser (SFA) (3039 Skalar, the Netherlands). pH was measured using a pH meter (Radiometer Analytical PHM210) with a glass electrode (Beckman 511084). Total C, N and organic matter in the soil samples were analysed using near infrared spectroscopy (NIR) (Reeves III, 2010; Soriano-Disla *et al.*, 2014).

5.2.7 Linear regression analysis

Single and multiple factor linear regression analyses were performed to correlate Se content in grass and soil parameters measured in samples from the 2011 field experiment in order to identify soil parameters that determine Se content in grass. Since the Se contents in grass were not normally distributed, the data were transformed into Log(10) before used in the regression analyses.

5.3 Results and discussion

5.3.1 Total and extractable Se content in soils

Results of Se measured in different soil extractions (0.01 M $CaCl_2$, 0.43 M HNO_3, hot water, ammonium oxalate and aqua regia) have been discussed in detail in our previous study using 83 soil samples, largely from other grasslands and arable lands in the Netherlands (21 soil samples used in the current study overlap with data in that paper) (Supriatin *et al.*, 2015b). For the samples used in the current work (n = 21; Table 1), the range and average fraction of Se in each extraction follow the order of aqua regia (120 to 1400 µg kg^{-1}; taken as 100%) > ammonium oxalate (18 to 203 µg kg^{-1}; 14 ± 2% of total Se (aqua regia)) > hot water (13 to 85 µg kg^{-1}; 8 ± 2%) > HNO_3 (7 to 60 µg kg^{-1}; 5 ± 3%) > $CaCl_2$ (3 to 23 µg kg^{-1}; 1.7 ± 0.6%) (Table 1 and A.1). In general, Se concentrations in all extractions of organic rich soils (i.e. clay-peat and peat soils) are higher than those in the other more mineral soil types (Table 1 and A.1), due to the high organic C content in the former soils. On average only 4 ± 4% of total Se (aqua regia) is present as inorganic Se (mainly selenite) as measured in ammonium oxalate extraction using HPLC - ICP-MS (Table A.2). Our previous study showed that Se in similar grassland soils is mostly present in organic form, i.e. on average 87 ± 4% of total Se (aqua regia), and the extractability of Se follows to a large extent the extractability of organic C in all the soil extractions (Supriatin *et al.*, 2015b). Both the total Se measured in aqua regia and Se in the other extractions (ammonium oxalate, hot water and $CaCl_2$ extractions) are mainly present in organic form, except that Se in HNO_3 extraction is mainly present as inorganic Se (Supriatin *et al.*, 2015b).

5.3.2 Selenium content in grass

Selenium content in grass samples from the 2011 field experiment (without NPK application) ranges from 16 to 115 µg kg^{-1}, with an average of 42 µg kg^{-1} (Fig. A.1.1), comparable to the range of Se content in grass samples (without Se fertilization) from Germany, i.e. 11 to 123 µg kg^{-1} (n = 304) (Hartfiel and Bahners, 1988). These grassland soils from Germany also contained low total Se, i.e. 0.035 to 0.65 mg kg^{-1} (n = 304) (Hartfiel and Bahners, 1988). Within different soil types, Se content in grass samples is between 33 µg kg^{-1} (on sandy soils) to 74 µg kg^{-1} (on clay-peat soils) (Fig. 1A). Figure 1A clearly shows that without Se fertilizer application, Se content in the grass is mostly below the adequate amount of Se for dairy cattle intake, i.e. 150 to 200 µg Se kg^{-1} dry weight of grass (Gierus *et al.*, 2002), indicating that the Se bioavailability in grassland soils in the Netherlands, like those in Germany referred to above, is low.

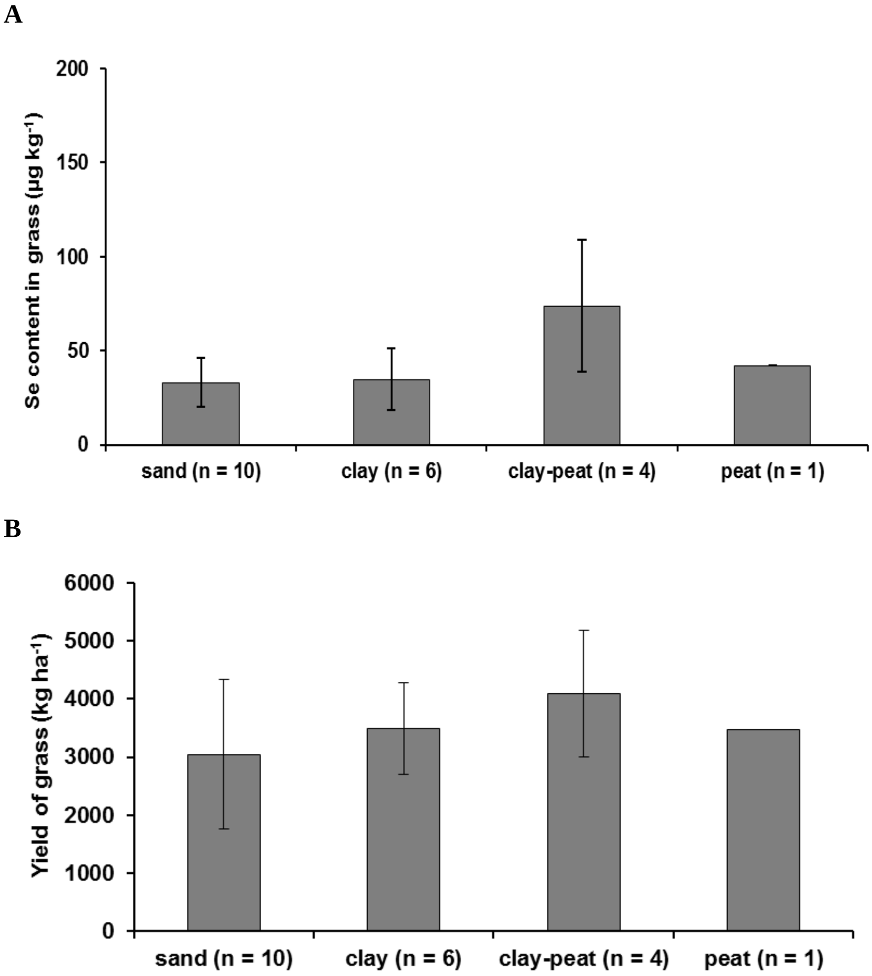


Fig. 1. Selenium content (A) and yield (B) of grass obtained from 2011 field experiment without NPK application (n = 21). The data are presented as means and standard deviations per soil type.

The yield of grass varies between 1300 to 5300 kg dry weight ha^{-1} (Fig. A.1.1). No significant difference of the average yield of grass grown on different soil types was found (Fig. 1B). The correlation between yield and Se content in grass (µg Se kg^{-1} dry biomass) was weak ($R^2 = 0.22$, $p < 0.05$, Fig. A.1.1), indicating that Se is not a limiting factor for plant growth. The variation in yield of grass grown on different soil types is smaller (4 times) compared to the variation in Se content in grass (7 times). The Se content in grass (in µg Se kg^{-1} dry biomass) correlates well with the total Se uptake per hectare (Se content x yield; in g Se ha^{-1}) ($R^2 = 0.87$, $p < 0.001$, Fig. A.1.2), suggesting that the yield effect on Se plant uptake is small. Therefore, for the purpose of comparison among soils, there is not much difference whether the absolute Se content or total Se uptake is used.

5.3.3 Soil parameters determining Se uptake in grass

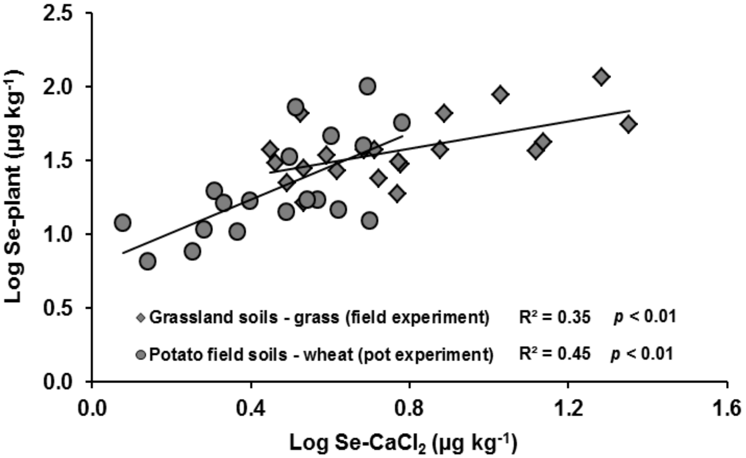
To identify which soil parameters are important in determining Se uptake in grass grown under field conditions, initially, single factor regression analysis was performed by correlating Se content in grass and each individual soil parameter (Table 3). Among the soil properties measured, clay content and amorphous Fe-(hydr)oxide content are positively correlated to Se content in grass, whereas other soil properties, such as pH measured in 0.01 M $CaCl_2$ extraction (pH-$CaCl_2$), pH measured in hot water extraction (pH-hot water), organic matter content, C:N ratio and amorphous Al-(hydr)oxide content are not correlated to Se content in grass (Table 3). In the previous pot experiment using soils from potato fields, Se content in wheat shoots was also found to be positively correlated with soil clay content (Supriatin *et al.*, 2016). However, the positive correlation with pH and negative correlation with soil organic matter content and C:N ratio found in our previous study were not observed in this grassland field study. The range of Se in plants in this study (16 to 115 Se µg kg^{-1} grass) is similar to that in the previous study (7 to 101 Se µg kg^{-1} wheat shoots), but the grassland soil samples used in this work contain a larger amount of organic matter (2.3 to 25% versus 1.4 to 15%) and a narrower range of C:N ratio (9 to 18 versus 8 to 23) than the potato field soil samples used previously, which may explain partly the lack of correlation to these parameters.

Table 3. Regression parameters of the equation: Log_{10}(Se content in grass, µg kg^{-1}) = a + b*Log_{10} (soil parameter). The soil and grass samples were obtained from 2011 field experiment without NPK application.

Soil parameter	Intercept (a)	Coefficient (b)	R^2	*p* value
pH-$CaCl_2$	1.64	-0.01	0.003	> 0.05
pH-hot water	1.69	-0.02	0.007	> 0.05
Clay (%)	1.37	0.21	**0.33**	< 0.01
Organic matter (%)	1.31	0.28	0.16	> 0.05
Soil C:N ratio	2.17	-0.55	0.05	> 0.05
Amorphous Fe-(hydr)oxide (mg kg^{-1})	0.48	0.31	**0.38**	< 0.01
Amorphous Al-(hydr)oxide (mg kg^{-1})	1.26	0.10	0.04	> 0.05
Se-$CaCl_2$ (µg kg^{-1})	1.21	0.46	**0.35**	< 0.01
Se-HNO_3[a] (µg kg^{-1})	1.29	0.23	0.08	> 0.05
Se-hot water (µg kg^{-1})	0.74	0.57	**0.41**	< 0.01
Se-ammonium oxalate (µg kg^{-1})	0.95	0.36	**0.33**	< 0.01
Total Se (aqua regia) (µg kg^{-1})	0.67	0.35	**0.31**	< 0.01
Selenite[a] (µg kg^{-1})	1.26	0.28	**0.24**	< 0.05
DOC-$CaCl_2$ (mg kg^{-1})	0.84	0.32	**0.22**	< 0.05
DOC-hot water (mg kg^{-1})	1.53	0.29	0.17	> 0.05
S-$CaCl_2$ (mg kg^{-1})	1.30	0.27	**0.22**	< 0.05
S-hot water (mg kg^{-1})	0.89	0.44	**0.27**	< 0.05
S-ammonium oxalate (mg kg^{-1})	0.86	0.38	**0.28**	< 0.05
P-$CaCl_2$ (mg kg^{-1})	1.43	0.28	0.08	> 0.05
P-hot water (mg kg^{-1})	1.27	0.24	0.05	> 0.05
P-ammonium oxalate (mg kg^{-1})	0.37	0.45	**0.25**	< 0.05
PO_4-hot water (mg kg^{-1})	1.50	0.07	0.009	> 0.05

[a] The samples that have concentrations below the determination limit were not included in the regression analysis.

A



B

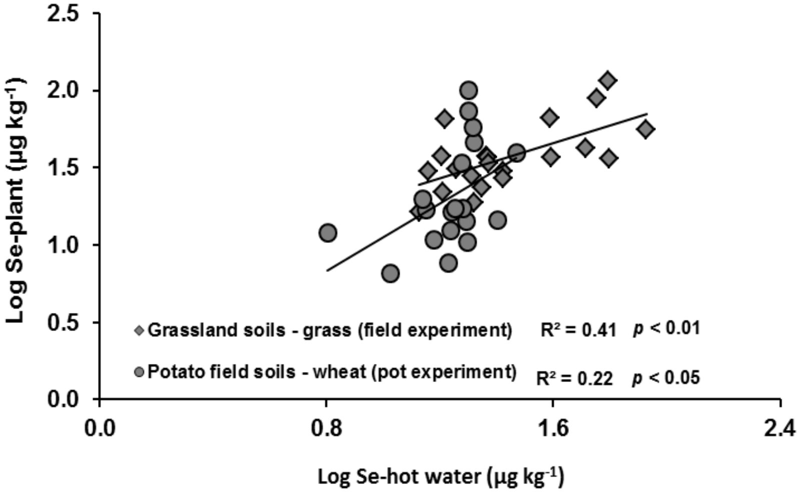


Fig. 2. Correlations between (A) Se in 0.01 M $CaCl_2$ extraction and Se content in plant and (B) Se in hot water extraction and Se content in plant. For the field experiment, the soil and grass samples were obtained from 2011 experiment without NPK application.

Among Se measured in different extractions, Se in 0.01 M $CaCl_2$ extraction (Se-$CaCl_2$), hot water extraction (Se-hot water), ammonium oxalate extraction, aqua regia digestion and selenite content in soils are positively correlated to Se content in grass, whereas Se in 0.43 M HNO_3 extraction is not correlated to Se content in grass (Table 3). In the previous pot experiment using soils from potato fields, positive correlations were only found between Se uptake and Se-$CaCl_2$ or Se-hot water, whereas the correlations with Se in other extractions were negative or insignificant (Supriatin *et al.*, 2016). In both studies, the relationships between Se in plant and Se-$CaCl_2$ or Se-hot water show a similar trend (Fig. 2), indicating that the soluble and labile organic Se forms control the bioavailability of Se. In the grassland soil samples of the current study, a positive correlation between Se-$CaCl_2$ or Se-hot water and Se in other extractions was found (Table A.3), whereas in the potato field soils used in the pot experiment of the previous work (Supriatin *et al.*, 2016), Se-$CaCl_2$ and Se-hot water were hardly correlated with Se in other extractions (Table A.3). This observation explains the difference between the two studies regarding the correlation between Se plant uptake and Se in extractions other than $CaCl_2$ and hot water extraction.

In addition, other soil parameters measured, such as DOC in 0.01 M $CaCl_2$ extraction, S in 0.01 M $CaCl_2$, hot water and ammonium oxalate extractions, and P in ammonium oxalate extraction are positively correlated to Se content in grass, whereas DOC in hot water extraction, P in 0.01 M $CaCl_2$ and hot water extractions, and P-PO_4 in hot water extraction are not correlated to Se content in grass (Table 3). Among the single soil parameters that are correlated to Se content in grass, Se in hot water extraction correlates most strongly with Se content in grass ($R^2 = 0.41$, $p < 0.01$; Table 3). These results indicate that in general the single soil parameters included in the current study can explain less than 50% of the Se variability in grass grown under field conditions.

In addition to the single factor regression analysis, multiple factor regression analysis was performed by combining two or three soil parameters, which have been found significantly correlated with Se content in grass in the single regression analysis (Table 3). Combining two or three soil parameters hardly increased the description of the variability of Se content in grass, with the maximum adjusted R^2 of only 0.38 (Table A.4). In general, combination of Se in hot water extraction with other soil parameters in two or three factors regression led to the highest adjusted R^2 of 0.35 to 0.38 (Table A.4). These findings indicate that the major part of the Se variability in grass grown under field conditions cannot be described by the soil parameters included in the current study.

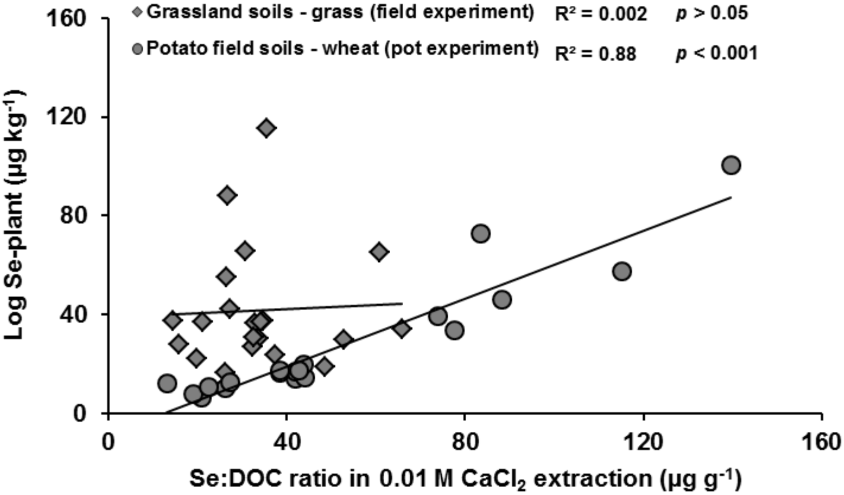


Fig. 3. Correlation between Se to DOC ratio in 0.01 M $CaCl_2$ extraction and Se content in plant. For the field experiment, the soil and grass samples were obtained from 2011 experiment without NPK application.

In the previous pot experiment using potato field soils, it has been demonstrated that the Se to DOC ratio in $CaCl_2$ soil extraction correlates strongly to Se plant uptake (Supriatin *et al.*, 2016). This Se to DOC ratio can be interpreted as the content of soluble Se-rich organic molecules in soils (Supriatin *et al.*, 2016). When the Se measured in the grass of this study is plotted as a function of Se to DOC ratio in the $CaCl_2$ soil extraction, hardly any correlation is observed, in contrast with the strong correlation found in the previous study (Fig. 3). This difference is attributed to the lower range of Se to DOC ratio in $CaCl_2$ extraction of grassland soils than that in potato field soils with similar range of Se content in grass and wheat shoots (Fig. 3). In addition, at a relatively low Se to DOC ratio in $CaCl_2$ extraction (i.e. < 40 $\mu g\ g^{-1}$), the amount of Se in grass is larger than Se in wheat shoots at the same Se to DOC ratio.

These findings show that labile organic Se in hot water extraction correlates most strongly to Se content in grass ($R^2 = 0.41$, $p < 0.01$; Table 3), suggesting that labile organic Se in hot water extraction can be used to a certain extent as an indicator of bioavailable Se in low Se grassland soils with predominantly organic Se under field conditions. Our previous study has shown that dissolved organic matter (DOM) extracted in $CaCl_2$ and labile organic matter extracted in hot water are richer in Se than other soil organic matter (acid-soluble, amorphous metal oxide associated and the majority of soil organic matter) (Supriatin *et al.*, 2015b). Moreover, mineralization or dissolution of Se-rich labile organic matter in soil solid phase is found to determine the amount of Se-rich DOM in soil solution and subsequent Se plant uptake in low Se (potato field) soils with predominantly organic Se (Supriatin *et al.*, 2016). These observations confirm the controlling influence of labile organic Se on the bioavailability and plant uptake of Se in low Se (grassland) soils with predominantly organic Se as shown in the current study. The content of clay, soil organic matter, and amorphous Fe- and Al-(hydr)oxides has a positive effect on the amount of labile organic Se in hot water extraction (Table 4). Among these soil properties, amorphous Fe-(hydr)oxide content correlates most strongly with Se in hot water extraction ($R^2 = 0.85$, $p < 0.001$), subsequently followed by clay content ($R^2 = 0.74$, $p < 0.001$) (Table 4), which correspond to the finding that Se content in grass are also positively correlated to amorphous Fe-(hydr)oxide content ($R^2 = 0.38$, $p < 0.01$) and clay content ($R^2 = 0.33$, $p < 0.01$) (Table 3). In soil, adsorption of organic matter on Fe-(hydr)oxide or clay surfaces (Anderson *et al.*, 1981; Oades, 1988; Gu *et al.*, 1994; Kaiser and Guggenberger, 2003; Tombácz *et al.*, 2004) may include the adsorption of Se-containing organic matter as well, suggesting that adsorption and desorption processes on soil mineral surfaces control the bioavailability of labile organic Se in the soils.

Table 4. Correlation between Se in hot water extraction and basic soil properties. The soil samples were obtained from 2011 field experiment without NPK application.

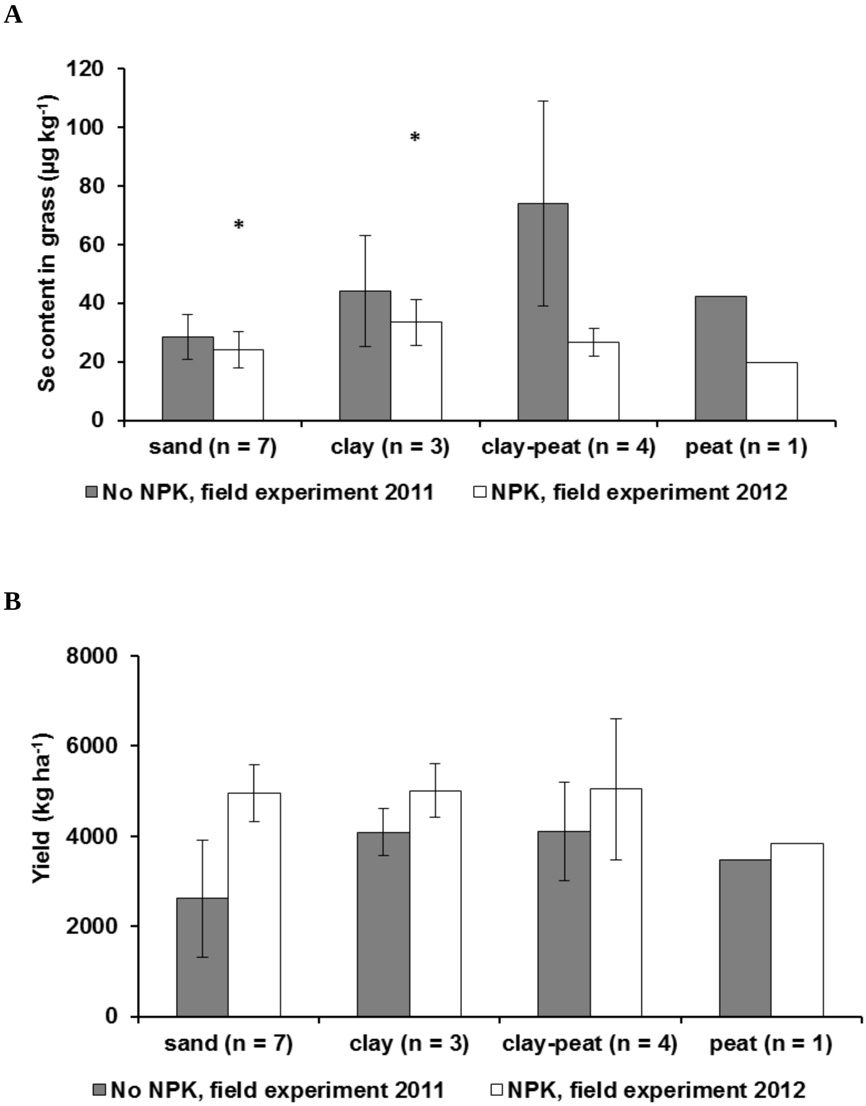
Soil parameter	Log Se-hot water (µg kg^{-1}) = a + b*Log(soil parameter)			
	Intercept (a)	Coefficient (b)	R^2	*p* value
pH-hot water	1.89	-0.07	0.08	> 0.05
Clay (%)	1.10	0.36	**0.74**	< 0.001
Organic matter (%)	0.88	0.62	0.62	< 0.001
Soil C:N ratio	1.92	-0.44	0.02	> 0.05
Amorphous Fe-(hydr)oxide (mg kg^{-1})	-0.36	0.51	**0.85**	< 0.001
Amorphous Al-(hydr)oxide (mg kg^{-1})	0.46	0.34	0.33	< 0.01

5.3.4 Mass balance estimation

The total uptake of Se in grass ranges from 0.02 to 0.61 g ha^{-1} (Fig. A.1.2). If we calculate the amount of dissolved Se in pore water based on the soluble Se concentration measured in 0.01 M CaCl$_2$ extraction of moist soils (0.03 to 0.12 µg L^{-1}, Supriatin *et al.* 2015a) and the soil moisture content in the field (22% to 146%), the amount of dissolved Se in the top 10 cm of the grassland soils (using a bulk density of 1 g cm^{-3} for clay-peat and peat soils and 1.4 g cm^{-3} for sandy and clay soils) is 0.01 to 0.11 g ha^{-1}. With this calculation, the total amount of Se taken up by grass accounts for 130% to 1100% of the dissolved Se in pore water. This finding indicates that to supply the amount of Se taken up by grass during 52 days grass growth (period between soil sampling and first cut of grass) the dissolved Se in the pore water needs to be replenished about 1.3 to 11 times, corresponding to a total turn-over time of 5 to 39 days, with an average of 22 ± 16 days. The amount of Se taken up in wheat from the potato field soils in the previous pot experiment is in similar range as Se taken up in grass in the current study, i.e. 0.8 to 14 times of the amount of dissolved Se in pore water, which corresponds to turn-over time of 4 to 67 days, with an average of 17 ± 10 days during 53 days of wheat growth (Supriatin *et al.*, 2016). This calculation showed that the turn-over of soluble organic Se in grassland soils is similar to that in potato field soils, although labile organic Se in hot water is more relevant as indicator of bioavailable Se in the grassland soils instead of Se-rich DOM in $CaCl_2$ extraction (Table 3 and Fig. 3).

5.3.5 Effect of NPK application on Se uptake in grass

The effect of NPK application on Se content in grass grown on different soil types was evaluated by comparing Se content in grass grown on soils without NPK application (2011 field experiment) with that in grass grown on the same fields with NPK application (2012 field experiment) (n = 15; Table 2). In general, NPK application tends to decrease Se content in grass grown on all the soil types, especially on organic rich soils (Fig. 4A and C). The decrease of Se content in grass grown on organic rich soils (on average 56% and 53% for clay-peat and peat soils, respectively) is more pronounced than that in grass grown on the more mineral soils (on average 12% and 24% for sandy and clay soils, respectively) (Fig. 4C). Two grass samples from fields with NPK application, which contain relatively high Se (i.e. 67 and 97 µg kg^{-1}; Fig. 4A), were not included in the comparison because the Se content in grass with NPK application is higher than that in grass without NPK application of the corresponding fields, which is in contrast to the general trend of the NPK effect on Se plant uptake. The relatively high Se content in grass of these two samples is probably due to contamination of Se fertilizer applied on the neighbouring plots with Se application (2012 field experiments: NPK vs. NPK plus selenate; Chapter 6). On the other hand, NPK application tends to increase the yield of grass grown on all soil types, especially on sandy soils (Fig. 4B and C). The small decrease of Se content in grass grown on sandy and clay soils upon NPK application can be attributed to the dilution effect of the higher yield, but the large decrease of Se content in grass on clay-peat and peat soils cannot be fully explained by dilution due to the yield increase (Fig. 4C).


A
Se content in grass (µg kg-1)
120
100
80
60
40
20
0
*
*
sand (n = 7)
clay (n = 3)
clay-peat (n = 4)
peat (n = 1)
No NPK, field experiment 2011
NPK, field experiment 2012
B
Yield (kg ha-1)
8000
6000
4000
2000
0
sand (n = 7)
clay (n = 3)
clay-peat (n = 4)
peat (n = 1)
No NPK, field experiment 2011
NPK, field experiment 2012

C

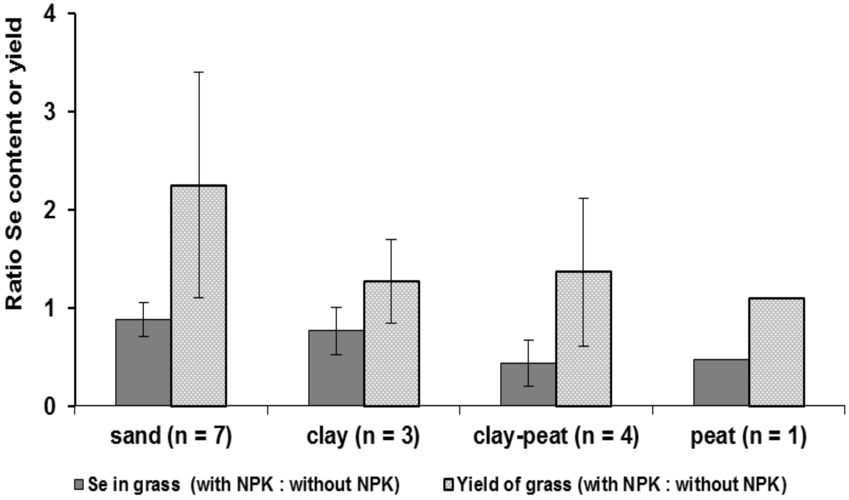


Fig. 4. Selenium content (A), yield (B) and ratio of Se content or yield (C) of grass obtained from plots with NPK application and from plots without NPK application with similar locations and soil types (n = 15). * = outliers, consist of two grass samples with relatively high Se content from two different fields with NPK application. These outliers were not included in the Figure 4C. The data in the figures are presented as means and standard deviations per soil type.

The Se content in grass grown on soils applied with NPK decreases with the increase of soil organic matter content, clay content and amorphous Fe-(hydr)oxide content (Fig. 5 and A.2.1 and A.2.4), whereas other soil properties such as pH, C:N ratio and amorphous Al-(hydr)oxide content, hardly influence Se content in grass (Fig. A.2.2, A.2.3 and A.2.5). The negative effect of clay content and amorphous Fe-(hydr)oxide content on Se plant uptake in soils applied with NPK (Fig. A.2.1 and A.2.4) might be caused by a correlation with soil organic matter content (clay: $R^2 = 0.45$, $p < 0.05$; amorphous Fe-(hydr)oxide: $R^2 = 0.78$, $p < 0.001$; data not shown). The findings suggest that NPK application and soil organic matter result in a synergetic effect in reducing Se bioavailability in soils. A possible explanation is that NPK application increases soil microbial activity to reduce Se into a lower valence state, and the reduced Se further incorporates into soil organic matter. Previous studies have shown that application of mineral NPK fertilizers increased soil microbial biomass and activity (enzyme activity) compared to unfertilized soils (Weber *et al.,* 1985; Böhme *et al.,* 2005; Chu *et al.,* 2007), which may be related to the increase of crop residue, root biomass and root exudates upon fertilization (Chu *et al.*, 2007; Gong *et al.*, 2009). However, the mechanism of Se incorporation into soil organic matter through microbial activity is still unclear. The studies of Gustafsson and Johnsson (1994) and Copin *et al.* (2006) indicated that most of ^{75}Se (as selenite) added to forest floors or mineral soils was found in the humic substances of soil organic matter upon equilibration or incubation. Moreover, other studies found an increase of ^{75}Se (as selenite) incorporation into soil organic matter (Gustafsson and Johnsson, 1994; Darcheville *et al.*, 2008) and an increase of elemental Se (Se^0) and residual Se fractions in a non-sterilized soil sample compared to those in a sterilized sample (Darcheville *et al.*, 2008), suggesting that soil microorganisms play a role in the process of Se immobilization into soil organic matter and residual fraction (probably as resistant organic matter). In addition to Se incorporation into soil organic matter, another possible explanation is that NPK fertilization may reduce the mineralization of organic Se into available Se forms in soils. However, this hypothesis is in contrast with the results of previous studies, which indicated that long-term balanced NPK fertilization in general increased soil organic C and N mineralization compared to unbalanced N, NP, PK, or NK fertilization and unfertilized control (Rudrappa *et al.*, 2006; Manna *et al.*, 2007; Yan *et al.*, 2007; Gong *et al.*, 2009). The increase of soil organic matter mineralization upon NPK fertilization is attributed to the increase of soil microbial biomass and activity induced by an increase of crop residue and root exudates (Gong *et al.*, 2009).

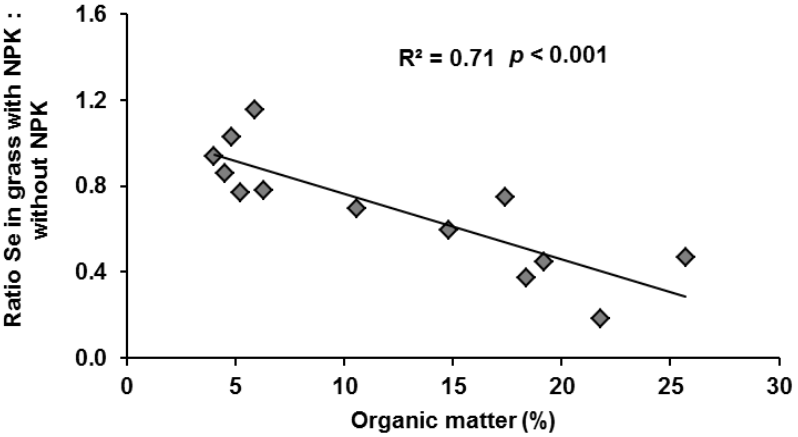


Fig. 5. Correlation between ratio of Se content in grass from fields with NPK application to Se in grass from the same fields without NPK application and soil organic matter content measured in soil samples from 2012 field experiment.

5.4 Conclusions

Total Se content (aqua regia) in grassland soils of the Netherlands in this field study was found to be in the range of 0.12 to 1.40 mg kg^{-1}, with an average of 0.50 mg kg^{-1}. Soluble Se in 0.01 M $CaCl_2$ extraction of these soils is between 3.0 and 23 µg kg^{-1}, which accounts for on average 1.7 ± 0.6% of total Se. Both the total Se and soluble Se consist of mainly organic Se forms. The Se content in grass grown on these soils (without NPK application and without Se fertilization) under field conditions is between 16 and 115 µg kg^{-1}, with an average of 42 µg kg^{-1} (plant dry weight), which is far below the adequate amount of Se for dairy cattle intake, i.e. 150 to 200 µg Se kg^{-1} dry weight.

Among the soil parameters measured in this study, labile organic Se in hot water extraction correlates most strongly with Se content in grass ($R^2 = 0.41$, $p < 0.01$), suggesting that labile organic Se is related to a certain extent to the bioavailable Se in low-Se grassland soils. In addition, these findings indicate that > 50% of the Se variability in grass grown under field conditions cannot be described by the soil parameters included in the current study. The content of amorphous Fe-(hydr)oxide and clay in soils has a positive effect on the amount of labile organic Se, which corresponds to the finding that Se content in grass is also positively correlated to amorphous Fe-(hydr)oxide content and clay content (when no NPK is applied).

The application of NPK fertilizers decreased Se content in grass, and this effect becomes stronger with increasing soil organic matter content, suggesting that NPK application and soil organic matter exert a synergetic effect in reducing Se bioavailability in soils. On organic rich soils (clay-peat and peat soils), the decrease of Se content in grass is more pronounced, i.e. on average 50% decrease, than that on the other soils (sandy and clay soils), i.e. 12% and 24%, respectively. The small increase of grass yield upon NPK application, especially on organic rich soils, cannot explain the large decrease of the Se content in grass. A possible explanation is that NPK application increases soil microbial activity, which reduces Se into a lower valence state that is subsequently immobilized into soil organic matter.

The results of this study suggest that Se bioavailability in Dutch grassland soils, which are characterized by low Se content with predominantly organic Se, and Se-poor dissolved organic matter (compared to potato field soils), is partly determined by the amount of labile organic Se. In these kinds of soils, soil organic matter plays an important role in determining the bioavailability of Se.

Appendices

Table A.1. Amount and fraction of Se extracted in 0.43 M HNO_3, hot water and ammonium oxalate. The data are presented as mean ± standard deviation per soil type. Values in brackets are ranges of the Se amount and fraction in each extraction per soil type. The soil samples were obtained from 2011 field experiment without NPK application.

Soil type	Se extracted ($\mu g\ kg^{-1}$)			Se extraction efficiency (% of total Se-aqua regia)		
	Se-HNO_3	Se-hot water	Se-ammonium oxalate	Se-HNO_3	Se-hot water	Se-ammonium oxalate
Sand (n =10)	16.8 ± 7.8[a] (6.9 – 31.4)[a]	18.8 ± 4.4 (13.4 – 26.6)	35.5 ± 14.7 (18.3 – 67.5)	7 ± 3[a] (5 – 13)[a]	9 ± 2 (5 – 14)	15 ± 2 (11 – 19)
Clay (n = 6)	9.0 ± 2.1 (7.3 – 12.8)	28.5 ± 8.3 (21.0 – 39.3)	42.9 ± 17.4 (25.3 – 72.2)	3 ± 1 (1.4 – 4)	8 ± 2 (7 – 11)	12 ± 1 (9 – 13)
Clay-peat (n = 4)	31.7 ± 9.9 (20.1 – 40.3)	66.5 ± 12.5 (56.6 – 84.8)	170.0 ± 23.0 (152.2 – 203.0)	3 ± 1 (1.5 – 3)	5 ± 1 (5 – 7)	13 ± 0.8 (13 – 15)
Peat (n = 1)	59.6	51.7	168.0	5	5	15
All samples (n = 21)	**19.7 ± 14.3**	**32.2 ± 19.9**	**69.5 ± 59.6**	**5 ± 3**	**8 ± 2**	**14 ± 2**

[a] Soil samples that have Se concentrations below the determination limit were excluded from the table.

Table A.2. Selenium speciation in ammonium oxalate extraction measured using HPLC - ICP-MS. The soil samples were obtained from 2011 field experiment without NPK application.

Soil type	Total Se ammonium oxalate (µg kg^{-1})		Inorganic selenite[a]/total Se ammonium oxalate (%)		Organic Se/total Se ammonium oxalate (%)		Total Se aqua regia (µg kg^{-1})	Inorganic selenite[a]/total Se aqua regia (%)
	Range	Average ± SD[b]	Range	Average ± SD[b]	Range	Average ± SD[b]	Average ± SD[b]	Average ± SD[b]
Sand (n = 10)	14 – 71	35 ± 15	0 – 72	19 ± 23	28 – 100	81 ± 23	229 ± 73	3 ± 5
Clay (n = 6)	30 – 72	48 ± 15	16 – 67	42 ± 18	33 – 84	58 ± 18	358 ± 152	6 ± 4
Clay-peat (n = 4)	155 – 216	180 ± 26	18 – 31	26 ± 7	69 – 82	74 ± 7	1262 ± 110	4 ± 1
Peat (n = 1)	201	-	13	-	87	-	1142	2
Average ± SD[b]		**74 ± 66**		**26 ± 21**		**74 ± 21**	**506 ± 435**	**4 ± 4**

[a] Inorganic selenite as extracted with ammonium oxalate.

[b] Standard deviation.

Table A.3. Correlation between Se extracted (µg kg^{-1}) in different soil extractions (R^2). The correlations include Se concentrations measured in grassland soil samples from 2011 field experiment (without NPK application) of the current study and in potato field soil samples used in the pot experiment of the previous study (Supriatin *et al.,* 2016)**.**

	HNO_3	Hot water	Ammonium oxalate	Aqua regia
Grassland (n = 21)				
$CaCl_2$	0.37**	0.91***	0.74***	0.81***
HNO_3		0.38[a]**	0.53[a]***	0.46[a]**
Hot water			0.83***	0.89***
Ammonium oxalate				0.98***
Potato field (n = 19)				
$CaCl_2$	0.002^{ns}	0.50***	0.12[a] ^{ns}	0.0007[a] ^{ns}
HNO_3		0.003^{ns}	0.18[a] ^{ns}	0.20[a] ^{ns}
Hot water			0.08[a] ^{ns}	0.03[a] ^{ns}
Ammonium oxalate				0.61[a] ***

[a] The samples that contain Se concentrations below the determination limit were excluded from the correlations.

* indicates $p < 0.05$; ** indicates $p < 0.01$; *** indicates $p < 0.001$ and ^{ns} indicates not significant.

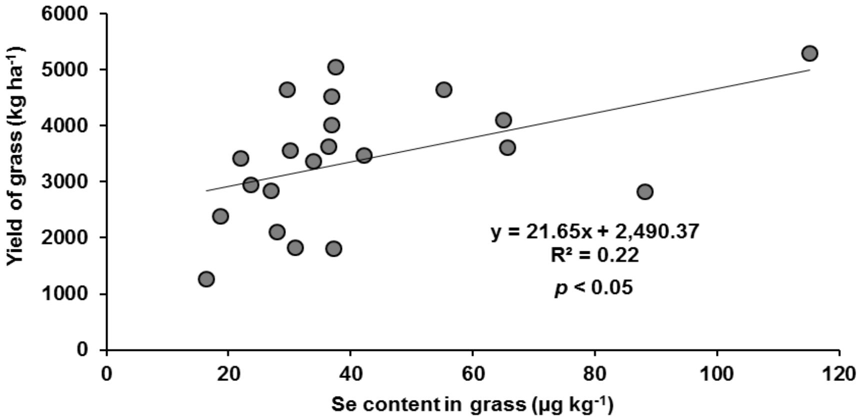
Table A.4. Results of multiple (two to three factors) linear regressions between Log_{10}(Se in grass, µg kg^{-1}) as a dependent parameter (Y) and Log_{10}(soil parameters) as independent parameters (X_1, X_2, ...), with an adjusted $R^2 > 0.30$ (n = 21). The soil and grass samples were obtained from 2011 field experiment without NPK application. Unit of independent parameters: Amorphous Fe-oxide, DOC-$CaCl_2$, S-$CaCl_2$, S-ammonium oxalate, S-hot water, P-ammonium oxalate (mg kg^{-1}); clay content (%); Se-$CaCl_2$; Se-hot water, Se-ammonium oxalate, total Se-aqua regia (µg kg^{-1}).

Model	Intercept (a)	Coefficient and independent parameter			Adjusted R^2	*p* value
		b_1 (X_1)	b_2 (X_2)	b_3 (X_3)		
Model 1	0.69	0.23 (Fe-oxide)	0.07 (clay)		0.33	< 0.05
Model 2	0.45	0.28 (Fe-oxide)	0.06 (DOC-$CaCl_2$)		0.32	< 0.05
Model 3	0.67	0.22 (Fe-oxide)	0.16 (Se-$CaCl_2$)		0.33	< 0.05
Model 4	0.64	0.09 (Fe-oxide)	0.42 (Se-hot water)		**0.35**	< 0.01
Model 5	0.55	0.24 (Fe-oxide)	0.11 (Se-amm. Ox.)		0.33	< 0.05
Model 6	0.47	0.27 (Fe-oxide)	0.06 (Se-aqua regia)		0.32	< 0.05
Model 7	0.50	0.30 (Fe-oxide)	0.01 (S-$CaCl_2$)		0.32	< 0.05
Model 8	0.48	0.25 (Fe-oxide)	0.12 (S-hot water)		0.33	< 0.05
Model 9	0.49	0.27 (Fe-oxide)	0.06 (S-amm. Ox.)		0.32	< 0.05
Model 10	0.40	0.28 (Fe-oxide)	0.07 (P-amm. Ox.)		0.32	< 0.05
Model 11	1.24	0.11 (clay)	0.28 (Se-$CaCl_2$)		0.32	< 0.05
Model 12	0.36	0.03 (clay)	0.51 (Se-hot water)		**0.35**	< 0.01
Model 13	1.08	0.13 (clay)	0.21 (Se-amm. Ox.)		0.33	< 0.05
Model 14	0.79	0.16 (clay)	0.23 (P-amm. Ox.)		0.31	< 0.05
Model 15	0.64	-0.12 (Se-$CaCl_2$)	0.71 (Se-hot water)		**0.35**	< 0.01

Table A.4. Continued.

Model	Intercept (a)	Coefficient and independent parameter			Adjusted R^2	*p* value
		b_1 (X_1)	b_2 (X_2)	b_3 (X_3)		
Model 16	0.73	0.67 (Se-hot water)	-0.08 (Se- amm. Ox.)		**0.35**	< 0.01
Model 17	0.96	1.05 (Se-hot water)	-0.35 (Se-aqua regia)		**0.38**	< 0.01
Model 18	0.66	0.70 (Se-hot water)	-0.10 (S-$CaCl_2$)		**0.36**	< 0.01
Model 19	0.66	0.76 (Se-hot water)	-0.10 (S-hot water)		**0.35**	< 0.01
Model 20	0.78	0.70 (Se-hot water)	-0.12 (S-amm. Ox.)		**0.35**	< 0.01
Model 21	0.75	0.58 (Se-hot water)	-0.005 (P-amm. Ox.)		**0.35**	< 0.01
Model 22	0.68	0.09 (Fe-oxide)	0.02 (clay)	0.39 (Se-hot water)	**0.32**	< 0.05
Model 23	0.54	0.09 (Fe-oxide)	-0.13 (Se-$CaCl_2$)	0.56 (Se-hot water)	**0.32**	< 0.05
Model 24	0.70	0.02 (clay)	-0.11 (Se-$CaCl_2$)	0.65 (Se-hot water)	**0.31**	< 0.05
Model 25	0.63	0.09 (Fe-oxide)	0.52 (Se-hot water)	-0.07 (Se-amm. Ox.)	**0.32**	< 0.05
Model 26	0.87	0.07 (Fe-oxide)	0.91 (Se-hot water)	-0.34 (Se-aqua regia)	**0.35**	< 0.01
Model 27	0.76	0.01 (clay)	0.63 (Se-hot water)	-0.06 (Se-amm. Ox.)	**0.31**	< 0.05
Model 28	0.91	-0.04 (clay)	1.20 (Se-hot water)	-0.40 (Se-aqua regia)	**0.35**	< 0.01
Model 29	1.72	0.19 (clay)	0.79 (Se-amm. Ox.)	-0.66 (Se-aqua regia)	0.32	< 0.05
Model 30	0.64	-0.12 (Se-$CaCl_2$)	0.79 (Se-hot water)	-0.07 (Se-amm. Ox.)	**0.31**	< 0.05
Model 31	0.88	-0.10 (Se-$CaCl_2$)	1.14 (Se-hot water)	-0.34 (Se-aqua regia)	**0.34**	< 0.05

1



2

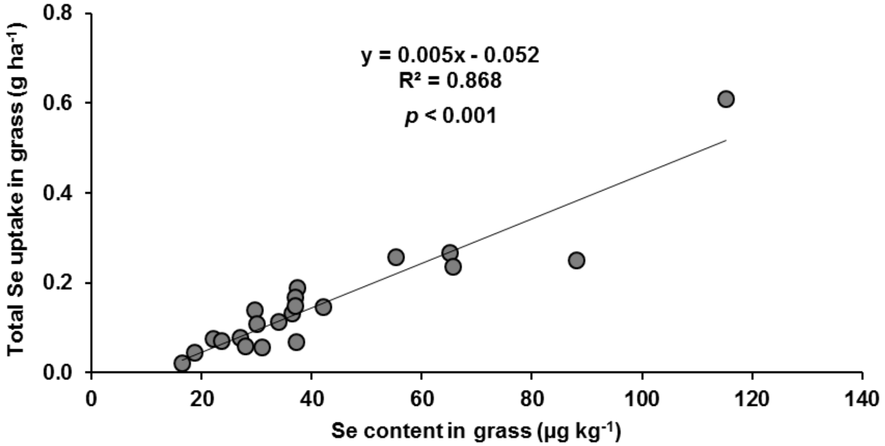
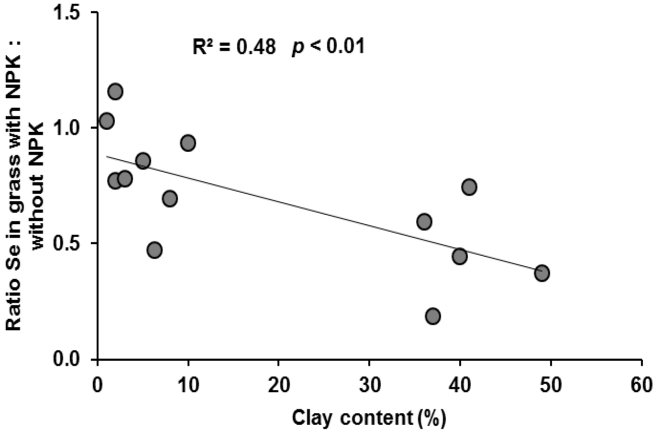
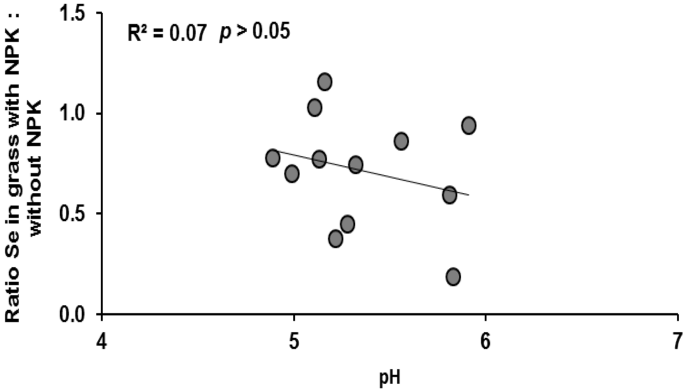


Fig. A.1. Correlation between (1) yield and Se content in grass and (2) total Se uptake and Se content in grass obtained from 2011 field experiment without NPK application.

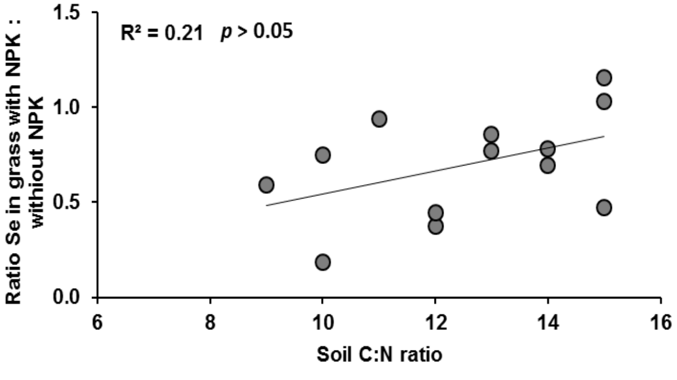
1


R² = 0.48 p < 0.01
Ratio Se in grass with NPK : without NPK
0.0
0.5
1.0
1.5
0
10
20
30
40
50
60
Clay content (%)

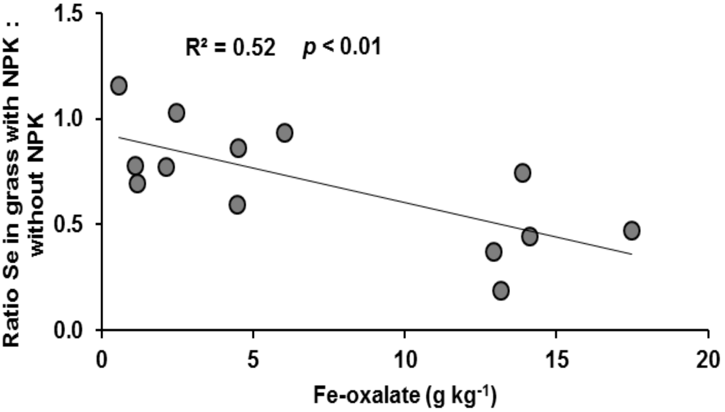
2


R² = 0.07 p > 0.05
Ratio Se in grass with NPK : without NPK
0.0
0.5
1.0
1.5
4
5
6
7
pH

3


R² = 0.21 p > 0.05
Ratio Se in grass with NPK : withiout NPK
0.0
0.5
1.0
1.5
6
8
10
12
14
16
Soil C:N ratio

4



5

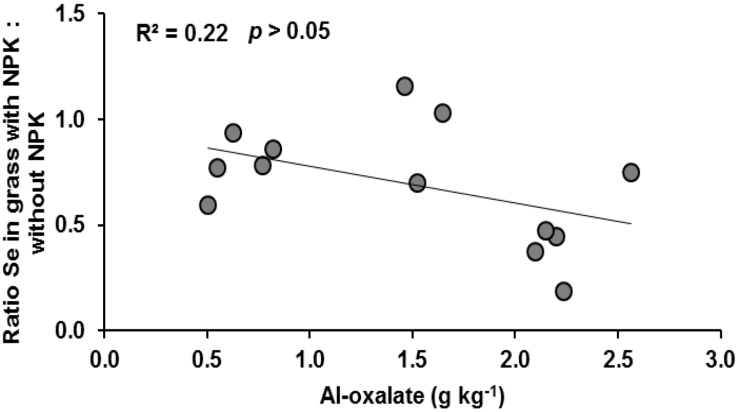


Fig. A.2. Correlations between ratio of Se content in grass from fields with NPK application to Se in grass from the same fields without NPK application and (1) pH, (2) clay content, and (3) soil C:N ratio measured in soil samples from 2012 field experiment, (4) amorphous Fe-(hydr)oxide content and (5) Al-(hydr)oxide content measured in soil samples from 2011 field experiment. Amorphous Fe- and Al-(hydr)oxide content in the soil samples from 2012 field experiment were not measured.

Chapter 6

Effects of Selenate Fertilization on Selenium Uptake in Grass Grown on Grassland Soils with Different Soil Properties

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Under preparation

Abstract

Two field experiments on 20 and 26 grassland fields, respectively, in the Netherlands consisting of different soil types were conducted in 2007 (N + cattle slurry and Se + N + cattle slurry) and in 2012 (NPK and Se + NPK). Without Se fertilization, Se content in grass ranged from 13 to 139 µg kg^{-1} in the fields with N plus slurry applications (2007) and from 16 to 107 µg kg^{-1} in the fields with NPK application (2012). In both experiments, Se content in grass is higher in clay soils with clay content ≥ 20% and pH > 6 than in the other soil types, and Se content of grass grown on the same soil types is comparable in the two experiments, suggesting little difference between N plus slurry and NPK treatment in terms of Se uptake. Selenium (as selenate) fertilization in both experiments increased Se content in grass by 3 to 6 fold, except that in grass grown on clay soils with clay content ≥ 20% and pH > 6 the increase was only 1.3 to 2 fold. Grasses grown on these clay soils already contained a relatively high amount of Se without Se fertilization. Upon Se fertilization, Se in grass was 38 to 266 µg kg^{-1} in the fields with N plus slurry applications (2007) and 86 to 213 µg kg^{-1} in the fields with NPK application (2012), which are still mostly below the recommended amount for cattle intake (150 to 200 µg kg^{-1}). At a relatively low dosage of Se fertilization (0.32 to 2.0 g Se ha^{-1}), Se content in grass is to a large extent related to the dose of Se applied. At a high dosage of Se fertilization (4.4 g Se ha^{-1}), Se content in grass is in general comparable for all the soil types. The effectiveness of Se fertilization is in general comparable for all the soil types in both experiments, suggesting a minor influence of soil properties on the effectiveness of selenate fertilization. Nevertheless, the effectiveness of Se fertilization on sandy soils tends to have a larger range and a higher mean than that on clay soils and organic rich soils (clay-peat and peat soils) in both experiments, probably due to Se adsorption on clay surfaces in clay soils, and Se immobilization into soil organic matter via microbial processes in organic rich soils. Only < 22% of Se applied to the soils was recovered in the grass for one season, indicating that the rest of Se is probably accumulated in the soils or lost via leaching and volatilization.

6.1 Introduction

Selenium (Se) is an essential micronutrient for humans and animals (livestock), but not for plants, therefore, optimum crop yields can still be obtained on soils with very low available Se (Gupta *et al.*, 2008). Large areas of soils on Earth have a relatively low Se content, and the Se levels in crops and grasses are often below the adequate amount for human or animal intake (Hartikainen, 2005). Contents of Se in crops or grasses can be increased by Se fertilization via soils or foliar application. The bioavailability and plant uptake of both native soil Se and fertilized Se are influenced by many factors. Gissel-Nielsen *et al.* (1984) suggested that Se content in plants is influenced by plant species (accumulator vs. non-accumulator plants), soil properties, fertilization treatments, Se speciation in soils, and metabolism of Se in plants. Understanding the effects of these factors, especially soil properties and fertilization treatments, on Se uptake in crops may help us to choose appropriate management practices on agricultural soils to maintain or increase Se bioavailability.

The predominant soil properties, such as soil organic matter content, clay content, metal oxide content and pH affect the effectiveness of Se fertilization (Gissel-Nielsen *et al.*, 1984; Mikkelsen *et al.*, 1989). Previous studies have shown that uptake of Se from selenate or selenite fertilizer added to soils by plants grown under controlled conditions (pot experiments) decreased with an increase of soil organic matter content and clay content (Bisbjerg and Gissel-Nielsen, 1969; Gissel-Nielsen, 1971; Yläranta, 1983; Johnsson, 1991), which was attributed to Se fixation or immobilization by soil organic matter and clay (Gissel-Nielsen and Hamdy, 1977; Hamdy and Gissel-Nielsen, 1977; Gustafsson and Johnsson, 1992). The solubility and availability of Se in soils can also be lowered due to Se (mainly selenite) adsorption on Fe-(hydr)oxide surfaces (Hamdy and Gissel-Nielsen, 1977; Balistrieri and Chao, 1990). However, the nature of Se fixation by soil organic matter is different from that by phyllosilicate clays and Fe-(hydr)oxides (Gissel-Nielsen *et al.*, 1984; Mikkelsen *et al.*, 1989), and more recent studies suggested that soil organic matter has a greater fixation capacity for Se than clay and Fe-(hydr)oxide minerals (Coppin *et al.*, 2009). Selenium fixation by clays and Fe-(hydr)oxides is mainly attributed to adsorption via anion exchange, forming outer-sphere complexes, or via ligand exchange, forming inner-sphere complexes (Balistrieri and Chao, 1990), whereas the mechanism of Se fixation in soil organic matter is still unclear. Selenium can be fixed in soil organic matter via either adsorption to Fe-(hydr)oxide surfaces present in soil organic matter (Coppin *et al.*, 2009) or via Se reduction by soil microorganisms and further incorporation into soil organic matter structure (Gustafsson and Johnsson, 1994). Soil pH also plays an important role in determining Se availability in soil (Mikkelsen *et al.*, 1989), in which Se availability and plant uptake increase in general with the increase of pH due to less adsorption of Se (mainly selenite) on clay or Fe-(hydr)oxide surfaces.

Further, the availability of major anions in soils, such as phosphate and sulphate, also influences Se uptake by plants (Mikkelsen *et al.*, 1989). Previous studies have shown that there is an increase of Se uptake in plants upon phosphate fertilization (Carter *et al.*, 1972; Liu *et al.*, 2004), which can be either due to a competition effect between phosphate and Se (mainly selenite) for the adsorption on soil mineral surfaces, releasing the adsorbed Se into soil solution (Carter *et al.*, 1972; Liu *et al.*, 2004; Eich-Greatorex *et al.*, 2010) or due to the increase of root proliferation, resulting in more root-soil contact to take up Se (Carter *et al.*, 1972). On the other hand, Se uptake by plants can be increased or decreased upon sulphate fertilization (Cartes *et al.*, 2006; Stroud *et al.*, 2010b), depending on the plant S status (i.e. S deficient or sufficient) and on whether Se is native or fertilized (selenite or selenate) (Stroud *et al.*, 2010b). Cartes *et al.* (2006) indicated that when selenite and sulphate fertilizer were applied together in a pot experiment, an up to 40% decrease of Se plant uptake was observed. Another study showed that when sulphate and selenate were applied together, sulphate increased Se plant uptake when the plant contained sufficient S, whereas a decrease of Se uptake was observed when the plant was S deficient (Stroud *et al.*, 2010b). Hopper and Parker (1999) indicated that competition between sulphate and selenate for plant uptake is stronger than that between phosphate and selenite. Compared to selenite, selenate binds much weaker to soil mineral surfaces, and there is little competition effect between sulphate and selenate for adsorption to soil minerals. Therefore, the increase of selenate plant uptake when sulphate is added can probably be attributed to inhibition of microbial selenate reduction into less available Se by sulphate because sulphate is then used in place of selenate as substrate for reduction (Stroud *et al.*, 2010b). On the other hand, sulphate fertilization decreased plant uptake of native soil Se, probably due to competition for the uptake dominating that for the adsorption or reduction of selenate in soil (Stroud *et al.*, 2010b).

In addition to soil factors, common fertilization practices of farmers, including manure or organic residue application and mineral NPK fertilization, may also influence Se uptake in plants. Previous studies have shown that addition of organic materials to soils, such as cattle manure or crop residue, reduced the uptake of added Se (selenate or selenite fertilizer) by plants, due to immobilization of Se into organic fertilizer (Ajwa *et al.*, 1998; Funwie, 2012). Another study of Sharma *et al.* (2011) showed that application of organic fertilizers from poultry manure, farmyard manure, and sugar cane press mud reduced Se accumulation in wheat and oilseed rape grains grown on a seleniferous soil. Our previous study also showed that mineral NPK fertilization, especially on organic rich soils, decreased Se plant uptake (Chapter 5).

In general, farmers apply mineral NPK and/or animal manure or slurry on grasslands to increase the yield of grass. Study on Se (as selenate) fertilization in combination with common fertilization practices of NPK and/or animal manure or slurry on various soil types under field conditions is necessary to obtain insight to design efficient Se fertilization strategies. The objectives of this study are to evaluate the range of Se content in grass grown on different soil types fertilized with N plus cattle slurry or with NPK, and to evaluate the effect of Se (selenate) fertilization on Se plant uptake in soils with different properties in corresponding fields with N plus cattle slurry or NPK applications.

6.2 Materials and Methods

6.2.1 Field experiments

6.2.1.1 Field experiments with Se, N and cattle slurry applications

In 2007, field experiments were conducted on 20 grasslands in the Netherlands, consisting of four soil types, i.e. sand, clay, loess and peat soils (Table 1). Each grassland field consisted of two plots, i.e. a plot with mineral N and cattle slurry applications and a corresponding plot with mineral Se, mineral N and cattle slurry applications. The size of each plot was 8 m x 1.5 m. Selenium fertilizer, N fertilizer and cattle slurry were applied in February or March 2007 before the first cut of grass. Selenium was applied as Na_2SeO_4-enriched N fertilizer. The fertilization dosages are 0.32 to 2.0 g Se ha^{-1}, 24 to 108 kg N ha^{-1} and 20 to 32 m^3 cattle slurry ha^{-1} following the scheme of individual farmers (Table 2). Soil samples (top soil 0 to 10 cm) were taken in January or February 2007 before fertilization. The soil samples from these fields have been used in a previous study on speciation of Se and DOC in soil solution and their relation to Se bioavailability (Weng *et al.*, 2011). Grass samples were harvested from the first cut in April or May 2007.

6.2.1.2 Field experiments with Se and NPK applications

In 2012, field experiments were performed on 26 grasslands in the Netherlands, consisting of sand, clay, clay-peat, and peat soils (Table 1). Among the fields, 16 fields were located at the same fields as those used in the field experiment of 2011 (Chapter 5), whereas the other 10 fields were located at new fields. Each grassland field consisted of two plots, i.e. a plot with mineral NPK application and a corresponding plot with mineral Se and NPK applications. The size of each plot was 8 m x 1.5 m. Mineral Se (as Na_2SeO_4-enriched N fertilizer; 4.4 g Se ha^{-1}), N (120 kg N ha^{-1}), P (31 kg P_2O_5 ha^{-1}), and K (60 kg K_2O ha^{-1}) fertilizers were applied on the corresponding plots in March or April 2012. In addition to Se, other micronutrients, i.e. Cu (3.5 kg CuO ha^{-1} in the form of $CuSO_4$), B (0.9 kg B ha^{-1} in the form of H_3BO_3) and Mn (1 kg MnO ha^{-1} in the form of $MnSO_4$) were also applied to the plots with Se application but are not a subject of the present study. Soil samples (top

Table 1. Characteristics of soil samples, total Se content (aqua regia) and soluble Se concentrations in soil samples from field experiments. Results are presented as mean ± standard deviation per soil type. Values in brackets are ranges of the parameters per soil type. The clay soils were further divided into two groups due to the differences in Se uptake.

Soil type	pH[a]	Organic matter (%)	Clay (%)	Fe-oxalate[b] (g kg^{-1})	Al-oxalate[b] (g kg^{-1})	Se-aqua regia (mg kg^{-1})	Se-$CaCl_2$ (µg kg^{-1})
Field experiment in 2007							
Sand (n = 8)	5.4 ± 0.5	4.6 ± 1.5	2.0	n.d.[c]	n.d.[c]	n.d.[c]	4.7 ± 1.7 (2.8 – 8.2)
Clay $\geq$ 20%, pH > 6 (n = 3)	7.2 ± 0.2	5.0 ± 1.2	26.0 ± 8.2	n.d.[c]	n.d.[c]	n.d.[c]	7.7 ± 0.7 (7.0 – 8.3)
Other clay (n = 3)	5.6 ± 0.5	4.4 ± 0.6	29.3 ± 4.2	n.d.[c]	n.d.[c]	n.d.[c]	6.2 ± 1.3 (4.8 – 7.4)
Loess (n = 3)	5.8 ± 0.2	4.3 ± 1.0	10.0 ± 1.7	n.d.[c]	n.d.[c]	n.d.[c]	6.6 ± 0.2 (6.5 – 6.7)
Peat (n = 3)	5.3 ± 0.1	25.7 ± 4.7	2.0	n.d.[c]	n.d.[c]	n.d.[c]	7.8 ± 2.3 (5.3 – 9.7)
Field experiment in 2012							
Sand (n = 13)	5.4 ± 0.8[e]	7.2 ± 3.8	3.4 ± 2.0	1.8 ± 1.3[d]	1.2 ± 0.4[d]	0.36 ± 0.21[d] (0.17 – 0.72)[d]	4.4 ± 1.7[e] (2.0 – 7.3)[e]
Clay $\geq$ 20%, pH > 6 (n = 3)	6.6 ± 0.4	9.2 ± 5.9	31.7 ± 3.2	4.5[d]	0.5[d]	0.79 ± 0.13[d] (0.51 – 1.06)[d]	6.2 ± 0.3 (5.9 – 6.5)
Other clay (n = 4)	6.6 ± 0.8	6.6 ± 5.5	17.8 ± 12.2	5.3 ± 1.1[d]	0.6 ± 0.1[d]	0.46 ± 0.13[d] (0.37 – 0.56)[d]	4.6 ± 1.1 (4.0 – 6.2)
Clay-peat (n = 4)	5.4 ± 0.3	19.2 ± 1.9	41.8 ± 5.1	13.5 ± 0.6[d]	2.3 ± 0.2[d]	1.26 ± 0.11[d] (1.16 – 1.40)[d]	13.8 ± 2.2 (11.8 – 16.1)
Peat (n = 2)	5.0[e]	23.0 ± 3.9	7.2 ± 1.2	17.5[d]	2.2[d]	1.00 ± 0.20[d] (0.86 – 1.14)[d]	9.3[e]

[a] Measured in 0.01 M $CaCl_2$ extraction at soil to solution ratio 1:10.

[b] Measured in ammonium oxalate-oxalic acid (pH 3) extraction at soil to solution ratio 1:20.

[c] Not determined.

[d] The values were derived from 16 soil samples obtained from the same fields in 2011. Especially for Se (aqua regia), other 6 soil samples obtained from the new fields in 2012 were analysed and included in the table.

[e] One soil sample was not analysed.

Table 2. Dosages of selenate, mineral N and cattle slurry applied to different soil types in the 2007 field experiment. The sandy soils were further divided into three groups due to the differences in Se dosages.

Soil type	Selenate (g Se ha^{-1})	Mineral N (kg N ha^{-1})	Cattle slurry (m^3 ha^{-1})
Sand1 (n = 3)	0.6	24.5	25
Sand2 (n = 2)	1.4	48.6; 86.4	30
Sand3 (n = 3)	1.85	100	30
Clay ≥ 20%, pH > 6 (n = 3)	2.0	108; 104; 104	32; 32; 20
Other clay (n = 3)	0.32; 0.36; 0.40	67.5; 75; 82.5	25
Loess (n = 3)	1.4	75.6	30
Peat (n = 3)	1.5	81	30

soil 0 to 10 cm) were taken in March 2012 before fertilization. Grass samples were taken from the first cut in May 2012.

6.2.2 Pre-treatment of soil and grass samples

After removing the plant residues and gravel, the soil samples were oven-dried at 40^oC under forced air ventilation for 24 to 48 h, ground, and sieved with a 2 mm sieve for further analysis. The grass samples were oven dried at 70^oC for 72 h and milled for further Se content analysis. The yield of grass (kg dry matter ha^{-1}) was also determined. The grass samples taken from the fields were dominated by *Lolium perenne*.

6.2.3 Soil extractions

A 0.01 M $CaCl_2$ extraction (soil to solution ratio 1:10) was performed on all soil samples from field experiments of 2007 and 2012 using the procedure proposed by Houba *et al.* (2000). A more detailed description of the extraction method can be found in Supriatin *et al.* (2015b). Selenium concentration and pH were measured in the $CaCl_2$ extraction. Selenium concentrations in aqua regia digestion and Fe and Al concentrations in ammonium oxalate extraction for the soils of the 2012 field experiment were obtained from the analyses of 16 soil samples taken from the same fields in 2011 experiment (Table 1).

6.2.4 Plant digestion

The grass samples from 2007 field experiment were digested using HNO_3, according to the procedure described by Weng *et al.* (2011). About 0.35 g sample was digested using 4 mL of concentrated HNO_3 at 180^oC and 60 bars for 1.5 h in a microwave with a closed system. The grass samples of the 2012 field experiment were digested using HNO_3 and H_2O_2 also in a microwave with a closed system, according to the procedure described by Supriatin *et al.* (2016). About 0.4 g of plant sample was put into a Teflon destruction vessel and 5 mL of concentrated HNO_3 was added to the sample and mixed. Afterwards, the destruction vessel was closed and allowed to stand overnight at room temperature (20 ± 1^oC). After that, the vessel was put into a microwave to allow the first digestion step to take place. After the first digestion step was completed, the sample was taken out and cooled down. About 1 mL of H_2O_2 was added to the sample and the second digestion step in the microwave took place. At the end, the sample was transferred into a 50 mL grainer tube and the vessel was washed twice with ultrapure water. The volume of the sample was made up to 50 mL by adding ultrapure water. Selenium concentration in the sample was measured.

We expect that the plant samples digested using HNO_3 alone may give similar Se concentrations as those digested using a mixture of HNO_3 and H_2O_2. The study of Bańuelos and Akohoue (1994) showed that Se concentrations in the reference samples of wheat flour and mustard leaf tissue digested using HNO_3 and H_2O_2 using a microwave is only slightly higher than those digested using HNO_3 alone (i.e. 7% difference for wheat flour sample and 14% difference for mustard leaf tissue sample, respectively). Wu *et al.* (1997) suggested that addition of H_2O_2 in a microwave plant digestion was aimed to maintain a higher temperature under a pressure limit and to reduce organic C content in the digestates. However, no profound improvement in accuracy of elements analysed was found in their results. Addition of H_2O_2 was also aimed to reduce the toxic nitrous oxide (N_2O) gas produced after digestion of plant samples using HNO_3 (Gerlinde Vink, *Personal communication*).

6.2.5 Chemical analysis

Concentrations of Se in 0.01 M $CaCl_2$ soil extracts and plant digests were measured using High Resolution ICP-MS (Thermo Scientific, Element2). pH was measured using a pH meter (Radiometer Analytical PHM210) with a glass electrode (Beckman 511084).

6.3 Results and discussion

6.3.1 Selenium content in grass without Se fertilization

Selenium content in grass from fields with mineral N plus cattle slurry applications (2007 experiment) ranges from 13 up to 139 µg kg^{-1}, with an average of 44 µg kg^{-1}; and from fields with NPK application (2012 experiment) ranges from 16 up to 107 µg kg^{-1}, with an average of 35 µg kg^{-1} (Fig. A.1). The range of Se content in grass in this study is comparable to that in grass samples (without Se fertilization) from Sweden, i.e. 4.5 to 186 µg kg^{-1}, with an average of 25 µg kg^{-1} (n = 27), and from Germany, i.e. 11 to 123 µg kg^{-1} (n = 304) (Hartfiel and Bahners, 1988; Johnsson, 1992). The grassland soils from Sweden and Germany also contain low total Se, i.e. 0.12 to 1.93 mg kg^{-1}, with an average of 0.59 mg kg^{-1} (n = 27) and 0.035 to 0.65 mg kg^{-1} (n = 304), respectively (Hartfiel and Bahners, 1988; Johnsson, 1992). These results show that, without Se fertilization, Se content in grass grown on low Se soils is mostly below the adequate amount of Se for dairy cattle intake, i.e. 150 to 200 µg Se kg^{-1} dry weight of grass (Gierus *et al.*, 2002). Both in the fields with N plus slurry applications (2007) and fields with NPK application (2012), Se content in grass grown on clay soils with clay content ≥ 20% and pH > 6 is in general higher than that in grass grown on other soil types (Fig. 1A and 2A). Although the total Se (aqua regia) and soluble Se in 0.01 M $CaCl_2$ extraction of organic rich soils (clay-peat and peat soils) are the highest among all the soil types (Table 1), the Se content in grass grown on these soils is in general lower than that in grass grown on clay soils with clay content ≥ 20% and pH > 6 (Fig. 1A and 2A), indicating that the Se bioavailability in organic rich soils is relatively low. Without Se fertilization, the Se content in grass is largely comparable for grass grown on the same soil types in the 2007 experiment (with N plus slurry; the average Se content in sandy soils is 32 ± 12 µg kg^{-1}, n = 8) and in the 2012 experiment (with NPK), suggesting no difference between N plus slurry and NPK treatment in terms of Se uptake.

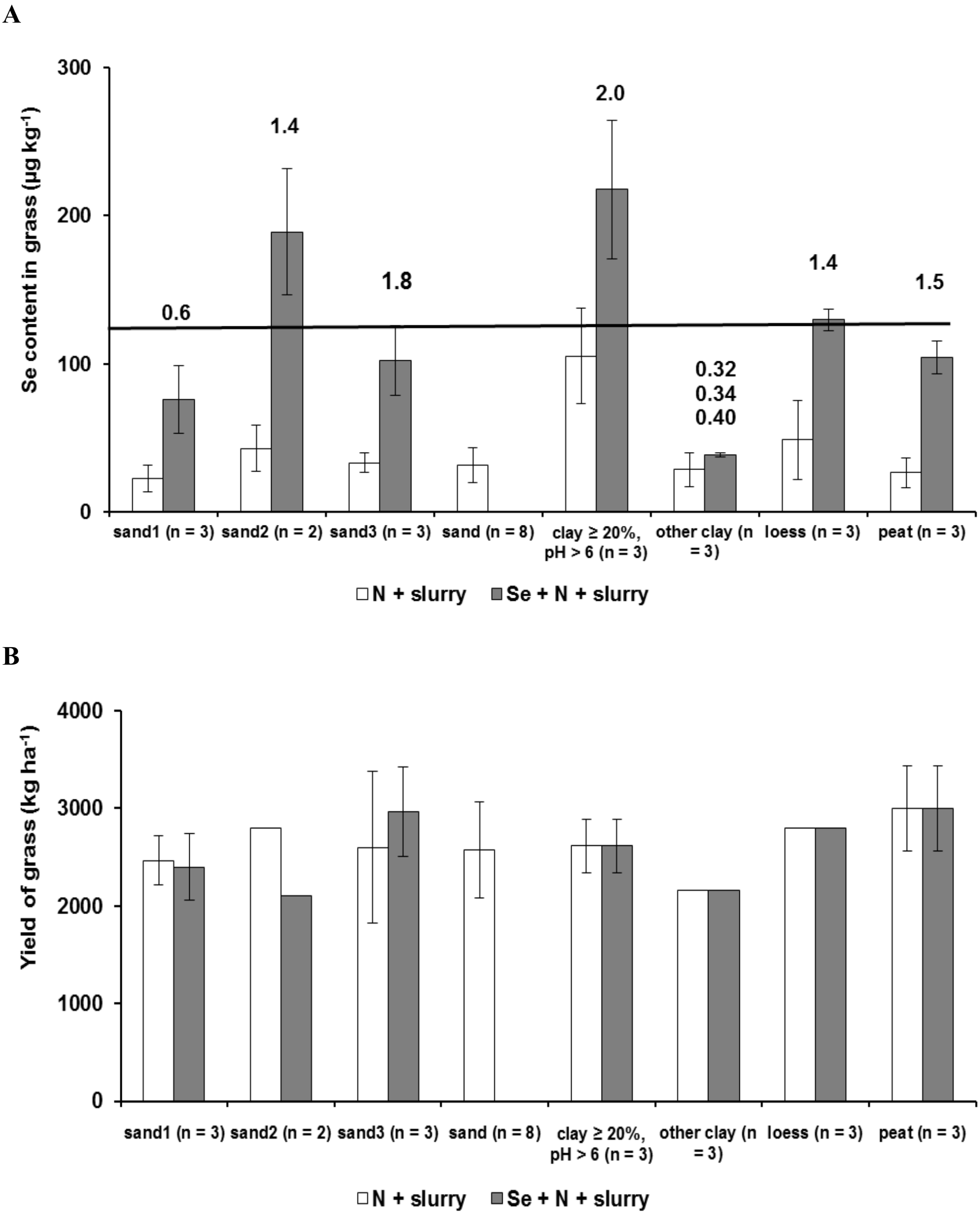
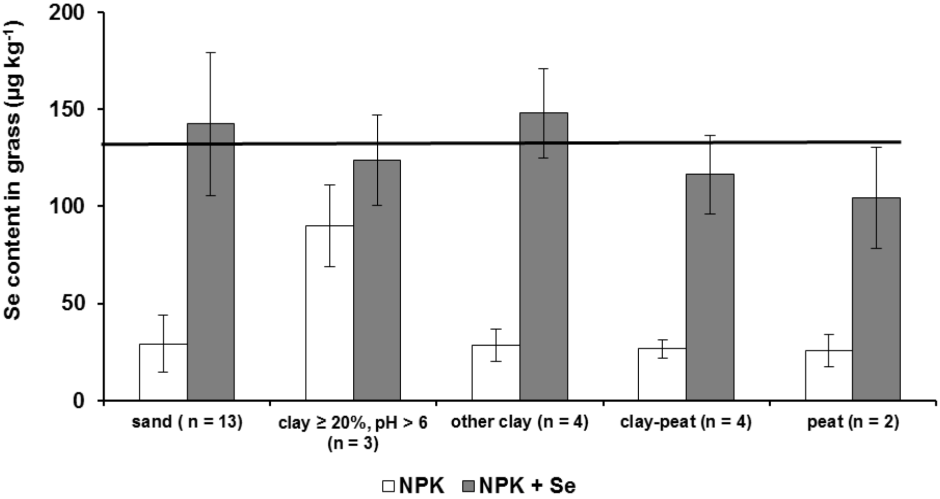


Fig. 1. Selenium content (A) and yield (B) of grass obtained from plots with N + cattle slurry applications and from plots with Se + N + cattle slurry applications obtained from the 2007 field experiment (n = 20). The data are presented as means and standard deviations per soil type. The line indicates the average Se content in

grass grown on soils applied with Se + N + cattle slurry (i.e. 119 µg kg^{-1}; all samples). Different amounts of Se (the values above the white bars in the unit of g ha^{-1}), mineral N and cattle slurry were applied on different fields (Table 2). The sandy soils were further divided into three groups according to the differences in Se dosages applied to the soils.

A



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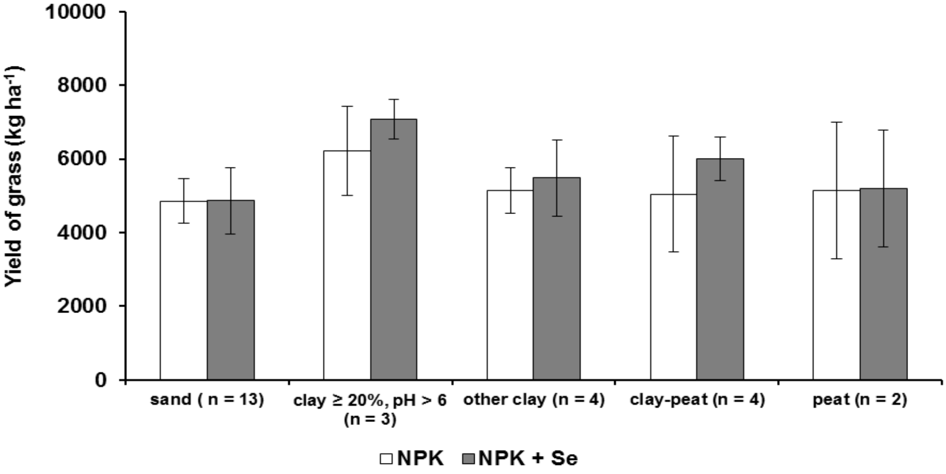


Fig. 2. Selenium content (A) and yield (B) of grass obtained from plots with NPK application and from plots with NPK + Se applications obtained from the 2012 field experiment (n = 26). The data are presented as means and standard deviations per soil type. The line indicates the average Se content in grass grown on soils applied with NPK + Se (i.e. 134 µg kg^{-1}; all samples). Similar amounts of Se, mineral N, P, and K were applied on different soil types (Section 6.2.1.2).

The yield of grass is 2100 to 3500 kg dry weight ha^{-1} and 2900 to 7200 kg dry weight ha^{-1} obtained from fields with N plus slurry applications (2007 experiment) and from fields with NPK application (2012 experiment), respectively (Fig. A.1). In general, no significant difference in the yield of grass grown on different soil types was found (Fig. 1B and 2B). No correlations between yield and Se content of grass were found both in fields with N plus slurry applications (2007) ($R^2 = 0.004$, $p > 0.05$) and fields with NPK application (2012) ($R^2 = 0.10$, $p > 0.05$) (Fig. A.1). The variation in yield of grass is smaller (2 to 3 times; Fig. A.1) compared to the variation in Se content in grass (7 to 11 times; Fig. A.1), and the Se content in grass (in µg Se kg^{-1} dry biomass) correlates well with the total Se uptake per hectare (Se content x yield; in g Se ha^{-1}) ($R^2 = 0.97$, $p < 0.001$ for 2007 experiment; and $R^2 = 0.90$, $p < 0.001$ for 2012 experiment) (Fig. A.2). Although the yield of grass with NPK application in 2012 is obviously higher than that with N plus slurry application in 2007 (Fig. 1B and 2B), no profound differences between the average Se content in grass grown on the corresponding soil types in both experiments were found (Fig. 1A (the average Se content in sandy soils is 32 ± 12 µg kg^{-1}, n = 8) and 2A), showing that the yield effect on Se plant uptake is small.

6.3.2 Effects of Se fertilization on Se uptake in grass

The effect of Se fertilization on Se content in grass grown on different soils applied with N plus cattle slurry (2007 experiment) or NPK (2012 experiment) was studied by firstly comparing the Se content in grass grown on soils with and without Se application. In general, Se application led to a significant increase of Se content in grass (Fig. 1A and 2A). Selenium content in grass upon Se fertilization ranges from

38 up to 266 µg kg^{-1}, with an average of 119 µg kg^{-1} in the fields with N plus slurry applications (0.32 to 2.0 g Se ha^{-1}, 2007), and from 86 up to 213 µg kg^{-1}, with an average of 134 µg kg^{-1} in the fields with NPK application (4.4 g Se ha^{-1}, 2012) (Fig. 1A and 2A). However, there was only a small increase of Se content in grass grown on "other clay" soils from fields with N plus slurry applications and on clay soils with clay content ≥ 20% and pH > 6 from fields with NPK application (Fig. 1A and 2A). In the fields with N plus slurry applications (2007), Se application on "other clay" soils did not increase significantly Se content in grass (Fig. 1A), probably due to the low amount of Se applied to these soils, i.e. 0.32 to 0.40 g Se ha^{-1} (Table 2). For the soils with clay content ≥ 20% and pH > 6 from fields with NPK application (2012), the increase of Se content in grass is relatively small, probably due to the increase of selenate adsorption on clay surfaces in the absence of organic acids from cattle slurry (without slurry application). Therefore, less amount of Se is taken up by grass with NPK application than that with N plus slurry application in this type of soil (Fig. 1A and 2A).

In the 2007 experiment with N plus slurry application, the amount of Se applied was varied (Table 2), therefore, the effect of Se application on Se content in grass grown on different soil types cannot be compared directly for this fertilization regime. However, the results show that at this relatively low Se dosage (0.32 to 2.0 g Se ha^{-1}), the Se content in grass is more or less proportional to the Se dose. In the 2012 experiment with NPK application, the average Se content in grass grown on different soil types applied with a relatively high dosage of 4.4 g Se ha^{-1} is not significantly different (Fig. 2A). In general, Se application hardly increased the yield of grass (Fig. 2B), indicating that Se is not a limiting factor for plant growth. This finding is in line with the study of Gupta *et al.* (2008) that suggested that optimum crop yields can still be obtained on soils with very low available Se.

To better compare the effectiveness of Se fertilization in increasing Se content in grass grown on different soil types in the two field experiments, the unit efficiency of Se fertilization was calculated as the increase of Se content in grass per unit

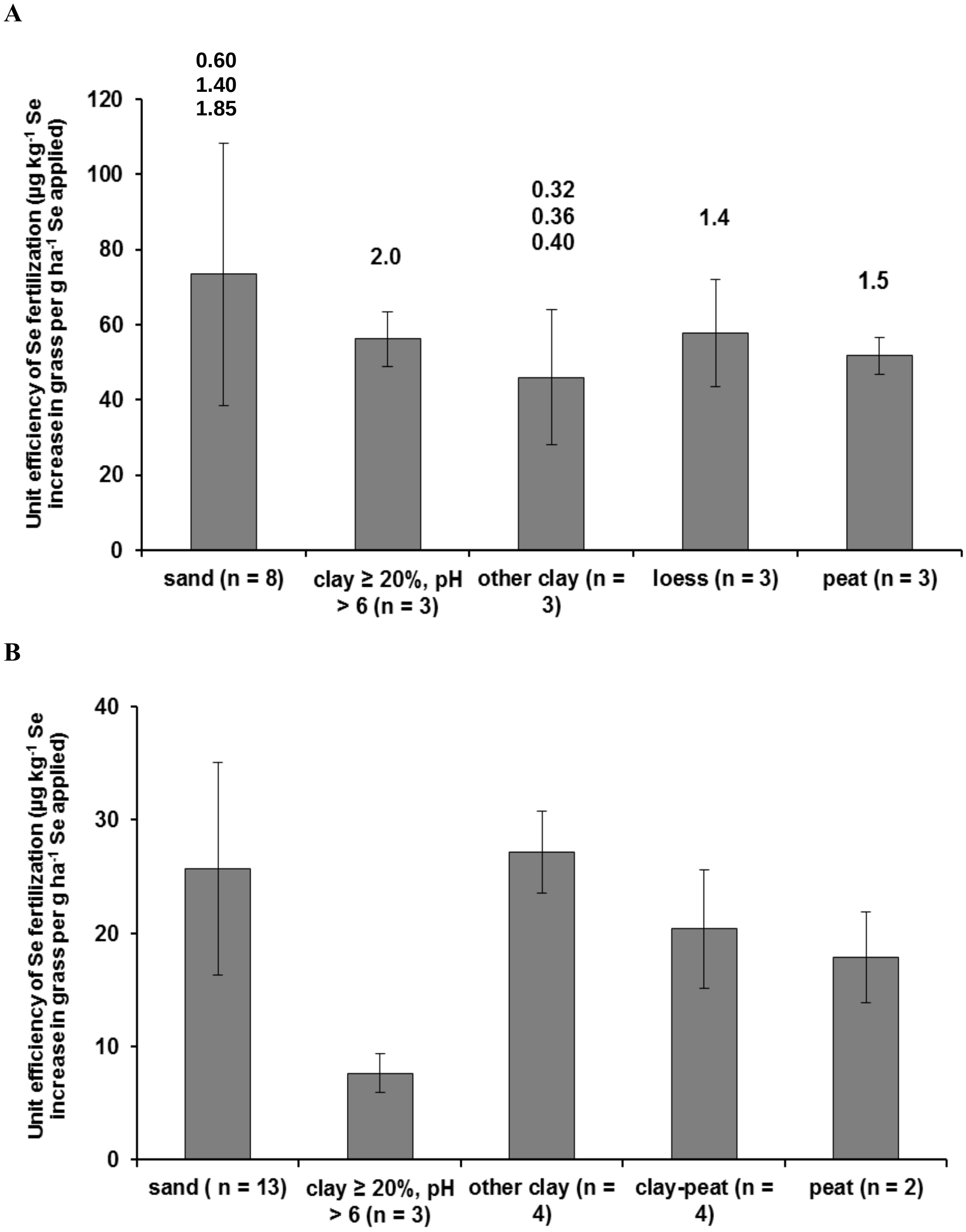


Fig. 3. Unit efficiency of Se fertilization in fields with N plus cattle slurry applications (2007 experiment) (A) and fields with NPK application (2012 experiment) (B). The data are presented as means and standard deviations per soil

type. Different amounts of Se (0.32 to 2.0 g Se ha^{-1}, the values above the grey bars) were applied on the fields with N plus cattle slurry applications (2007 experiment), whereas similar amounts of Se (4.4 g Se ha^{-1}) were applied on the fields with NPK application (2012 experiment).

amount of Se fertilizer applied. In general, the unit efficiency of Se fertilization on the fields with N plus slurry applications (2007) are higher than those on the fields with NPK application (2012) (Fig. 3A and B), which may be attributed to lower grass yield of the 2007 experiment than the 2012 experiment (Fig. 1B and 2B), non-linearity of Se fertilization efficiency (possibly higher efficiency at lower dosage in the 2007 experiment), or difference in fertilization (N plus slurry versus NPK). However, the first hypothesis is in contrast with our finding that the effect of yield on Se uptake in grass without Se application is small (see Section 6.3.1).

In both experiments, the unit efficiencies of Se fertilization are not statistically different among the soil types, except for the significantly lower unit efficiency for clay soils with clay content ≥ 20% and pH > 6 of fields with NPK application in 2012 (Fig. 3A and B). This finding confirms the results of a meta-analysis of Se fertilizations, showing that organic matter content, acidity and total Se content of the soils only had a minor influence on the effectiveness of Se fertilization (Ros *et al.*, 2016). Nevertheless, in sandy soils, the unit efficiency of Se fertilization tends to have a larger range and a higher mean than that in the clay soils and organic rich soils in both experiments (Fig. 3A and B), which may be due to Se adsorption to clay surfaces in clay soils and Se immobilization by soil organic matter via microbial processes in organic rich soils as discussed in Chapter 5.

In the fields with NPK application (2012), the unit efficiency of Se fertilization on clay soils with clay content ≥ 20% and pH > 6 is much lower than that for the other soil types (Fig. 3B), which is not the case in the fields with N plus slurry applications (2007) (Fig. 3A). This difference is probably explained by the decreased binding of selenate on clay surfaces of clay soils with clay content ≥ 20%

and pH > 6 in the presence of low molecular weight organic acids from cattle slurry (Øgaard *et al.*, 2006) of the fields with N plus slurry applications, resulting in more selenate uptake by grass in the fields with N plus slurry applications (2007) than that in the fields with NPK application (2012) (Fig. 1A and 2A). The study of Øgaard *et al.* (2006) has shown that application of selenate and cattle slurry together on peat and loam soils increased Se content in wheat grain, especially at soil pH > 6 (pH 6.1 and 6.8 for the loam soil and peat soil, respectively), in contrast to that no effect was found at lower pH soils (pH 5.4 and 6.0 for the loam soil and peat soil, respectively). In the fields with NPK application (2012), the lower Se fertilization efficiency on clay soils with clay content ≥ 20% and pH > 6 than for other soil types (Fig. 3B) can be due to a higher amount of selenate adsorbed on clay surfaces in the absence of organic acids from cattle slurry (without slurry application), therefore limits selenate availability for plant uptake. Previous studies showed that Se uptake in plants grown on low-organic matter soils applied with selenite or selenate fertilizer decreased with an increase of clay content both at low pH and high pH soils (Bisbjerg and Gissel-Nielsen, 1969; Gissel-Nielsen, 1971; Johnsson, 1991), similar to our finding that the Se fertilization efficiency was higher in low clay content soils (i.e. sandy soils and “other clay” soils) than that in clay soils with clay content ≥ 20% and pH > 6 in the fields with NPK application (2012) (Fig. 3B).

In general, the recovery of Se fertilizer, calculated as percentage of the additional amount of Se in grass upon Se fertilization relative to amount of Se applied, was very low, i.e. 7% to 22% on the fields with N plus slurry applications (2007 experiment) and 3 to 19% on the fields with NPK application (2012 experiment) (data not shown), although the Se content in grass in both fields increased significantly upon Se fertilization (Fig. 1A and 2A). These findings indicate that most of selenate fertilizer applied was probably lost from the soils through leaching and volatilization or reduced into selenite or other Se species and subsequently adsorbed or incorporated into the soils. A similar range of use efficiency of selenate fertilizer applied to Finnish soils was observed, i.e. 5 to 20% (Yläranta, 1985 *in* Keskinen *et al.* 2011), in which most of the residual selenate fertilizer was recovered as adsorbed, organically associated, and recalcitrant pools both in the mineral soils (pH 4.0–5.8) and organic soils (pH 4.1–5.3) with low pH range (Keskinen *et al.*, 2011).

6.4 Conclusion

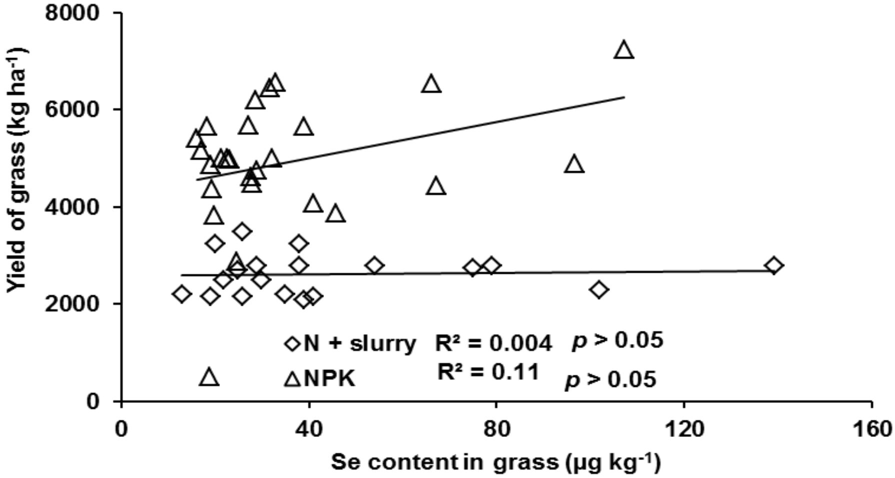
Selenium content in grass from fields with N plus cattle slurry applications ranges from 13 to 139 µg kg^{-1} and from fields with NPK application ranges from 16 up to 107 µg kg^{-1}, which are all below the adequate amount of Se for dairy cattle intake, i.e. 150 to 200 µg Se kg^{-1} dry weight of grass. In both experiments, Se content in grass grown on clay soils with clay content ≥ 20% and pH > 6 is in general higher than that in grass grown on other soil types, when no Se was fertilized. The Se content in grass is comparable in the same soil types in the experiment with N plus slurry and in the experiment with NPK, indicating no difference between N plus slurry and NPK fertilization in terms of Se plant uptake.

Application of Se in the form of selenate (0.32 to 2.0 g Se ha^{-1} to the fields with N plus slurry applications and 4.4 g Se ha^{-1} to the fields with NPK application) in general significantly increased Se content in grass, which ranges from 38 up to 266 µg kg^{-1} in the fields with N plus slurry applications, and from 86 up to 213 µg kg^{-1} in the fields with NPK application. However, the majority of Se content in grass remained below the recommended amount for cattle intake. At the low dosage of Se applied (0.32 to 2.0 g Se ha^{-1}), Se content in grass is to a large extent proportional to the Se dose. At the same relatively high Se dosage (4.4 g Se ha^{-1}), Se content in grass is in general comparable for different soil types.

The effectiveness of Se fertilization on different soil types, which is indicated by the increase of Se in grass per unit of Se applied, is in general comparable for different soil types in both experiments, except for the significantly lower effectiveness for clay soils with clay content ≥ 20% and pH > 6 of fields with NPK application. These findings suggest a minor influence of the soil properties on the effectiveness of selenate fertilization. Nevertheless, the Se fertilization efficiency on sandy soils tends to have a larger range and higher mean than that on clay soils and organic rich soils in both experiments, probably partly due to Se adsorption on clay surfaces in clay soils, and Se immobilization by soil organic matter via microbial processes in organic rich soils, which decrease Se availability for plant uptake. Moreover, the recovery of Se fertilizer in grass is very low, i.e. 3 to 22% of the total Se applied for one season, indicating that most of selenate applied was probably leached out from the soils or volatilized or adsorbed and incorporated into the soils.

Appendix

1



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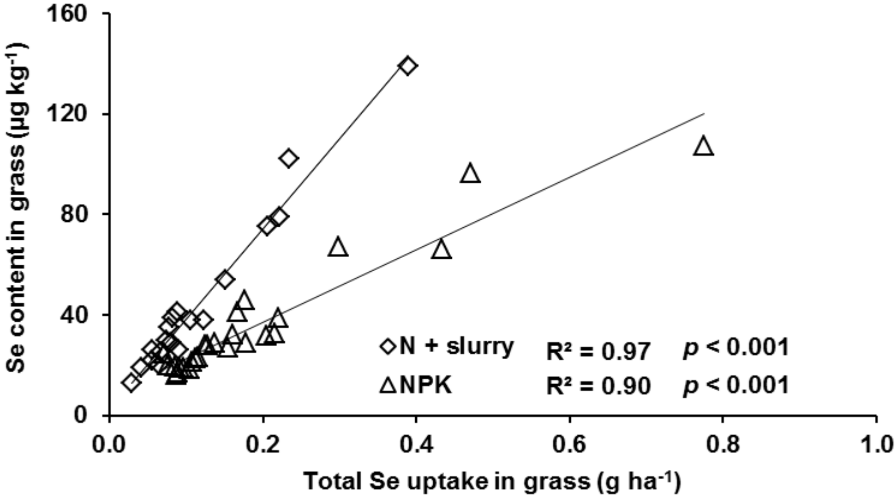

Fig. A.1. Correlation between (1) yield and Se content in grass and (2) total Se uptake and Se content in grass obtained from fields with N plus cattle slurry applications and fields with NPK application.

Chapter 7

General Discussion

7.1 Introduction

Selenium (Se) content and bioavailability in soils are the main factors determining Se level in the food chain (plant – animal – human). In contrast to studies on Se fertilization, research on the relationship between native Se in soil and Se plant uptake, with either field experiments or pot experiments, has not been performed intensively. Understanding the mechanisms and factors influencing Se bioavailability in soil for plant uptake is important for designing appropriate agricultural management practices that can maintain and/or improve Se bioavailability in soils and avoid environmental pollution.

In this thesis, comprehensive research on Se content, speciation, bioavailability and fertilization for plant uptake in agricultural soils in the Netherlands was performed. In Chapter 2, the amount and speciation of soluble Se in 0.01 M $CaCl_2$ extraction of moist and dried soil samples were studied, which has given insight into the effects of soil sample pre-treatment (i.e. drying and rewetting) on the solubility and speciation of Se in soil solution. The concentration and speciation of Se in 0.01 M $CaCl_2$ extraction were further used in this thesis as indicators to predict Se uptake in plants. In Chapter 3, the amount, extractability and speciation of Se in soils were studied using a number of single step soil extractions. From this study, a better understanding regarding the level, distribution and speciation of Se in different Dutch agricultural soils was achieved. In Chapter 4, correlations between soil parameters including Se in different extractions and soil properties, and Se plant uptake were studied with pot experiment, using soils from potato fields and wheat as the testing plant. In these correlations, the best indicator of Se plant uptake was identified and the influences of soil properties on Se uptake were discussed. In Chapter 5, a correlation study similar to that in Chapter 4 was conducted, but the experiments were carried out on grass grown under field conditions instead of in a greenhouse pot experiment. The best soil indicator of Se plant uptake and the relationships between soil properties and Se bioavailability were investigated. In addition, the effect of NPK application on Se plant uptake was discussed. In Chapter 6, a Se (as selenate) fertilization study was performed on grassland fields with different soil properties applied with N plus cattle slurry or with NPK, and efficiency of Se fertilization in increasing the Se content in grass was investigated. In the current chapter (Chapter 7), the main findings from all previous chapters will be discussed in a general context, and the implications of the findings for utilization and future research will be presented.

7.2 The role of soil organic matter in determining the amount, solubility and speciation of Se in soils

Chapter 2 and 3 of this thesis have shown that soil organic matter plays a major role in determining the amount, solubility and speciation of Se in agricultural soils (both grassland and arable land) in the Netherlands. A 0.01 M $CaCl_2$ extraction (soil to solution ratio 1:10), which has been proposed to estimate total soluble elements in soil solution and to evaluate bioavailability of the elements in soils (Houba *et al.*, 2000), was used to determine solubility of Se in the soils. An increase of Se solubility in 0.01 M $CaCl_2$ soil extraction upon soil drying was observed and associated with the increase of DOC, although the increase of Se was more pronounced than that of DOC (Chapter 2). The increase of Se-containing organic matter either from soil microorganism or plant origin is likely responsible for the increase of soluble Se and DOM upon soil drying. Other findings, which indicate that Se solubility is associated with the solubility of soil organic matter, are that Se in 0.01 M $CaCl_2$ extraction from both moist soils and dried soils is mainly present as colloidal sized organic Se, and that the distribution of Se between the hydrophilic acid and fulvic acid fraction of DOM in 0.01 M $CaCl_2$ extraction of dried soils corresponds to the distribution of DOC between these fractions.

Further study on Se speciation and extractability in soils also showed that Se in soils is mainly associated with soil organic matter (Chapter 3). The majority (on average 82%) of total Se in soils is present as organic Se oxidizable with NaOCl and only small fractions are present as inorganic Se (5%, mainly as selenite) and residual Se (13%). Moreover, the extractability of Se in different reagents employed in this study follows in general the extractability of soil organic C, which supports the conclusion that Se in soil is predominantly present in organic form. Among soil organic matter in different extractions, the dissolved (0.01 M $CaCl_2$), labile (hot water) and humic substances (0.1 M NaOH) organic matter are richer in Se (on average 31 ± 14; 23 ± 13; 19 ± 8 µg Se g^{-1} C, respectively) than soil organic matter in other extractions, i.e. acid-soluble (0.43 M HNO_3), metal oxide associated (ammonium oxalate), and total organic matter (NaOCl and aqua regia) (10 ± 3; 14 ± 7; 9 ± 4; 12 ± 6 µg Se g^{-1} C, respectively). In addition, total Se content is positively related to total soil organic matter content. All of these findings support the conclusion that Se and soil organic matter are associated both in the soil solution and solid phase.

A low Se level in the soil parent materials and a relatively high soil organic matter content in Dutch agricultural soils (i.e. 2 to 54% in grassland soils, 4 to 41% in maize field soils, and 1.4 to 15% in potato field soils of samples studied in this thesis) are likely responsible for the predominance of organic forms of Se (Chapter 3 and 4). The predominance of organic Se species in soils was also found in the studies of Yamada *et al.* (2009) and Kang *et al.* (1993) for Japanese soils, in which the soil organic matter content is 0.36 to 13% and 3 to 30%, respectively, and the total soil Se content (0.05 to 2.80 mg kg^{-1} and 0.17 to 1.23 mg kg^{-1}, respectively) is similar to that in this study (0.12 to 1.97 mg kg^{-1} in grassland soils, 0.20 to 1.20 mg kg^{-1} in maize field soils, and 0.22 to 0.73 mg kg^{-1} in potato field soils; Table 1). Keskinen *et al.* (2009) also indicated the predominance of organic Se in organic rich Finnish soils, although the exact fraction of organic Se has not been studied in these soils so far. Moreover, a positive relation between soil organic matter content and total soil Se content was observed for low Se soils from Japan, Sweden, Scotland, Spain (Mediterranean), and France with total Se content of 0.05 to 2.80 mg kg^{-1} and soil organic matter content of 0.12 to 36% (Johnsson, 1992; Yamada *et al.*, 2009; Fordyce *et al.*, 2010; Roca-Perez *et al.*, 2010; Tolu *et al.*, 2014), and for soils with relatively high Se from China (soil organic matter content of 0.07 to 2.4%, with an average of 0.7% and total soil Se of 0.31 to 7.65 mg kg^{-1}, with an average of 1.50

Table 1. Amounts of Se and organic C and ratios of Se to organic C in different extractions of soil samples from different land uses.

Extraction	Se extracted (µg kg^{-1})			Organic C extracted (g kg^{-1})			Ratio Se:C (µg g^{-1})
	Min.	Max.	Mean ± SD[a]	Min.	Max.	Mean ± SD[a]	Mean ± SD[a]
Grassland (n = 27)							
0.01 M $CaCl_2$	2.1	40.2	8.9 ± 9.5	0.1	0.9	0.3 ± 0.3	34 ± 13
0.43 M HNO_3	6.9[b]	73.3[b]	22.7 ± 17.7[b]	0.6	8.6	2.4 ± 2.1	10 ± 3[b]
Hot water	13.4[c]	84.8[c]	32.2 ± 19.9[c]	0.3[c]	4.5[c]	1.7 ± 1.2[c]	23 ± 13[c]
Ammonium oxalate	18.3	267.2	77.5 ± 72.9	1.0	28.6	6.3 ± 7.3	14 ± 7
Aqua regia	117.6	1968.0	570.7 ± 555.9	11.5[d]	272.0[d]	50.6 ± 51.9[d]	10 ± 4
Maize field (n = 15)							
0.01 M $CaCl_2$	2.6	11.3	6.4 ± 2.9	0.1	0.5	0.3 ± 0.1	30 ± 16
0.43 M HNO_3	10.9	53.5	27.4 ± 14.6	0.8	5.5	2.7 ± 1.6	11 ± 4
Hot water	n.m.[e]	n.m.[e]	n.m.[e]	n.m.[e]	n.m.[e]	n.m.[e]	n.m.[e]
Ammonium oxalate	30.0[b]	179.9[b]	94.0 ± 46.0[b]	0.9	25.1	9.4 ± 7.3	14 ± 10[b]
Aqua regia	200.9	1195.3	651.1 ± 382.7	19.6[d]	206.6[d]	78.0 ± 60.8[d]	10 ± 4
Potato field (n = 21)							
0.01 M $CaCl_2$	1.2	6.0	3.3 ± 1.3	0.04	0.18	0.07 ± 0.03	55 ± 34
0.43 M HNO_3	7.0	32.0	14.4 ± 7.3	0.3	1.7	0.9 ± 0.5	19 ± 8
Hot water	6.4	29.5	18.8 ± 5.3	0.3	2.2	0.7 ± 0.5	36 ± 23
Ammonium oxalate	38.0	146.0	78.6 ± 34.4	0.5	9.9	3.7 ± 2.7	31 ± 25[b]
Aqua regia	223.6[b]	733.6[b]	398.5 ± 159.5[b]	7.0[d]	72.5[d]	26.8 ± 20.8[d]	19 ± 9[b]

[a] Standard deviation.

[b] Soil samples that contain Se concentrations below the determination limit were excluded from the table.

[c] Six soil samples were not measured.

[d] The total organic C was calculated from the measured soil organic matter content with an assumption that soil organic matter contains 50% organic C.

[e] Not measured.

mg kg^{-1}) (Xing *et al.*, 2015). The studies of Qu *et al.* (1997) and Zhang *et al.* (2002) (*in* Chen *et al.* (2010)) reported that Se in low Se soils in China is mainly present as Se associated with soil organic matter. Moreover, several studies have demonstrated that in low Se environments organic matter is the dominant factor controlling soil Se content and availability (Johnson *et al.*, 2000; Fordyce *et al.*, 2010).

In addition to soil organic matter content, the properties of soil organic matter, which are influenced by land uses (grassland, maize field, and potato field), determine to a great extent the richness of Se in different soil organic matter pools extracted using different reagents employed in this study (Table 1). A compilation of data extracted from previous chapters (Chapter 2, 3 and 4) is presented in Table 1, representing Se extractability in different soil extractions and Se richness in different organic matter pools of soils from different land uses. The amounts of Se in all the extractions of (dried) soils from grassland and maize field are in general higher than those in potato field soils, which is in agreement with a higher extractability of organic C in the grassland and maize field soils than in the potato field soils. Inclusion of organic rich soils (i.e. clay-peat and peat soils) from grassland and maize field (Chapter 2 and 3) has led to a higher amount of average extractable organic C and Se concentration than those in potato field soils that consist of non-organic rich soils (i.e. sand, clay, loess, and reclaimed peat soils) (Chapter 4). However, the organic matter in grassland soils and maize field soils contain in general lower Se (lower Se to organic C ratio in all extractions) than that in potato field soils (Table 1), even after excluding organic rich soils from grassland samples (ratios Se to organic C (µg g^{-1}): 35 ± 14 ($CaCl_2$), 10 ± 3 (HNO_3), 24 ± 15 (hot water), 13 ± 6 (ammonium oxalate), 10 ± 4 (aqua regia)) and maize field samples (36 ± 17 ($CaCl_2$), 13 ± 4 (HNO_3), 18 ± 12 (ammonium oxalate), 11 ± 4 (aqua regia)). Upon soil drying, an increase of Se to DOC ratio in $CaCl_2$ extraction of grassland soils and maize field soils was observed, in contrast with a constant Se to DOC ratio in $CaCl_2$ extraction of potato field soils (Chapter 2), suggesting that the richness of Se in soil organic matter from different land uses reflects the nature properties of Se-containing soil organic matter. Nevertheless, the properties of soil organic matter from different land uses hardly affect the Se speciation in soils. No profound differences in Se speciation in soils from different land uses were observed (Chapter 3 and 4).

7.3 Selenium-rich dissolved and labile organic matter are related to Se bioavailability in soils

In Chapter 3, the Se-rich soil organic matter pools, including dissolved (extracted in 0.01 M $CaCl_2$), labile (hot water) and humic substances (especially the hydrophobic organic neutral fraction, 0.1 M NaOH) organic matter, have been identified. These pools may play an important role in supplying Se to plants in low Se soils with predominantly organic Se. However, specific organic Se compounds present in those pools were not identified in this study. A possible schematic pathway for Se-rich organic matter in soil solution and solid phase to supply Se for plant uptake was presented in Chapter 4 and Figure 1 in this chapter. The labile organic matter may represent the Se-rich solid organic matter, which releases the Se-rich DOM into soil solution via mineralization and/or dissolution.

The concentration of Se-rich DOM, which can be represented by the Se to DOC ratio in 0.01 M $CaCl_2$ extraction, is a good indicator of Se plant uptake in the potato field soils, under controlled conditions (pot experiment) (Chapter 4). On the other hand, the labile organic Se extracted in hot water is the best soil indicator found to predict Se plant uptake in the grassland soils under field conditions, although the prediction power is < 50% (Chapter 5). These different indicators identified for Se plant uptake between potato field soils and grassland soils can be caused by difference in the range of soil organic matter quality (i.e. soil C:N ratio). The variation of soil C:N ratio is larger for the potato field soils (8 to 23) than for the grassland soils (9 to 18) (Chapter 4 and 5). The large variation in soil organic matter quality (soil C:N ratio) of the potato field soils corresponds to a large variation in their Se content in organic matter (Se to organic C ratio), and there is a negative correlation between soil C:N ratio and Se to organic C ratio in different soil extractions (R^2 = 0.51 to 0.79, $p < 0.001$), in contrast to a lack of such a correlation in grassland soils (R^2 = 0.04 to 0.16, $p > 0.05$) (Table 2). In the potato field soils, soil C:N ratio is negatively correlated to Se plant uptake (R^2 = 0.41, $p < 0.01$; Chapter 4), in contrast to a lack of such a correlation for the grassland soils (R^2 = 0.05, $p > 0.05$; Chapter 5). Therefore, Se to DOC ratio in the 0.01 M $CaCl_2$ extraction was found to be the best indicator of Se plant uptake from the potato field soils, which is not the case for grassland soils. In grassland soils, labile organic Se measured in hot water extraction was found as the best indicator for Se uptake. These findings suggest that in the soils with Se-rich organic matter (i.e. potato field soils, Table 1) soil organic matter quality (soil C:N ratio) and the content of Se-rich DOM control Se plant uptake, whereas in the soils with Se-poor organic matter (i.e. grassland soils, Table 1) labile organic Se controls to a certain extent Se plant uptake. In conclusion, the amount and/or properties of Se-containing soil organic matter determine Se bioavailability and plant uptake in soils with a low total Se level with predominantly organic Se.

Table 2. Correlation between soil C:N ratio and Se to organic C ratio in different soil extractions (R^2) measured in grassland soils taken from 2011 field experiment (without NPK application) (Chapter 5) and potato field soil samples used in the pot experiment (Chapter 4).

Ratio Se:organic C in soil extraction	Soil C:N ratio grassland		Soil C:N ratio potato field	
	R^2	*p* value	R^2	*p* value
$CaCl_2$	0.11	> 0.05	0.51	< 0.001
HNO_3	0.10	> 0.05	0.52	< 0.001
Hot water	0.10	> 0.05	0.64	< 0.001
Ammonium oxalate	0.16	> 0.05	0.79	< 0.001
Aqua regia	0.04	> 0.05	0.51	< 0.001

The data of soil C:N ratio and Se:organic C ratio were transformed into Log_{10}.
The unit of Se to organic C ratio is $\mu g\ g^{-1}$.

It has been estimated that the total amount of Se taken up by plants in both pot experiment using the potato field soils and field experiment using the grassland soils is in similar range, i.e. 0.8 to 14 times and 1.3 to 11 times, respectively, of the dissolved Se in the soil pore water (Chapter 4 and 5). These amounts of Se taken up by plants require replenishment of dissolved Se in the pore water, with a turnover time of 4 to 67 days (on average 22 days) in the potato field soils and of 5 to 39 days (on average 17 days) in the grassland soils (Chapter 4 and 5). It has been shown that the turnover of free amino acids in soil is in the magnitude of hours (Jones and Kielland, 2002), whereas the turnover of up to 40% of DOM in soil solution is in the order of days to months (Kalbitz *et al.*, 2000). Our study has shown that most of soluble Se in the soils studied presents as Se associated with colloidal sized DOM (Chapter 2), which has similar order of turnover time (days to two months) estimated above as that in the study of Kalbitz *et al.* (2000). These findings suggest that the turnover of soluble Se in the potato field soils is mainly determined by the quality of soil organic matter (i.e. soil C:N ratio), whereas the turnover of soluble Se in the grassland soils is dependent on the buffering pool size of labile organic Se measured in hot water extraction (see above). The pool size of labile organic Se (in hot water extraction) is on average 6 and 5 times of that of soluble Se (in 0.01 M $CaCl_2$ extraction) in the potato field soils and the grassland soils, respectively (Table 1). All of these findings indicate that in soils with a low total Se level, which is dominated by organic Se, the solubility and lability of Se-containing soil organic matter determine Se bioavailability and plant uptake (Chapter 4 and Fig. 1 in this chapter), although different organic Se pools or species are the limiting factors for soils from different land uses.

Further research is needed to confirm the generality of the conclusion above, because firstly the two experiments were carried out under different conditions (pot experiment and field experiment), using different plant species (wheat and grass) and covering different soil types (sand, clay, loess, and reclaimed peat soils versus sand, clay, clay-peat, and peat soils). Secondly, the Se-rich DOM and Se-rich labile organic matter are strongly correlated as shown by the correlations between soluble Se in 0.01 M $CaCl_2$ extraction and labile organic Se in hot water extraction in both potato field soils and grassland soils (Table 3). Both the soluble Se and labile organic Se show a positive correlation to Se plant uptake in the potato field soils and grassland soils (Chapter 4 and 5).

Table 3. Correlation between Se extracted (μg kg^{-1}) in different soil extractions (R^2).

	HNO_3	Hot water	Ammonium oxalate	Aqua regia
Grassland (n = 27)				
$CaCl_2$	0.66^{a}***	0.91^{b}***	0.80***	0.87***
HNO_3		0.38^{ab}**	0.67^{a}***	0.65^{a}*** or $0.004^{ac\ ns}$
Hot water			0.83^{b}***	0.89^{b}***
Ammonium oxalate				0.98***
Potato field (n = 21)				
$CaCl_2$	0.004^{ns}	0.52***	$0.07^{a\ ns}$	$0.002^{a\ ns}$
HNO_3		$2*10^{-5ns}$	$0.18^{a\ ns}$	$0.20^{a\ ns}$
Hot water			$0.03^{a\ ns}$	$0.01^{a\ ns}$
Ammonium oxalate				0.61^{a}*** or $0.02^{ac\ ns}$

[a] The samples that contain Se concentrations below the determination limit were excluded from the correlations (HNO_3 extraction of grassland soils, ammonium oxalate extraction and aqua regia digestion of potato field soils).

[b] Twenty one samples were included in the correlations because six soil samples were not analysed for hot water extraction.

[c] The R^2 after removing three extreme values from the correlation.

*indicates $p < 0.05$; ** indicates $p < 0.01$; *** indicates $p < 0.001$ and ^{ns} indicates not significant.

7.4 Soil properties determining potential bioavailable Se in soils

In Chapter 4 and 5 the soil properties that are correlated to Se plant uptake were discussed. In potato field soils under controlled conditions (pot experiment), soil pH and soil C:N ratio are the major soil properties correlated to Se bioavailability (Chapter 4). In contrast, in grassland soils under field conditions, the Fe-(hydr)oxide content and clay content are found the most important properties (Chapter 5). This difference can be explained by the different limiting factor of Se plant uptake in the potato field soils and in the grassland soils. As mentioned above, in the potato field soils, the content of Se-rich DOM in soil pore water determines Se plant uptake, whereas in the grassland soils, the amount of labile organic Se is limiting the Se plant uptake. The solubility of Se-rich DOM and Se plant uptake in potato field soils increase with the increase of pH and decrease of soil C:N ratio (Chapter 4), whereas the amount of labile organic Se and Se plant uptake in grassland soils increase with the increase of Fe-(hydr)oxide content and clay content (Chapter 5).

Further correlation analysis was performed to elucidate if similar soil properties influence the amount of potential bioavailable forms of Se (i.e. Se-rich DOM in 0.01 M $CaCl_2$ extraction and labile organic Se in hot water extraction) in both grassland soils and potato field soils. The content of Se-rich DOM, which is represented by the Se to DOC ratio in 0.01 M $CaCl_2$ extraction, is positively correlated to pH of potato field soils ($R^2 = 0.66$, $p < 0.001$) and grassland soils ($R^2 = 0.50$, $p < 0.001$), and negatively correlated to C:N ratio of potato field soils ($R^2 = 0.51$, $p < 0.001$), but hardly correlated to C:N ratio of grassland soils ($R^2 = 0.11$, $p > 0.05$). On the other hand, the labile organic Se in hot water extraction is positively related to clay content of grassland soils ($R^2 = 0.74$, $p < 0.001$) and potato field soils ($R^2 = 0.28$, $p < 0.05$), and Fe-(hydr)oxide content of grassland soils ($R^2 = 0.85$, $p < 0.001$), but hardly correlated to Fe-(hydr)oxide content of potato field soils ($R^2 = 0.0004$, $p > 0.05$). In summary, the soil pH and C:N ratio determine the amount of Se-rich DOM in solution of potato field soils, whereas in grassland soils the soil pH is more relevant than the soil C:N ratio (Fig. 1). In contrast, the adsorption capacity of the soil, especially clay content, determines the amount of labile organic Se in both grassland soils and potato field soils (Fig. 1). The strong correlation between labile organic Se in hot water extraction and Fe-(hydr)oxide content in grassland soils may be caused by the fact that clay content and Fe-(hydr)oxide content are well correlated ($R^2 = 0.52$, $p < 0.001$ or $R^2 = 0.87$, $p < 0.001$ if one outlier was removed), and that Fe-(hydr)oxide can adsorb natural organic matter. Labile organic matter in soils largely consists of carbohydrates and N-containing compounds, such as N-amino species and amide (Leinweber *et al.,* 1995), which mainly come from soil microbial biomass and root exudates (Sparling *et al.*, 1998) and (Feller *et al.* (1991) *in* Ghani *et al.* (2003)). Proteins, polyamino acids and polysaccharides derived from soil microbial extracellular exudates can be adsorbed on clay and iron oxide surfaces (Ding and Henrichs, 2002; Cao *et al.*, 2011), in which proteinacueous constituents are preferentially adsorbed on clay minerals than on iron oxide (Cao *et al.*, 2011). This finding is in line with our observation that clay content has a positive effect on the amount of labile organic Se in both grassland soils and potato field soils, which is probably associated with proteins and amino acids (Kang *et al.*, 1991b). In addition, the adsorption of labile (dissolved) organic matter on soil mineral surfaces is weaker than that of recalcitrant (dissolved) organic matter (Leinweber *et al.*, 1995; Kaiser and Guggenberger, 2000), supporting our finding that the labile organic Se is potentially available for plant uptake (see Section 7.3).

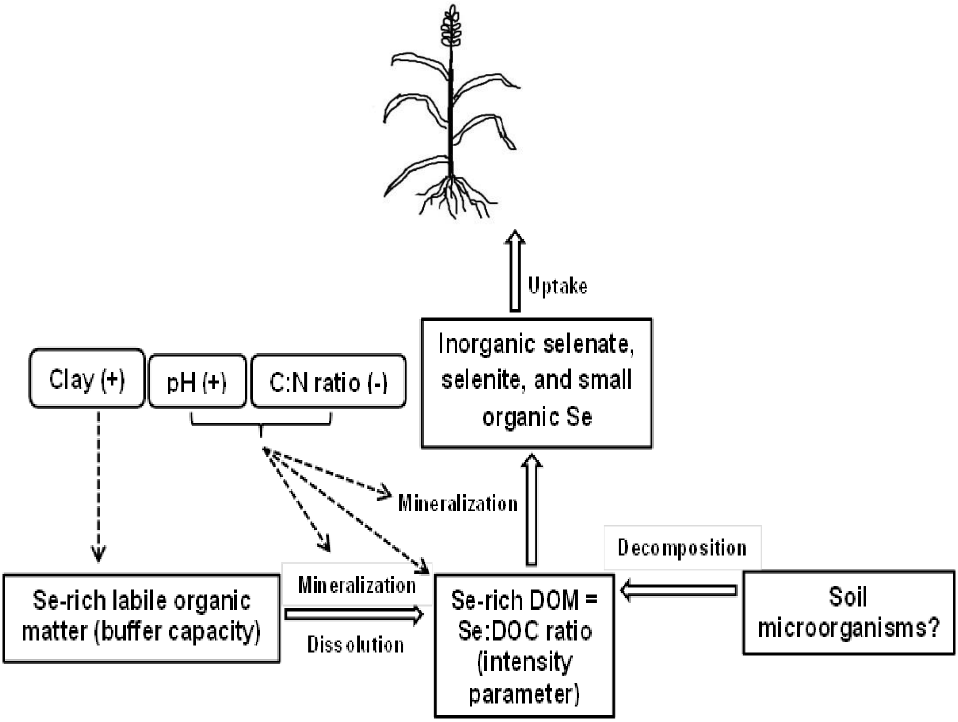

Fig. 1. Schematic pathways between Se in the soil solid phase, soil solution, and plants, and the basic soil properties influencing these processes in soils. In soils that contain low total Se with predominantly organic Se, Se-rich labile organic matter in the soil solid phase, as buffer capacity, provides Se-rich DOM in the soil solution upon mineralization/dissolution. The decay of soil microorganisms may also contribute to Se-rich DOM. The Se-rich DOM, as an intensity parameter, can either

be directly taken up by plants (in case of Se-amino acids), or after being mineralized into chemical forms that are directly available (selenate, selenite, small organic Se). Both Se-rich labile organic matter and Se-rich DOM are potential bioavailable Se in soils for plant uptake. In general, clay content has a positive effect on the amount of Se-rich labile organic matter, whereas soil pH has a positive effect (and soil C:N ratio a negative effect) on the amount of Se-rich DOM, the mineralization/dissolution of Se-rich labile organic matter into Se-rich DOM, and the mineralization of Se-rich DOM into directly available Se (selenate, selenite, small organic Se).

7.5 Other factors influencing Se plant uptake in the field

The study under field conditions (without NPK application) in Chapter 5 revealed lower predictability of Se plant uptake using soil parameters than that under controlled conditions (pot experiment, Chapter 4) (41% versus 88% of the variability of Se content in plant, respectively). We consider the following factors as potentially important in modifying Se content in grass under field conditions in addition to soil properties: (1) The location of the field that leads to differences in soil moisture content (and thus differences in redox potential) and soil temperature, which influence the mineralization and turnover rate of Se-rich organic molecules in the soils, and consequently the bioavailability of Se; (2) The location of the field that leads to differences in Se air deposition, in which high level of Se deposition is associated with industrial and coastal zones (Haygarth, 1994); (3) Differences in fertilization history of each field.

A review by Ros *et al.* (2014) suggested that organic fertilizer application (manure or slurry), phosphate fertilizer application (may contain Se), irrigation water and atmospheric deposition can be the sources of Se inputs in Dutch grassland ecosystems. However, during the growing season of the pot experiment in Chapter 4 and the field experiment in Chapter 5 used for correlation studies (correlation between soil factors and Se plant uptake), neither manure nor slurry was applied. In relation to Se input from phosphate application, as we discussed in Chapter 5, application of NPK can reduce Se plant uptake especially in organic-rich soils. The effects of NPK application on Se plant uptake increase with the increase of soil organic matter content. The decrease of Se bioavailability is larger in organic rich soils with organic matter content of > 15% than that in other soil types (i.e. sandy and clay soils). The NPK effect can be possibly attributed to the increase of soil microbial activity to reduce Se into a lower valence state and further incorporation of the reduced Se into soil organic matter. Nevertheless, no NPK fertilizer was applied during the year in the field experiment of grassland, which was used for the correlation studies (Chapter 5). In the pot experiment, NPK fertilizer has been applied before field soil sampling. The potato field soils contain 1.4 to 14.5% organic matter, in which 15 out of 19 soil samples used contain < 6% organic matter (Chapter 4). Therefore, we expect a negligible effect of the NPK application on Se plant uptake in the pot experiment using potato field soils of Chapter 4.

The study of Ros *et al.* (2014) estimated that the supply of Se from irrigation or sprinkling water in Dutch grassland system is low, i.e. maximally 0.36 g Se ha^{-1}. Atmospheric deposition of Se is dependent on the distance to the sea, the amount of precipitation and the magnitude of emission from local and foreign sources (Johnsson, 1992). We estimated the distance of the locations of grassland fields used in the current study to the sea (i.e. Wadden sea or North sea) to see if there is a correlation between the distance of the grassland fields to the sea and Se content in grass (Table 4) because sea spray contains a relatively high amount of Se (Haygarth, 1994). The results show that Se contents in grass taken from grassland fields close to the sea are not consistently higher than those in grass taken from grassland fields faraway from sea area (Table 4), and Se content in grass and the average distance of grassland field to the sea are hardly correlated ($R^2 = 0.04$, $p > 0.05$), indicating a small contribution of Se from deposition of sea spray to the Se content in grass. Haygarth *et al.* (1991) estimated that the input of Se from atmospheric deposition (mainly via wet deposition in the form of soluble H_2SeO_3)

Table 4. The average distance of the grassland fields from sea area and Se content in grass taken from the corresponding fields (without NPK and Se fertilization) (2011 field experiment, n = 21, Chapter 5). The distance was derived from the nearest distance between sea area and the grassland fields using Google maps.

Location (city, province)	Average distance from sea (Wadden Sea or North Sea) (km)	Soil type	Se content in grass (µg kg^{-1})
Thesinge, Groningen	28	Clay ≥ 20%, pH > 6	66
Thesinge, Groningen	28	Other clay	37
Drachstercompagnie, Friesland	39	Sand	22
Drachstercompagnie, Friesland	39	Sand	28
Tynaarlo, Groningen	44	Sand	16
Bantega, Friesland	51	Sand	37
Bantega, Friesland	51	Sand	30
Harmelen, Utrecht	52	Clay-peat	36
Harmelen, Utrecht	52	Clay-peat	115
De Meern, Utrecht	58	Clay-peat	55
De Meern, Utrecht	58	Clay-peat	88
Luttelgeest, Flevoland	64	Other clay	34
Luttelgeest, Flevoland	64	Sand	65
De Hoeve, Friesland	67	Peat	42
De Hoeve, Friesland	67	Sand	31
Swifterband, Flevoland	83	Other clay	24
Swifterband, Flevoland	83	Other clay	19
Woold Winterswijk, Gelderland	198	Sand	37
Woold Winterswijk, Gelderland	198	Sand	27

into soil-herbage system of the UK grasslands is 2 to 7 g ha^{-1} $year^{-1}$, and the total uptake of Se by grass via atmospheric deposition is about 0.01 to 0.035 g ha^{-1} $year^{-1}$, accounting for 10 to 34% of Se taken up by grass (Se content in grass is assumed 40 µg kg^{-1} and the grass yield is 2600 kg ha^{-1}). Only about 0.5% of atmospheric deposited Se per year is taken up by grass, whereas the rest is readily re-volatilized into the air (total volatilization losses from soil is estimated at about 0.1 to 10% of total soil Se $year^{-1}$), immobilized in the soil or leached out from the soil, therefore becomes unavailable for plant uptake (Haygarth *et al.*, 1991). The low contribution of atmospheric deposition to Se content in grass indicates that Se in soils is still the major source of Se uptake in crops (De Temmerman *et al.*, 2014).

The finding of low predictability of Se uptake in the field in the current study is similar to that in the study of De Temmerman *et al.* (2014), which concluded that the Se concentrations in low Se agricultural soils in Belgium (total Se content of 0.14 to 0.70 mg kg^{-1}) are far too low to generate a driving force for Se uptake in crops under field conditions, with low predictability of Se variability in crops, i.e. 9 to 48%. The fluctuation of soil moisture (water content), redox conditions, and temperature in the fields (Spadoni *et al.*, 2007; De Temmerman *et al.*, 2014) may influence the mineralization, turnover rate, and bioavailability of Se-rich organic molecules in soils. Previous studies indicated that the solubility of DOM in soils is positively correlated to soil temperature and soil moisture, which are attributed to the increase of biological activity in soils (Falkengren-Grerup and Tyler, 1993; Kalbitz *et al.*, 2000). Consequently, these factors may also influence the solubility of Se-containing DOM in soil.

7.6 Effectiveness of Se fertilization

Selenium (as selenate) fertilization was performed on a number of grasslands that consist of different soil types applied with N plus cattle slurry (2007 field experiment) and with NPK (2012 field experiment) (Chapter 6). The study revealed that the effectiveness of Se fertilization, which is indicated by the increase of Se in grass per unit amount of Se applied, is only weakly influenced by soil types both in the fields with N plus slurry applications (2007 experiment) and with NPK application (2012 experiment). The weak influence of soil properties on Se fertilization efficiency is probably due to the high selenate solubility in the soils (Yläranta, 1983). The findings in this study correspond to the results of a meta-analysis of Se fertilization that indicated that total Se content, acidity and organic matter content of the soils and crop species only had a minor influence on the effectiveness of Se fertilization (Ros *et al.*, 2016).

Nevertheless, the effectiveness of Se fertilization on sandy soils tends to have a larger range and a higher mean than that on clay soils and organic rich soils (clay-peat and peat soils) in both experiments, probably partly due to Se adsorption on clay surfaces in clay soils, and Se reduction and immobilization into soil organic matter in organic rich soils, which limit Se availability for plant uptake. In this study, Se was applied to the grassland fields as selenate. It is expected that, if the Se is applied in the form of selenite instead of selenate, the soil properties may influence the effectiveness of Se fertilization more significantly due to stronger adsorption of selenite to soil than selenate, and due to the easiness of Se reduction. Moreover, forms of Se fertilizer applied to soils also determine the translocation pattern of Se in plants (selenate is very mobile within plant tissue and easily transported from roots into shoots, whereas selenite tends to accumulate in the roots), thus the effectiveness of Se fertilization (Keskinen *et al.*, 2010). Although selenate fertilization in our study significantly increased Se content in grass grown on different soil types, however, the amount of Se in grass upon fertilization is still mostly below the optimum amount of Se in grass for cattle intake (i.e. 150 to 200 µg Se kg^{-1} dry weight). In addition, use efficiency of selenate by grass is very low, i.e. < 22% of Se applied to the soils for one season, indicating that most of selenate applied is leached out or volatilized from the soils or immobilized in the soils.

7.7 General Conclusions

This thesis has shown the evidences of association between Se and soil organic matter in low Se content agricultural soils in the Netherlands. The associations include: (1) the total Se content is positively correlated to soil organic matter content; (2) the solubility and extractability of Se in soils follow the solubility and extractability of soil organic C; (3) the majority of Se present in soils is in organic form, both in the soil solution and solid phase; (4) the distributions of Se and organic C in the different fractions of solid organic matter (i.e. humic acids, hydrophobic organic neutral, hydrophilic acids) and dissolved organic matter (i.e. hydrophilic acids and fulvic acids) are comparable; and (5) the Se richness in solid and dissolved organic matter are related to properties of soil organic matter from different land uses. The relatively high soil organic matter content in these low Se soils is likely responsible for these associations.

Different soil parameters determine Se plant uptake in low Se soils with predominantly organic Se, depending on the properties of Se-containing soil organic matter. The intensity parameter of Se-rich DOM in soil solution (i.e. Se to DOC ratio in 0.01 M $CaCl_2$ extraction) determines Se plant uptake in soils containing Se-rich organic matter (e.g. potato field soils), whereas the buffer capacity of labile organic Se to supply Se-rich DOM in soil solution limits Se plant uptake in soils containing Se-poor organic matter (e.g. grassland soils). Further research is needed to confirm the generality of the conclusion above, because the two experiments were carried out under different conditions (pot experiment and field experiment), using different plant species (wheat and grass) and covering different soil types from different land uses (potato fields and grassland). Site-specific properties in the field in addition to soil parameters included in the current study may largely (> 50%) determine Se uptake in grass under field conditions, which is in contrast with the results of the pot experiment in which the soil parameter explains 88% of Se uptake in wheat shoots. In general, the content of Se-rich DOM in soils increases with the increase of soil pH (and with the decrease of soil C:N ratio), and the amount of labile organic Se in soils that can resupply Se-rich DOM is determined by the content of clay (and Fe-(hydr)oxides). NPK fertilization, as one of the external factors, can reduce Se plant uptake, especially in organic rich soils.

Selenium (as selenate) fertilization on grassland with N plus cattle slurry or NPK application shows a positive effect to increase Se content in grass grown on different soil types with a large range of total Se, pH, clay content and organic matter content. Selenium content in grass grown on different soil types upon Se fertilization becomes more similar than before the fertilization. The results indicate that the effectiveness of Se fertilization is only weakly modified by soil properties, probably due to the high solubility of selenate in the soils. Nevertheless, the Se fertilization tends to be slightly more effective on sandy soils than on clay and organic rich soils.

This thesis has shown that the content and quality of soil organic matter play an important role in determining the amount, speciation and bioavailability of Se in low Se soils with predominantly organic Se as in the Netherlands. Soil parameter indicators for Se plant uptake have been identified, which can be used to predict Se status in soil testing. Effects of Se fertilization on Se level in grasses were also investigated. The results in this thesis can be used as guidance to develop soil testing and fertilization recommendation for efficient Se management, especially in Dutch agricultural soils.

7.8 Possible utilizations in practice

The utilizations that can be derived from the results in this thesis are presented below.

1) For soil-testing of Se status, I recommend use of dried soil samples. Concentrations of soluble Se extracted in 0.01 M $CaCl_2$ and labile organic

Se extracted in hot water are higher in the dried soils than in the moist soils, which make the measurement of Se concentration more reliable, especially when considering the very low concentrations of Se in the Dutch agricultural soils (Chapter 2). Further, dried samples are easier to homogenise and less vulnerable to storage changes. Nevertheless, because Se in the Dutch agricultural soils is mainly associated with soil organic matter, the soil samples should be analysed as soon as possible to avoid changes in Se concentrations and speciation during the storage of the soil samples due to organic matter decomposition, especially when the samples are stored at room temperature. If the samples need to be stored for a longer period, a low storage temperature (e.g. 4°C) is preferred over room temperature.

2) For determination of Se species, the inorganic Se can be measured in ammonium oxalate – oxalic acid (pH 3) extraction using anion exchange HPLC coupled with ICP-MS. The organic Se concentration in soils can be measured with 1 M NaOCl (pH 8) oxidation-extraction. Total soil Se can be measured after aqua regia digestion. The above method is relatively simple and reliable for Se speciation analysis in soils with low Se concentrations in comparison with commonly used sequential soil extractions.

3) The ratio of Se to DOC in 0.01 M $CaCl_2$ extraction can be used to predict Se plant uptake in soils containing Se-rich organic matter (e.g. potato field soils). Moreover, the soil pH and soil C:N ratio are the soil properties that determine the bioavailability of Se in these soils. On the other hand, Se in hot water extraction can be used as an indicator to predict Se plant uptake in the soils containing Se-poor organic matter (e.g. grassland soils), however, the prediction power is low (< 50%) for at least field conditions.

4) In evaluating Se bioavailability in soils, it should be considered that NPK fertilization, especially on organic rich soils, decreases Se bioavailability in soils for plant uptake.

5) The effectiveness of Se (as selenate) fertilization in combination with N plus cattle slurry or NPK application shows limited differences for all the soil types, however, the effectiveness tends to be slightly higher on sandy soils than on clay soils and organic rich soils. Moreover, Se fertilization up to 4.4 g Se ha^{-1} in the form of selenate on grassland soils does not lead to a Se content in grass that meets the optimum amount of Se in grass for cattle intake. This finding can be used further to design effective and efficient fertilization strategies for Se that would enable the optimum amount of Se in grass for cattle intake.

7.9 Future research

In this thesis, a first thorough study on content, speciation, extractability, bioavailability and fertilization of Se in Dutch agricultural soils was conducted and many new insights have been obtained, which form a sound basis for future research.

1) I have revealed that most of Se in the Dutch agricultural soils, both in the solution and solid phase, is in the organic form. However, more research is needed to show the molecular nature of organic Se in different soils.

2) I have conducted pot experiments with potato field soils and field experiments with grassland soils. Future research that focuses on exploring and validating the (soil) indicators of Se plant uptake is recommended, with additional pot and field experiments using different soil types and/or soils from other land uses.

3) Specific research focusing on the speciation, distribution and turnover of the organic Se pools that are related to Se bioavailability (i.e. Se-rich labile organic matter and Se-rich DOM), is recommended to develop better insight into bioavailability of organic-Se species in soils. This research can be combined with the research recommended in no 1.

4) In relation to fertilization treatments, research on the effects and mechanisms of NPK fertilization on Se bioavailability in soils with low and high amount of organic matter or soils from different land uses is necessary in order to find an effective and efficient fertilization strategy that can increase or maintain Se bioavailability in soils.

5) Study on the fate of Se fertilizer in soils and strategies to improve effectiveness of Se fertilization and evaluation on the long-term efficiency of Se fertilization are important to design a sustainable Se fertilization approach that enables optimum Se content in grass or crops for cattle and human intake and avoid environmental pollution.

These recommendations for future research would enable to reveal the (soil) indicator(s) that can be used to predict Se bioavailability and plant uptake in low Se soils with predominantly organic Se. In addition, understanding on the factors and mechanisms that influence specific organic Se pools that are related to Se bioavailability in this type of soils would be achieved. Moreover, effective and efficient fertilization approaches for Se can be developed for this type of soils in order to meet the optimum amount of Se in crops for cattle and human intake, and avoid negative effect on the environment and ecosystem.

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Summary

Selenium (Se) is an essential micronutrient for animals and humans. In the food chain, the intake of Se by animals and humans depends largely on Se content in plants, whereas the major source of Se in plants lies in the soil. Therefore, understanding Se bioavailability in soils for plant uptake and its controlling factors and mechanisms is important. So far, there is no comprehensive published research on the content, speciation, bioavailability, plant uptake and fertilization effect of Se in agricultural soils in the Netherlands, which contain low amount of Se in predominantly organic form. Moreover, the effects of land use, soil properties, and NPK fertilization on Se uptake in plants grown on different soils from the Netherlands have not been studied in depth either. Therefore, the objective of this thesis is to study the level, speciation, bioavailability, plant uptake and fertilization of Se in agricultural soils in the Netherlands and underlying controlling factors and mechanisms, to provide guidance for soil testing and fertilization recommendation for efficient Se management in agriculture.

In **Chapter 2**, the effects of soil sample pre-treatment, especially of soil drying and rewetting on soluble Se in 0.01 M $CaCl_2$ extraction was studied on various soil samples from different land uses (grassland, maize field, and potato field). Dissolved organic carbon (DOC) was also measured in the corresponding soil extractions because of the association between soluble Se and DOC. Selenium speciation analysis and dissolved organic matter (DOM) fractionation were performed using Donnan Membrane Technique (DMT) and DAX-8 resin. The results revealed that soil drying and rewetting increased both soluble Se and DOC concentrations in 0.01 M $CaCl_2$ extraction, in which the increase of soluble Se (1.3 to 34 fold) is more pronounced than that of DOC (1.3 to 11 fold). The increase of soluble Se upon soil drying is attributed to the increase of Se associated with colloidal sized DOM (i.e. colloidal sized organic Se). Upon drying, the Se to DOC ratio increased in most of the soils, especially in grassland soils and maize field soils (on average 18 ± 12 and 17 ± 11 μg Se g^{-1} C, respectively, in moist soils and

34 ± 13 and 30 ± 16 µg Se g^{-1} C, respectively, in dried soils), indicating enrichment of Se in DOM after drying, i.e. the Se increase is more pronounced than DOC increase. On the other hand, a constant Se to DOC ratio in soils from potato fields was found upon soil drying (62 ± 35 µg g^{-1} in moist soils and 64 ± 33 µg g^{-1} in dried soils). No consistent difference in the Se to DOC ratio of hydrophilic (Hy) and fulvic acid (FA) fractions of DOM in the dried soils was found. The distribution of Se in the hydrophilic and fulvic acid fractions of DOM in extractions of the dried soils (68% and 32%, respectively) is comparable to the distribution of DOC in the corresponding fractions of DOM (74% and 26%, respectively). This chapter has provided evidence of the association between soluble Se and DOC in soil solution.

In **Chapter 3**, a study on speciation and extractability of Se in various soil samples from grassland and arable land is reported. Three single step extractions, including ammonium oxalate – oxalic acid (pH 3) extraction coupled with speciation analysis using High Performance Liquid Chromatography (HPLC) – ICP-MS, 1 M NaOCl (pH 8) oxidation-extraction and aqua regia digestion, were performed on a number of selected soil samples from grassland and arable land to determine the amount and fraction of inorganic, organic and residual Se in the soils. In addition, seven single step extractions were conducted to study different Se pools and their speciation, and Se extractability in the soils, including 0.01 M $CaCl_2$, 0.43 M HNO_3, hot water, ammonium oxalate – oxalic acid (pH 3), 0.1 M NaOH, 1 M NaOCl (pH 8) and aqua regia. Total Se contents measured in aqua regia are between 0.12 and 1.97 mg kg^{-1} (on average 0.58 mg kg^{-1}; n = 83), in which the total Se contents of grassland soils (0.12 to 1.97 mg kg^{-1} with an average of 0.62 mg kg^{-1}; n = 42) and arable land soils (0.20 to 1.20 mg kg^{-1} with an average of 0.53 mg kg^{-1}; n = 41) are comparable. From these total contents, on average 82% of Se is in organic form, 5% is in inorganic form (mainly selenite; selenate was hardly measurable) and 13% is present in residual fraction. There is no profound difference of Se speciation between grassland soils and arable land soils. The amount and fraction of Se extracted both in grassland soils and arable land soils follow the order of aqua regia > 1 M NaOCl (pH 8) > 0.1 M NaOH > ammonium oxalate (pH 3) > hot water >

0.43 M HNO_3 > 0.01 M $CaCl_2$. The extractability of Se in each extraction follows the extractability of organic C, and the amount of Se in each extraction is positively correlated to extractable organic C, supporting the predominance of organic Se both in the soils as a whole and in each extraction, except for the 0.43 M HNO_3 extraction. In 0.43 M HNO_3 extraction, Se is expected mainly present as inorganic Se plus a small amount of organic Se. Based on the Se to organic C ratio, organic matter in the $CaCl_2$, hot water, and NaOH extractions (on average 31 ± 14 µg Se g^{-1} C, 23 ± 13 µg g^{-1}, and 19 ± 8 µg g^{-1}, respectively) appears to be more enriched with Se than in the other extractions (10 ± 3 µg g^{-1} in HNO_3 extraction; 14 ± 7 µg g^{-1} in ammonium oxalate extraction; 9 ± 4 µg g^{-1} in NaOCl extraction and 12 ± 6 µg g^{-1} in aqua regia). The hydrophobic neutral (HON) fraction (on average 30 ± 14 µg Se g^{-1} C) of soil organic matter in NaOH extraction is richer in Se, followed by the hydrophilic (Hy) fraction (22 ± 11 µg g^{-1}), whereas the humic acids (HA) (16 ± 7 µg g^{-1}), and probably also humin (not measured) and the fulvic acids (FA) (not measured) fraction are relatively poor in Se compared to the HON and Hy fractions. The findings in this study indicate a strong association between Se and organic C both in the solid phase and the solution of low Se Dutch agricultural soils.

In **Chapter 4**, a correlation study between Se plant uptake and soil parameters was conducted for low Se soils from arable land (i.e. potato field) with predominantly organic Se, in order to identify the soil parameters that are best related to Se plant uptake and to explore the mechanisms that control Se bioavailability. These soils contain (dissolved) organic matter richer in Se, relative to grassland soils used in the study of Chapter 5. A pot experiment was conducted with summer wheat (*Triticum aestivum* L.) using nineteen soil samples consisting of sand, clay, loess and reclaimed peat soils. Selenium in wheat shoots and soil parameters, including basic soil properties, C:N ratio, inorganic selenite content, and Se and organic C in different soil extractions (0.01 M $CaCl_2$, 0.43 M HNO_3, hot water, ammonium oxalate, aqua regia) were analyzed. The regression model showed that Se to DOC ratio in 0.01 M $CaCl_2$ extraction largely explains (88%) the variability of Se content in the wheat shoots, indicating that Se-rich DOM in soil solution is the main source of bioavailable Se in these low Se potato field soils with a low amount of soluble Se but with Se-rich DOM compared to the grassland soils. Soil pH, C:N ratio, clay content and organic matter content have been identified as the major soil properties related to the Se to DOC ratio in $CaCl_2$ extraction and Se plant uptake. Among these soil properties, soil pH correlates most strongly with Se to DOC ratio in $CaCl_2$ extraction and Se content in wheat shoots, which increased with the increase of soil pH. In addition to soil pH, our findings suggest that soil organic matter quality (i.e. soil C:N ratio) controls to a large extent the amount and release of Se-rich organic molecules from the soil solid phase to soil solution and subsequent uptake. In general, Se to organic C ratio in all soil extractions (0.01 M $CaCl_2$, 0.43 M HNO_3, hot water, ammonium oxalate, aqua regia) increased with a decrease of soil C:N ratio, indicating that at low soil C:N ratio soil organic matter is richer in Se. In contrast, the soil pH is strongly correlated with Se to organic C ratio in $CaCl_2$ and hot water extraction, but weakly correlated with Se to organic C ratio in other extractions, suggesting that the soil pH controls the amount of Se-containing organic matter in soluble and labile organic matter pools instead of the amount of Se-containing organic matter in soil solid phase. This chapter has shown the importance of Se-rich DOM in soil solution in supplying bioavailable Se, and the relation between richness of N and Se in soil organic matter (soil C:N ratio and Se to organic C ratio are related) in the soils under study.

In **Chapter 5**, a similar study as in Chapter 4 was conducted but under field conditions (field experiment) instead of under controlled conditions (pot experiment). The study was performed on a number of grassland fields (without NPK application), consisting of different soil types, i.e. sand, clay, clay-peat and peat soils. Similar to the potato field soils, the grassland soils in general contain low total Se with predominantly organic Se. However, these grassland soils contain poorer-Se organic matter and DOM than the potato field soils, with soil C:N ratio varying over a narrower range. Regression modelling showed that among all the soil parameters measured in this study, Se in hot water extraction correlates most strongly with Se content in grass, which explains partly (41%) the Se variability in grass grown under field conditions. Selenium in hot water extraction is mostly in organic form, representing the labile organic Se in soils. This finding indicates that > 50% of the Se variability in grass grown under field conditions is influenced by site-specific properties in the fields that were not considered in the current study. The amorphous Fe-(hydr)oxide content and clay content are the soil properties that correlate positively with both the amount of Se in hot water extraction and Se content in grass, suggesting that adsorption and desorption on soil mineral surfaces control the amount and availability of labile organic Se in the soils. The best predictor of Se uptake identified is different for the potato field soils (Se to DOC ratio in $CaCl_2$ extraction) and grassland soils (Se in hot water extraction), which may suggest that land use influences the limiting factors of Se bioavailability in soils, probably via properties of Se-containing organic matter. However, this difference can also be caused by variations in ranges of soil parameters of the samples chosen. Therefore, further research is needed to validate these findings. In this chapter, a second field experiment on similar grassland fields was performed to study the effect of NPK fertilization on Se plant uptake in grass. Application of NPK fertilizers decreased Se content in grass, especially on organic rich soils. This decrease becomes stronger with increasing soil organic matter content, suggesting that NPK application and soil organic matter result in a synergetic effect in reducing Se bioavailability in soils. The possible cause is immobilization of Se into soil organic matter due to Se reduction induced by an increase of soil microbial activity upon NPK application. This chapter confirms the role of (Se-containing) soil organic matter in determining Se bioavailability both in soils without and with NPK application under field conditions.

In **Chapter 6**, a study on Se (as selenate) fertilization on grassland fields with N plus cattle slurry application and with NPK application was conducted. Selenium contents in grass were analyzed in both control fields and fields with Se application, whereas soil samples were analyzed before fertilization (without any fertilizer applications). Selenium fertilization (0.32 to 4.4 g Se ha^{-1}) in both experiments (fields with N plus slurry application and fields with NPK application) increased Se content in grass by 3 to 6 fold, except in grass grown on clay soils with clay content ≥ 20% and pH > 6, in which the Se content in grass is already relatively high without Se fertilization, the increase was 1.3 to 2 fold. However, on the majority of the fields, Se content in grass is still below the recommended amount for cattle intake (i.e. 150 to 200 µg Se kg^{-1} dry weight of grass). The effectiveness of Se fertilization, which is indicated by the increase of Se content in grass relative to the amount of Se applied per ha (µg kg^{-1} Se increase in grass per g ha^{-1} Se applied), has small differences for different soil types in both experiments. However, the effectiveness tends to be slightly higher on sandy soils than on clay soils and organic rich soils (i.e. clay-peat and peat soils). The weak influence of soil properties on Se fertilization efficiency is probably due to the high solubility of selenate in the soils. The recovery of Se fertilized in grass for one season is low, i.e. 3 to 22% of the total Se applied. The findings in this study have shown the possibility to increase Se content in grass via Se (as selenate) fertilization. However, further research is recommended to investigate options to increase the effectiveness of Se fertilization on the fields which cover different soil types (sand, “other clay”, clay soils with clay content ≥ 20% and pH > 6, clay-peat, and peat soils), to understand the major factors controlling the effectiveness of Se fertilization and to avoid accumulation and pollution of Se in the environment, especially for the long term.

In this PhD thesis, a comprehensive study on the content, speciation, extractability, bioavailability and fertilization of Se in agricultural soils in the Netherlands was performed. New insights in the factors and mechanisms determining the speciation, extractability and bioavailability of Se in these low Se soils with predominantly organic Se have been obtained and discussed in detail in Chapter 2 to 6 and summarized in **Chapter 7**. In conclusion, this thesis has shown the central role of soil organic matter in determining the amount, speciation and bioavailability of Se in the low Se Dutch agricultural soils. In addition, this thesis has provided methods and recommendations for soil testing of Se status, Se speciation, soil indicators for Se plant uptake, and agricultural management practices including NPK and Se fertilization that can influence Se bioavailability in soils. These new insights form a good basis for further research and development of efficient Se management in Dutch agricultural soils.

Acknowledgements

This thesis was made through a long journey with learning, experiences, enjoyment, enthusiasm, struggling, patience, persistence and of course with the help of many people. In this page I would like to acknowledge the people for their help, support and companionship during this journey.

I would like to thank my promotor Rob Comans and my co-promotor Liping Weng for their supervisions, suggestions, ideas, contributions and supports during my PhD research and journey. I want to thank Rob for his constructive feedback on the manuscripts and his support during my PhD journey. I would like to thank Liping for her supervision, suggestions, constructive inputs, and support during PhD research and journey. I really appreciate her critical feedback and valuable inputs during the discussions and the writing process of the manuscripts, which brought the manuscripts to a better level. I also want to thank my colleague and officemate Cristian Terrones Cano for his contributions, ideas and help during the experiments, discussions and journey of the research project. I would like to thank Wim Bussink from Nutrient Management Institute NMI Agro, Wageningen for his contributions in the project and his help with soil and plant samples and important additional data and information on soil and plant samples. I am also thankful to my previous promotor Willem van Riemsdijk and the late Erwin Temminghoff and for their contributions and suggestions in the first year of the PhD research.

I also would like to acknowledge people from Chemical and Biological Soil Laboratory for their help and suggestions during the experiments. I would like to thank Peter Nobels for his valuable contributions and suggestions during the discussions regarding sampling preparations and analyses using ICP-MS, ICP-AES and HPLC. I also would like to thank Gerlinde Vink for her guidance and help during the experiments, and her valuable inputs and suggestions during the discussions for the preparation of the experiments. I want to thank Jaap Nelemans and Willeke van Doorn-van Tintelen for their help and suggestions for soil sample

pre-treatments and greenhouse pot experiment. I am thankful to Johan Uijtenbroek, Miranda Vlag, Wim Pape and Andre van Leeuwen for their help during my works in the laboratory. I also would like to acknowledge Marnella van der Tol and Esther van den Brug from Secretariat of Department of Soil Quality for their help and support related to administration stuff during my PhD at Department of Soil Quality.

I want to thank to all my colleagues from Department of Soil Quality for nice working environment during my PhD. I want to thank my officemates Cristian, Qian and Susan for nice talk, sharing cookies or some food, and staring outside our window together in order to have a small break during our working time in the office. I also thankful to Tatiana, Paulo, Edvaldo, Yunyu, Karst, Jingmeng, Xinxin, Ma Lin, Jana, Bastiaan, Mart, Inge, and Wei for companionship, talk and sharing story about our daily PhD life. I also want to thank my Indonesian friends in Wageningen for their companionship, help and sharing the laugh, food, story, experiences during my stay in Wageningen. Hikmah, Novi, Indra, Mbak Nurmi, Mbak Nani, Mbak Aisyah, Kang Asep, Mbak Yayu, Kang Apri, Mbak Yessy, Mbak Ovi, Pak Maman, Farida, Tresty, Atik, Mbak Yuni, Diah, Bu Eva, Mbak Ria, Mbak Juliana, Pak Dikky, Pak Fajar, Pak Dadan, Teh Nia, Yuda, Mbak Vivi, Titis, Tika, Kang Dasep, Mbak Pini, Mbak Shinta and others, thank you for your warm friendship to escape from the loneliness during my stay in Wageningen.

Last but not least, a big thank to my father Turmudi, my mother the late Sutinah, my sisters Dede and Nining, my brother Nanang, and my relatives the late Lek Yusuf, Mbak Endang, Mbak Wal, Mbak Wiwi, Lek Nono, Mbak Neneng, Kus and Mbak Ipah for their full support, prayer, love, care, and help in every single thing during my PhD journey. “Terimakasih yang tak terhingga atas semua kasih sayang, doa dan dukungan yang telah kalian berikan kepada saya sehingga saya dapat menyelesaikan disertasi and studi S3 saya ini.” Thank you! Terimakasih! Dank je wel!

Curriculum Vitae

Supriatin was born on 19th December 1979 in Bandar Lampung, Indonesia. She obtained her BSc degree in Soil Science from University of Lampung, Indonesia in 2003. For her bachelor thesis, she studied about “The activity of phosphatase in earthworm casts at coffee fields under different cover crop management”. In 2005, she started her career as a junior lecturer and researcher at Department of Soil Science, University of Lampung, Indonesia. In 2007, she continued her Master study at Wageningen University in an MSc program of Soil Science with specialization in soil quality. For her master theses, she conducted a major thesis about “Understanding the roles of dissolved and soluble organic nitrogen in N mineralization using ^{15}N isotope tracer”, and a minor thesis about “Developing a new method in measuring selenium bioavailability in soils”, both at Department of Soil Quality, Wageningen University. The latter thesis was a preliminary study for her PhD research. In 2009, she obtained her MSc degree in Soil Science, and afterwards she continued her work at University of Lampung, Indonesia. In 2010, she went back to Wageningen and started her PhD research within an *Stichting Technische Wetenschappen* (STW) project “Micronutrient Management for Sustainable Agriculture and Environment: A New Innovative Approach”, which was focused on selenium and copper. The results of selenium research were presented in this thesis. After finishing her PhD, she will continue her work at Department of Soil Science, University of Lampung, Indonesia.

List of publications

Weng, L., Vega, F.A., **Supriatin, S.**, Bussink, W., Van Riemsdijk, W.H., 2011. Speciation of Se and DOC in soil solution and their relation to Se bioavailability. Environ. Sci. Technol. 45, 262-267.

Supriatin, S., Terrones, C.A., Bussink, W., Weng, L., 2015a. Drying effects on selenium and copper in 0.01 M calcium chloride soil extractions. Geoderma 255-256, 104-114.

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Supriatin, S., Weng, L., Bussink, W., Comans, R.N.J. Soil parameters infuencing selenium uptake in grass grown under field conditions. *In preparation*.

Supriatin, S., Weng, L., Bussink, W., Comans, R.N.J. Effects of selenate fertilization on selenium uptake in grass grown on different soil types. *In preparation*.

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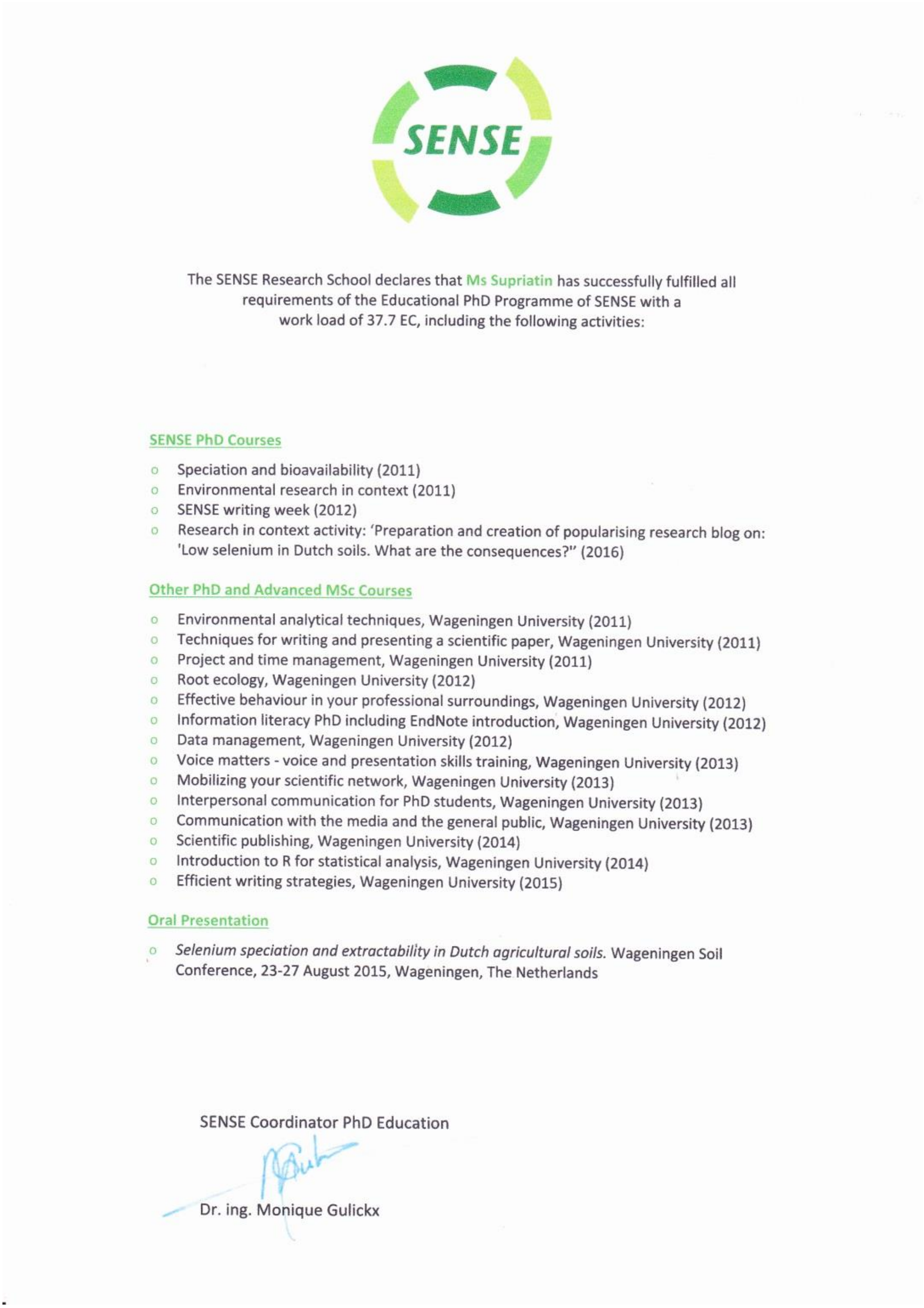

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- Effective behaviour in your professional surroundings, Wageningen University (2012)
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- *Selenium speciation and extractability in Dutch agricultural soils.* Wageningen Soil Conference, 23-27 August 2015, Wageningen, The Netherlands

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The research described in this thesis was financially supported by *Stichting Technische Wetenschappen* (STW), the Netherlands under the project "Micronutrient Management for Sustainable Agriculture and Environment: A New Innovative Approach" (project number 10707).

Financial support from Wageningen University for printing this thesis is gratefully acknowledged.

Cover design by Asep Bayu Ekawijaya and Supriatin Supriatin
Photo in cover by Nani Maryani and Supriatin Supriatin