Clever Cover Cropping

Litter trait diversities and elemental flows

Rima J. Porre

Propositions

1. Contrary to the popular assumption, it is not possible to predict litter mixing effects on decomposition
(this thesis)

2. Fertilising cover crops is unnecessary and poses a risk to the environment
(this thesis)

3. Scientists can only be true to science when they support freedom of speech

4. The human body is built to run long distances

5. Scientists should be allowed to focus solely on science

6. Current science funding prohibits progress in science

7. Media coverage rather than facts govern our perception of risk

8. Facing hardship is crucial to be content with life

Propositions belonging to the thesis, entitled:

‘Clever Cover Cropping – Litter trait diversities and elemental flows’

Rima Jasmijn Porre
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Clever Cover Cropping

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Thesis

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Chapter 1

General introduction

Rima J. Porre

1.1 Problem description

The increasing global population forces intensive agricultural practices in order to sustain the ever greater demand in food supply (Schmidt-Traub et al., 2019; Tilman et al., 2011). The large amounts of N and P used for intensive agriculture have led to a major disruption of the N and P cycle and a decline in biodiversity and climate change (Steffen et al., 2015). The large majority of the atmosphere's nitrogen exists in the inert form of N_2. However, since the Haber-Bosch process has been developed in 1908 humans have been able to convert N_2 to reactive nitrogen, NH_3 (Erisman et al., 2008). This lead to a dramatic increase in global agricultural productivity (Galloway et al., 2017). Sadly, efficiency of N use is low, this causes a whole suite of environmental risks such as eutrophication, biodiversity loss, ozone depletion and climate change (Erisman et al., 2013). Now, in alignment with the sustainable development goals (United Nations General Assembly, 2015) action plans are proposed with the aim to achieve efficient and resilient agricultural systems, conservation and restoration of biodiversity and food security and healthy diets (Schmidt-Traub et al., 2019). A major goal here is to increase food production without losing biodiversity, *i.e.* sustainable intensification (Crist et al., 2017). Moreover, intensive agricultural practices have left the soil depleted of nutrients and soil organic matter (SOM) (Janzen, 2006; Poeplau and Don, 2015). Especially SOM is important for the soil structure, the water holding capacity and is a source of nutrients (N and P) and is thus essential for crop biomass production and its quality (Liu et al., 2006). Therefore, the SOM content needs to be stabilised or increased in order to maintain many of these soil functions (Lehmann and Kleber, 2015; Reeves, 1997; Six et al., 2002; von Lützow et al., 2006).

1.2 Cover crops

A commonly used agricultural practice that can prevent nutrient leaching as well as increase the soil fertility is cover cropping (Abdalla et al., 2019; Blanco-Canqui et al., 2015; Jian et al., 2020; Wittwer et al., 2017). Cover crops replace bare fallows in between cash crop growing seasons, and the cover crop biomass is incorporated into the soil before the following cash crop (Blanco-Canqui et al., 2015). For this reason cover crops are often referred to as green manures because they provide additional soil organic matter and nutrients to the soil in spring (Blanco-Canqui et al., 2015). Additionally they are commonly called catch crops since they scavenge remaining nutrients from the soil that may have been left behind after the main growing season and that will become mineralised from the SOM through ongoing soil microbial activity, and therefore prevent N leaching (Thorup-Kristensen et al., 2003). In addition to increasing SOM cover crops are also known to increase N mineralisation in the spring (Tribouillois et al., 2015), reduce mineral N leaching in the autumn (Tribouillois et al., 2015), prevent soil erosion (Blanco-Canqui et al., 2015; Poeplau and Don, 2015), reduce weed growth (Haramoto and Gallandt, 2004), increase arbuscular mycorrhizal fungi (AMF) activity (Blanco-Canqui et al., 2015; Dabney et al., 2001), suppress diseases (Kruger et al., 2013), reduced P leaching (Maltais-Landry and Frossard, 2015), increase P availability (Maltais-Landry and Frossard, 2015), stimulate soil (microbial) life (Blanco-Canqui et al., 2015) and improve soil structure (Abdollahi and Munkholm, 2014). However, the extent to which each of these functions are realised does depend on the cover crop characteristics. Cover crops are commonly from the Fabaceae, Poaceae or Brassicaceae families (Tribouillois et al., 2016). Cover crops from the Fabaceae family are legumes and can thus fix atmospheric N through biological N fixation (Fageria et al., 2005), this generally results in a high biomass N concentration and can thus supply a large amount of N to the soil and subsequent cash crop (Tribouillois et al., 2015). Crops from the Poaceae or Brassicaceae family are fast growing and have a high N requirement (Kaye et al., 2019), these are thus more often used as catch crops because they prevent N leaching by taking up large amounts of N in their biomass (Holmes et al., 2019). Additionally brassica cover crops are often used for disease suppression (van Leeuwen-Haagsma et al., 2019)

1.3 Proposed benefits of mixing cover crops

Until now, cover crops across the globe are usually grown in monocultures (Finney et al., 2016). Nevertheless, the potential role of using cover crop mixtures to promote cover crop functions for the soil and to aid diversification of agroecosystems is growing. This attention is motivated by the fact that biodiversity is declining worldwide (Cardinale et al., 2012; Tscharntke et al., 2012; Wall et al., 2015) and one of the sustainable development goals is increasing biodiversity in agriculture (United Nations General Assembly, 2015). Additionally studies have shown that a higher plant diversity can increase primary productivity (Cardinale et al., 2007), promote soil C storage (Isbell et al., 2017; Lange et al., 2015) and increase soil fertility (Isbell et al., 2017; Tilman et al., 1996). Thus growing cover crops in mixtures could potentially be a simple tool to increase biodiversity in agriculture while simultaneously increasing soil C storage and soil fertility. The growing interest in the use of cover crop mixtures is especially prominent in Europe as well as in the USA. In the Netherlands (Dijksma, 2014; RVO, 2015) as well as in several other European countries farmers are stimulated to grow cover crops in mixtures as part of new greening measures. This interest in cover crop mixtures has led to several studies on cover crop mixtures in recent years. Recent studies have indeed shown that cover crop mixtures can have a higher yield than expected from monocultures (Finney et al., 2016). Yet for a farmer it is more important that a mixture produces more biomass than the best performing monocrop. Moreover, not only biomass quantity, but also biomass quality and rooting patterns are important when considering the effect of cover crop mixtures on soil C and N cycling.

1.4 Biomass production in mixtures

1.4.1 (Transgressive) overyielding

The concept of overyielding, based on the relative yield (RY) and relative yield total (RYT), originated in Wageningen (De Wit, 1960). The RY is calculated as the single species yield multiplied by the initial % in the mixture. The RYT is the sum of the RY of the individual species in the mix. The species mixtures exhibits overyielding, when a mixture produces a higher biomass than expected based on the average of the single species *i.e.* with a RYT > 1 (Hector, 2006). However this does not signify that the mixture has a higher yield than the most productive monocrop. Transgressive (or transitive) overyielding indicates when the mixture produces a higher biomass than the most productive single species (Hector, 2006).

1.4.2 Mechanism

The mechanisms involved in (transgressive) overyielding are complementarity, which consist of facilitation and niche differentiation (Loreau and Hector, 2001), and the selection effect (Tilman et al., 1997). Facilitation occurs when a plant directly or indirectly improves the biotic or abiotic conditions for the other plant and thus stimulates its growth (Hauggaard-Nielsen and Jensen, 2005). Facilitation between a legume and non-legume if often reported where the legume fixes N_2 and thus more N is available in the soil for the non-legume (Tribouillois et al., 2016). Niche differentiation occurs when plants in a mixture do not directly compete for the same resources (Loreau and Hector, 2001). Vertical belowground niche differentiation is possible when mixtures contain shallow and deep rooting species (Hoekstra et al., 2015). Aboveground species complementarity is often in terms of light capture (Evers et al., 2019) whereas belowground complementarity is in terms of nutrient acquisition, i.e. vertical root distribution (Heuermann et al., 2019) or increasing nutrient availability by root exudates or N_2 fixation of legumes (Finn et al., 2013) as well as reduced species specific pathogen pressure (Schnitzer et al., 2011). Lastly the selection effect refers to the probability of one species in the mixture doing well in a certain environment and thus dominating the biomass (Loreau and Hector, 2001; Tilman et al., 1997).

1.4.3 From natural to agricultural systems

A meta-analysis by Cardinale et al. (2007) reported that overyielding of mixtures in natural systems is often (70%) shown in natural systems yet transgressive overyielding is rarely found (12%). However, a study on 31 intensively managed grasslands found that transgressive overyielding occurred in 60% of the sites (Finn et al., 2013). Finn et al. (2013) proposed that the much higher incidence of transgressive overyielding in their study could be attributed to the selection of complementary species. Two recent meta-analyses showed that overyielding in intercropping systems is common and most often caused by temporal niche differentiation (Li et al., 2020; Yu et al., 2015). Yet transgressive overyielding is rare (Wendling et al., 2017; Yu et al., 2015). Selecting complementary cover crop mixtures, such as a combination of a legume and a non-legume could potentially give similar results. It remains unclear if transgressive overyielding in cover crop mixtures is likely since it is such a short growing season, when you compare it to grasslands where transgressive overyielding is often found. The best performing single grass species very often changes from year to year whereas mixtures have been shown to be more stable (Elhakeem et al., 2019; Finn et al., 2013). In short, complementary cover crop mixtures should consistently exhibit transgressive overyielding over the years in order to maximise their effect on ecosystem services such as N leaching reduction and C sequestration.

1.5 Nitrogen losses

1.5.1 During cover crop growth

Cover crops have been shown to reduce nitrate leaching by 6 to 94%, depending on the type of cover crop (Kaspar and Singer, 2011). High biomass producing cover crops (such as cereals and crucifers) are efficient in preventing N leaching, whereas leguminous cover crops, which fix N, are generally not (Blanco-Canqui, 2018; Thapa et al., 2018). Leaching of N is generally most reduced under cover crops with a high biomass (Thapa et al., 2018), high N uptake (White et al., 2017) and deep roots (Thorup-Kristensen, 2001) are generally most efficient at reducing N leaching. Since cleverly chosen cover crop mixtures (*i.e.* species with complementary growing strategies and resource uptake) are expected to produce a higher biomass, which requires additional N and water uptake, than monocrops, N leaching in mixtures during cover crop growth might be further decreased. Additionally, root architecture (rooting depth, root length distribution and diameter) varies widely among cover crop species (Bukovsky-Reyes et al., 2019) and rooting-based complementarity of cover crop species could therefore lead to a higher nutrient uptake by cover crop mixtures than by monoculture cover crops, and thus reduce

NO_3^- leaching more effectively (Brooker et al., 2015; Hauggaard-Nielsen and Jensen, 2005; Miyazawa et al., 2010).

1.5.2 After cover crop incorporation

After termination and incorporation of cover crops into the soil, the residues mineralise and N will become available for crop uptake. A higher N uptake by cover crops in autumn potentially results in larger N availability to the cash crop during its growing season. The rate at which this N in the cover crops becomes available for the cash crop depends on the cover crop biomass quality (Bichel et al., 2017). Cover crop with a low biomass C:N ratio, such as legumes, are expected to mineralise faster and thus release mineral N quicker (Crews and Peoples, 2005), possibly when mineral N availability still exceeds cash crop N demand. This can lead to losses of N to the environment in the form of nitrate leaching or N_2O emissions. Mitigation of N_2O is important since N_2O has a global warming potential 265 times stronger than CO_2 (Stocker et al., 2013). On the other hand cover crops with a high C:N ratio, such as grasses, decompose more slowly which can lead to immobilisation of N and can hamper cash crop growth (Thorup-Kristensen et al., 2003). Potentially mixtures of cover crops with different C:N ratios can have a more steady mineralisation of N and thus prevent N losses as well as promote cash crop growth. Or, interactions between the residues in the mixture could alter the decomposition and mineralisation rate and thus the release of nutrients to the soil (I will elaborate on this in the next paragraph). Besides the quality, biomass quantity is important to consider, since complementary cover crop species mixtures are expected to produce a higher biomass than single cover crops. Mixtures with a high biomass and a low C:N ratio might increase the risk of N leaching in spring, if the release of N is not at the same time when the cash crop has a high N demand.

1.6 Litter trait effects on carbon and nitrogen mineralisation

1.6.1 Controls of decomposition rate

To predict the timing of nutrient release of cover crop mixtures that are incorporating in the soil not only single residue but also mixed residue decomposition needs to be considered. It is known from natural systems that plant species mixtures are expected to affect decomposition rates as demonstrated by studies using leaf litter mixtures (Wardle et al., 1997). From litter decomposition studies it is known that the rate at which single litters decompose are controlled by inherent plant traits such as the C:nutrient ratio (Delgado-Baquerizo et al., 2015; Hättenschwiler and Jørgensen, 2010), while multiple studies have shown that the C:nutrient ratio alone cannot explain variations in decomposition rates (De Carvalho et al., 2012; de Carvalho et al., 2014; Hoorens et al., 2003; Pérez Harguindeguy et al., 2008). De Carvalho et al. (2012, 2014) suggest that organic C quality, other than C:N ratio is a good indicator of litter decomposition rates. Other litter chemical parameters looked at are total C, lignin, total N, total P, lignin:N ratio, cellulose, hemicellulose, sugar, starch and phenols (Delgado-Baquerizo et al., 2015; García-Palacios et al., 2013; Hättenschwiler and Jørgensen, 2010; Hoorens et al., 2003; Sariyildiz and Anderson, 2003; Veen et al., 2015).

1.6.2 Mixing effects

Litter species can interact in mixtures and subsequently affect the decomposition rate (Gartner and Cardon, 2004). Some litters contain compounds such as polyphenols which inhibit microbial growth and could thus slow down decomposition of the surrounding litters (Hättenschwiler et al., 2005). In contrast, litters with a low C:N ratio can facilitate decomposition of more recalcitrant litter via nutrient transfer and thus speed up decomposition rates (Hättenschwiler et al., 2005). However, to this date, results are inconclusive as all outcomes appear to be possible (faster, slower, no change in decomposition rate). The mechanisms underlying these changes in litter decomposition rates are not fully understood (Pérez Harguindeguy et al., 2008). Yet, it has been proposed that the degree of dissimilarity between the characteristics of the litters of species decomposing in mixtures can help to explain and predict synergistic effects of species mixtures on decomposition rates (Handa et al., 2014; Hättenschwiler et al., 2005). The question remains if litters decompose faster (synergistic mixing effects) or slower (antagonistic mixing effects) than their respective monocultures, and if these mixing effects can indeed be explained by litter trait diversity or if this would depend on the system (soil type, climate, nature/arable) in which the litters decompose.

1.7 Carbon sequestration

1.7.1 SOM fractions

Dead plant material is transformed to soil organic matter by biological, physical and chemical processes (Lehmann and Kleber, 2015). This organic matter will reside in the soil up to centuries depending on the stabilisation process. Organic matter exists as low molecular weight compounds in solution (dissolved organic matter; DOM), as particulate organic matter (POM) or as decomposition products of residues bound to minerals (mineral associated organic carbon; MAOM) (Lavallee et al., 2020). These fractions are either free in the soil or occluded in micro and macro- aggregates. The amount of these fractions present in the soil depends on the plant inputs (biomass as well as root exudates and leaf leachates), soil type and management practices. Generally, POM is positively related to biomass input (Haddix et al., 2020), aggregation is negatively related to the amount of tillage (Six and Paustian, 2014) and MAOM is positively related to the clay content of a soil (Six et al., 2002). Recent studies have shown that residues that are easily decomposed (low C:N ratio), stimulate microbial growth and the microbial necromass is subsequently stabilised by binding to minerals in the MAOM fraction (Cotrufo et al., 2013; Haddix et al., 2020).

1.7.2 Cover crop effect on SOM

The main management option to increase SOM is increasing the biomass input, which is done by incorporating cover crops in soil (Poeplau and Don, 2015). Indeed Poeplau and Don (2015) showed that single cover crops can increase the soil C content by 0.32 ± 0.08 Mg C ha^{-1} y^{-1}. However they mentioned only the total C pool and not the individual fractions. In order to sequester C ideally both the micro-aggregates and the MAOM fraction are increased since these are most stable in the soil, whereas DOM and POM persist for a shorter time and could thus release nutrients thereby increasing nutrient availability for the next crop. As mentioned previously Cotrufo et al. (2013) proposed that residues which are easily decomposed, such as cover crops could potentially increase the stable C fraction in soil. Yet this remains to be tested. Additionally, cover crop mixtures are hypothesised to have a higher biomass as well as a higher variation in residue composition relative to biomass from a monocrops. It is unclear if individual or cover crop mixtures increase the stable or the labile C fractions. And subsequently if this positively affects C sequestration as well as cash crop growth.

1.8 This thesis

1.8.1 Research question and hypotheses

In this thesis I aimed to answer the overall research question “Do cleverly chosen cover crop mixtures, with diverse litter traits improve element cycling in an agricultural rotation?”. To answer this question I examined if cover crop mixtures affect N cycling; N in the cover crops, main crops, in the soil as well as losses through N leaching and N_2O emissions. Besides I examined how litter trait diversities in cover crop (mixtures) affect the C cycle, in terms of decomposition rates, labile C and C sequestration in stable C fractions. My overall hypothesis is: Mixing cover crop species increases and diversifies biomass input in soil, which enhances soil organic matter content and mineralisation rates while reducing nutrient losses during and within the growing season (Fig 1.1).

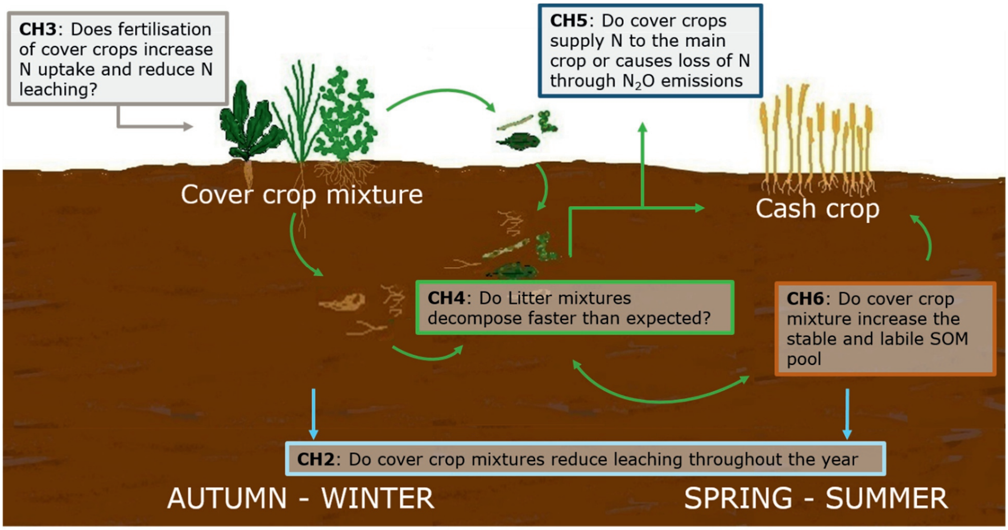

Figure 1.1 Framework of this PhD thesis which encompasses cover crop mixture effects on elemental flows during cover crop growth (autumn/winter), after cover crop incorporation (spring) and during cash crop growth (spring/summer). The boxes represent the 5 experimental chapters in this thesis.

Below I present the 5 main hypothesis I tested, each corresponding to the main focus of one of my 5 experimental chapters:

1) Cover crop mixtures are more effective at reducing N leaching during growth compared to single cover crops

2) Fertilising non leguminous cover crops with N will kick-start cover crop growth and thereby reduce N leaching, whereas fertilising leguminous cover crops will increase N leaching

3) Greater chemical litter dissimilarity in leaf litter mixtures will cause larger non-additive mass loss in litter mixtures compared to litter mixtures of species with high trait similarity

4) Mixtures of cover crop residues of different qualities will simultaneously provide N for plant growth and will limit N_2O emissions from unplanted soil, relative to soil with residues of the individual species

5) Cover crop (mixtures), by adding biomass of diverse quality, increase labile and stable soil organic C

1.8.2 Introduction of the long term field experiment

In order to test my hypotheses mentioned above I set-up a four year field experiment in collaboration with two other PhD projects. The PhD research of Ali Elhakeem focussed on productivity, resource capture, resource use efficiency and competitive relationships in cover crop mixtures during sub-optimal growth conditions in autumn, the PhD research of Sytske Drost examined if mixtures increase microbial functioning and soil quality. The field experiment consisted of three cover crop species, *Raphanus sativus*, *Avena strigosa* and *Vicia sativa.* These species were chosen based on the idea of "Clever over cropping", *i.e.* choosing cover crops that are complementary in terms of many traits (Fig 1.1). We chose a cover crop from the Brassicaceae family because it is a high yielding cover crop with a taproot system. The oat, from the Poaceae family, was equally high-yielding, yet is more winter hardy with a higher C/N ratio and a fibrous root system. Vetch, from the Fabaceae family, is a legume and thus can fix its own N, it has a lower C/N ratio compared to grasses and non N_2-fixing forbs and generally produces the lowest biomass of the three species in our species pool.

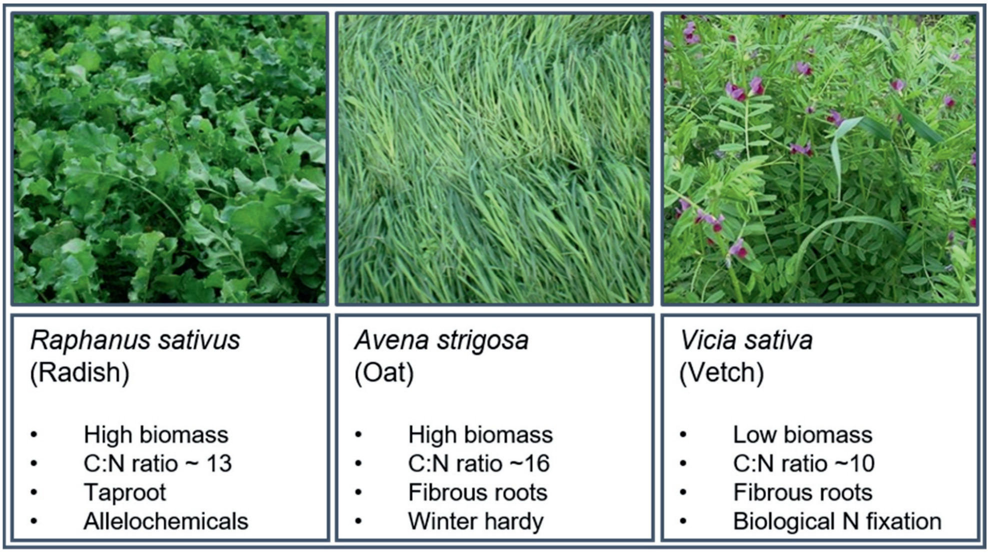

Figure 1.2 Cover crop selection for the Clever Cover Cropping field experiment (2016-2020).

These species were grown in monoculture as well as in all possible 2-species and a 3-species mixture. Besides from these treatments we also added a fallow. This came to a total of 8 treatments which were replicated 5 times and sown in a complete randomised block design. Cover crops were sown in August of each year, starting in 2016, and they were incorporated into the soil in February or March of the next year. In spring a cash crop was planted which rotated each year (maize 2017, potato 2018, barley 2019 as a common rotation). The experiment was located on a sandy soil close to Wageningen university (The Netherlands).

1.8.3 Chapter outline

In *chapter 2* I used the long term field experiment to assess if cover crops reduced leaching over time. I quantified N leaching during cover crop growth in autumn and winter as well as during cash crop growth in spring and summer. In order to explain differences in leaching between the cover crops I linked N leached to the cover crop above- and belowground biomass as well as the N concentration in the crop and in the soil.

In *chapter 3* I used another field experiment, with the same crop species (2018-2019) to examine if fertilising cover crops is necessary in order to kick-start growth. I analysed if biomass as well as nett N capture was increased by increasing fertilisation in steps from 0 to 90 kg N/ha. Additionally we assessed if applying extra N fertiliser did not unnecessarily increase leaching of N.

In *chapter 4* I used a meta-analysis on litterbag mixing studies to assess if mixing litters, of any species, consistently affects decomposition rates. Here I tested if litter trait differentiation affected the size and direction of litter mixing effects.

In *chapter 5* I examined, in a greenhouse experiment, if (mixing) cover crop residues affect the release of mineral N in the soil over time. Additionally the effect of the release of mineral N on subsequent cash crop growth and N_2O emissions was tested.

In *chapter 6* I tested if soil organic matter quantity and quality was changed due to the incorporation of cover crops in an agricultural rotation after 3 and 4 years of cover crops on a sandy tilled soil. The SOM quality was assessed by measuring different fractions of C ranging from labile to stable.

Chapter 2

Are cover crop mixtures more effective than cover crop monocultures at reducing N leaching losses?

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Abstract

There is a large risk of N leaching losses in autumn, the period after the cash crop harvest. Growing cover crops during this period can prevent or reduce these leaching losses. Complementary plant species growing in mixtures, are commonly reported to produce a higher average yield and capture more nutrients than monoculture cover crops and could perhaps reduce leaching losses of N even more. Therefore we hypothesised that cover crop mixtures reduce N leaching more than cover crop monocultures during their growth in autumn. Additionally we expected that cover crop mixtures had a higher root length density at depth compared to monoculture cover crop species. Finally we hypothesised that cover crop (mixtures) with a low C:N ratio and a high biomass production will lead to the largest N losses through leaching in spring after cover crop incorporation in the soil. Three monoculture cover crop species: radish (*Raphanus sativus*; a crucifer), vetch (*Vicia sativa*; a legume) and oat (*Avena strigosa*; a grass), as well as all possible 2- and 3-species mixtures were grown for 4 seasons in an agricultural rotation with a different cash crop each year. Leaching losses were estimated by analysing N in samples of pore water below the rooting zone and by modelling the volume of water leached per plot. We found that radish (containing mixtures) consistently reduced N leaching the most during the cover crop growing season. N leaching during cover crop growth was negatively related to the (root) biomass of the cover crops. Leaching losses during cash crop growth were not consistently higher after cover crops than after fallow soil. Yet, leaching could have occurred just after cover crop incorporation prior to the first spring sampling. Considering the (N) yield stability of mixtures, we showed that mixtures are the safest choice when looking at reducing N leaching of cover crop mixtures on sandy soils.

2.1 Introduction

In agricultural production, high fertilisation rates often come with N losses and environmental concerns (Foley et al., 2011; Quemada et al., 2013). A significant part of this N is often lost via leaching of nitrate (NO_3^-) as this form of N is mobile in the soil solution (Di and Cameron, 2002; Tilman et al., 2002). Autumn, the period after the cash crop harvest, is most prone to leaching losses in temperate regions (Di and Cameron, 2002). Growing cover crops during this period can prevent or at least reduce these leaching losses (Abdalla et al., 2019; Thapa et al., 2018). Cover crops will scavenge remaining nutrients via their root uptake from the soil and incorporate them into their biomass (Kaspar and Singer, 2011; Thorup-Kristensen et al., 2003). When the cover crops are terminated at the end of the winter or early spring the cover crop biomass is incorporated in the soil and serves as green manure. However, this could possibly cause leaching losses if N becomes available too soon, before the cash crop can take it up.

Mineral N enters the soil through fertilisation, SOM decomposition and through biological N fixation by free living N-fixing bacteria or in association with leguminous species. Due to the uptake of nutrients and soil pore water by crops, the concentration of nutrients but also the total downward water flow is lower compared to fields without vegetation (Blanco-Canqui, 2018; Kaspar and Singer, 2011). Cover crops have been shown to reduce nitrate leaching by 6 to 94%, depending on the type of cover crop (Kaspar and Singer, 2011). High biomass producing cover crops (such as cereals and crucifers) are generally most efficient in preventing N leaching, whereas leguminous cover crops, which fix atmospheric N_2, are generally less efficient (Blanco-Canqui, 2018; Thapa et al., 2018). A meta-analysis by Thapa et al. (2018) showed that non-legume cover crops reduced leaching on average by 56% while leguminous cover crops did not reduce leaching losses compared to fallow soil. Additionally, rooting patterns can have a large influence on the amount of N leached: crops that root deeper can intercept N further down the soil profile, while a high root length density facilitates uptake from a larger surface area in the soil (Thorup-Kristensen, 2001).

It is known from research in natural systems that increased plant species diversity can improve resource exploitation in the soil, which leads to an increased plant biomass C and N and to C and N accumulation in the soil (Cong et al., 2014; De Deyn et al., 2009; Fornara and Tilman, 2008; Tilman et al., 1996). The main mechanism underlying these plant diversity effects on C and N is complementarity between plant species in their growing strategies and resource acquisition which results in more efficient uptake of light, water and nutrients from the system (Wendling et al., 2017). Root architecture (rooting depth, root length distribution and diameter) varies widely among cover crop species (Bukovsky-Reyes et al., 2019) and rooting-based complementarity of cover crop species could therefore lead to a higher nutrient uptake by cover crop mixtures than by monoculture cover crops, and thus reduce NO_3^- leaching more effectively (Brooker et al., 2015; Hauggaard-Nielsen and Jensen, 2005; Miyazawa et al., 2010). Leguminous species can facilitate growth of neighbouring plants by increasing the N availability through biological N fixation (Blesh, 2018; Blesh et al., 2019; Bukovsky-Reyes et al., 2019). Because of this complementary resource use, where legumes can partly rely on fixed N_2, the companion species in the mixture is likely to have a higher biomass than expected from monocultures (Weidlich et al., 2018). This is mainly attributed to limited N and intraspecific competition for light in monocultures (Evers et al., 2019). Species mixtures could produce a higher biomass than expected (overyielding) or even a higher biomass than the most productive monoculture (transgressive overyielding) (Hector, 2006). When mixtures overyield, and thereby contain more nutrients in their biomass than monocultures (Cardinale et al., 2007), more nutrients will be returned to the soil in spring. In this case, cover crop mixtures have the potential to reduce N leaching in fall while also supplying the crop with adequate amounts of N in spring (Kaye et al., 2019).

After termination and incorporation of cover crops into the soil, the residues mineralise and N will become available for crop uptake. A higher N uptake by cover crops in autumn potentially results in larger N availability to the cash crop during its growing season. The rate at which this N in the cover crops becomes available for the cash crop depends on the cover crop residue quality (Bichel et al., 2017). Residues with a lower C:N ratio are expected to mineralise faster and thus release mineral N quicker, potentially resulting in leaching losses at the start of the cash crop growing season when N availability exceeds N uptake. On the other hand, residues with high C:N ratio lead to N immobilisation by the soil microbes thereby reducing N availability and suppressing crop growth (Thorup-Kristensen et al., 2003). Non-leguminous cover crops are usually worse than leguminous cover crops at supplying the soil with adequate amounts of nutrients in the spring (Kaspar and Bakker, 2015; Marcillo and Miguez, 2017; Thorup-Kristensen et al., 2003; Tonitto et al., 2006). Cereals in particular have a low N content, and thus decompose more slowly (Dabney et al., 2001). When cover crop decomposition is very slow, N immobilisation in the soil hampers cash crop growth (Marcillo and Miguez, 2017). Mixtures of legumes and non-legumes are expected to have a higher biomass production and N content. And, if the non-legume and legume in the mix decompose at different rates, these properties may prevent N immobilisation as well as N leaching.

In a four year field study, we aimed to test if cover crop mixtures reduce N leaching more than cover crop monocultures, during their growth in autumn, as well as after their termination during cash crop growth. We hypothesised that 1) during cover crop growth, N leaching is lower under cover crop mixtures than under sole cover crops, 2) cover crop mixtures have a higher root length density at depth compared to monoculture cover crop species, 3) N leaching losses in autumn are negatively correlated to cover crop biomass production, N uptake and root length density across monocultures and mixtures, and 4) cover crop (mixtures) with a low C:N ratio and a high biomass production lead to the largest N losses through leaching in spring.

2.2 Materials and methods

2.2.1 Field design

The field experiment was conducted on a sandy soil (83% sand, 12% silt, 2% clay, pH 5.3, 1.09 g/kg total N, 58 mg/kg total P of which 2.2 mg/kg plant-available P, and 91 mg/kg K; Nergena, Wageningen, The Netherlands, 51°59′41.9″N, 5°39′17.5″E). The field had a history of a conventional arable rotation. In 2016, before the start of the field experiment, winter wheat was grown on the entire field. The field experiment consisted of 8 cover crop treatments: three monoculture species, all possible 2- and 3- species mixtures of these monoculture cover crops and an fallow treatment (no cover crop, thus not fertilised in august). The cover crop species were: radish (*Raphanus sativus*; R), vetch (*Vicia sativa*; V, a legume) and oat (*Avena strigosa*; O). The 8 treatments were replicated 5 times as a complete randomised block design in plots of 10 by 6 metres. The cover crops were sown with a row spacing of 12.5 cm and at a depth of 2 cm. Seeding rates for the monocultures species radish, vetch and oat were 30 kg/ha, 110 kg/ha and 90 kg/ha respectively, according to farmer practice. Species were mixed according to a replacement design, using, for each of the species, 50% and 33% (in the 2- and 3-species mixtures respectively) of the seed rate in the monocultures. To ensure establishment, cover crops were fertilised after sowing with calcium ammonium nitrate (CAN: 27% N - 6% CaO - 4% MgO; 30 kg N/ha) (Fig 2.1). The cover crop growing season lasted from August until February of the next year. Subsequently the cover crops were mechanically terminated and incorporated into the soil up to a depth of 20 cm. Each year a different cash crop was grown after the cover crops (Fig 2.1). In 2017, maize (LG 31.211, supplied by Agrifirm) was fertilised with 300 kg K/ha (Kali60), 59 kg P/ha (Triple superphosphate) and 95 kg N/ha (CAN). Herbicide was applied as roundup to avoid weed growth between the rows. Due to a drought in the Netherlands the maize was irrigated on the 20-6-2017 with twice 15 mm. In 2018, potato (variety “eersteling”) was fertilised with 110 kg N/ha (CAN), 46 kg P_2O_5/ha and 60 kg K_2O/ha. In 2019, barley (variety “IRINA”) was fertilised with 60 kg N/ha (CAN).

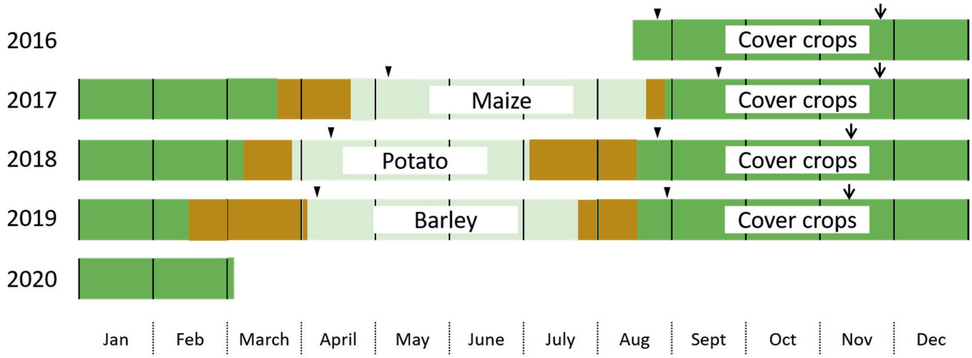

Figure 2.1 Timeline of the field experiment which started in August of 2016 and ended in March of 2020. Dark green bars indicate the cover crop growing season, light green bars the cash crop growing season. Brown bars indicate time during cash crops and cover crops, at which soil was prepared for the following crop. Arrows indicate the moment of cover crop subsampling. Triangles indicate the timing of fertilisation of the cover crop and cash crop. Cover crops were fertilised with 30 kg N/ha each year. Maize, potato and barley was fertilised with 95, 110 and 60 kg N/ha respectively.

2.2.2 Cover crop and cash crop biomass

Cover crop aboveground biomass was sampled 12 weeks after sowing (peak standing biomass; Fig 2.1). Biomass was sampled from two randomly allocated 1 m^2 squares at the centre of each plot by cutting just above the soil surface. Sampled biomass was washed and dried at 70 °C for 48h. In 2017-2019, biomass of mixtures were separated per species. Species were not separated in 2016. In 2016, cover crop root biomass was estimated by sampling with a 12 cm × 30 cm × 30 cm metal frame, which was placed perpendicular on the rows so multiple rows were included in the sample. In 2017-2019, four root cores (10 cm Ø, 30 cm depth) were extracted, two cores were sampled in the rows and two cores between the rows. Each core was divided into four depth layers, 0-5 cm, 5-10 cm, 10-20 cm and 20-30 cm. Samples from each plot were pooled together by depth. Root biomass was rinsed with water, in order to remove adhering soil and organic matter particles, and dried at 70 °C for 48 h. A root subsample from each plot was stored in alcohol and coloured with “Natural Red” dye, and analysed for root length density and specific root surface area using WinRhizo (WinRHIZO Pro 2013e) (Bouma et al., 2000).

Maize, potato and barley biomass was determined by harvesting a net plot (excluding border rows) of 18, 18 and 11 m^2 for the three respective crops. From each plot, a subsample was randomly collected from the harvested area and oven dried at 70°C to determine the cash crop dry weight. After drying, cover crop above- and belowground biomass as well as cash crop aboveground biomass were subsequently ball milled. Concentration of C and N in shoot and root were determined using combustion in an ElementAnalyser (Flash EA 1112, Thermo Scientific).

2.2.3 Pore water collection

Pore water collection, and analysis of the concentration of mineral N in the pore water, was done on eighteen dates (Fig 2.2). Three ceramic cup samplers were placed in each field plot on an angle of 45° at a depth of 70 cm below the soil surface (Rhizosphere, 2017). Pore water samples were stored in the freezer and subsequently analysed for NO_3^- and NH_4^+ on an AutoAnalyzer (SEAL QuAAtro SFA system, Beun- de Ronde B.V. Abcoude, The Netherlands). The frequency of collecting leachates was around once per month, when sufficient rainfall was predicted (Fig 2.2). Sufficient rainfall meant at least 5 mm of rain predicted for the next day. This was not sufficient in the period of drought (2018), when there was a large water deficit in the soil.

2.2.4 Soil cover

Soil cover was determined according to Elhakeem et al. (2019). In short, photos were taken each week, starting 2 weeks after sowing. A metal frame (1×0.75 m^2) was placed near the top of the canopy to standardise the area of which the photo was taken. A camera was placed above the frame pointing vertically down in a fixed position (1 m distance from the canopy). To determine the percentage ground cover, photos were analysed using the DIPimage toolbox for image analysis in MATLAB (MathWorks, 2013). A logistic model was fitted to the observed ground cover data. The fraction of ground cover for each day was estimated as:

$$GC_T = \frac{GC_{max}}{1+\exp^{-s(T-T_{\_50\%})}},$$

where GC_T is a fraction of ground cover at day T, GC_{max} is the maximum ground cover fraction, $T_{50\%}$ is the time when 50% of maximum predicted soil cover was reached, while s is a parameter that describes the steepness of the function through $T_{50\%}$.

2.2.5 Leachate estimation

We used the Soil Water Atmosphere Plant (SWAP) model to estimate leaching losses at the plot scale (Kroes et al., 2017). SWAP simulates the water movement and root water extraction in the soil, which we used to estimate the total volume of water percolation to the subsoil for the entire cover crop growing season, and thus the total amount of N leached by accounting for the concentrations of mineral N in the leachates.

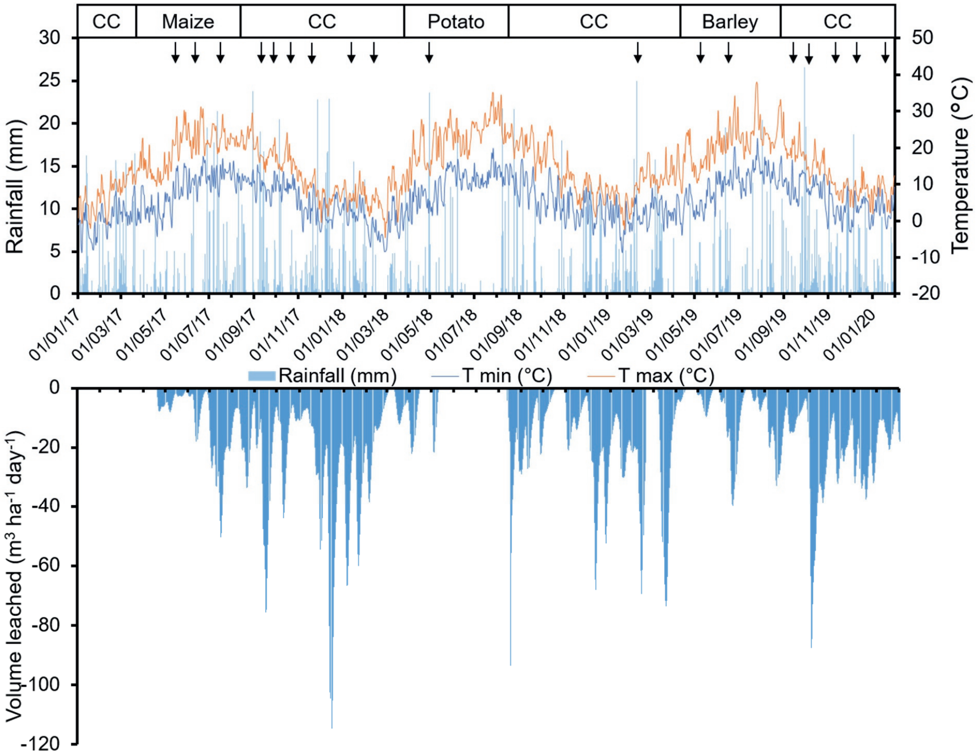

Figure 2.2 Timeline of the field experiment and the associated weather pattern and volume water leached. The top bar indicates the crop rotation with cover crops (CC) and three different main crops. The light blue bars indicate the rainfall in mm (primary positive axis), the blue line indicates the minimum and the orange line the maximum temperature in °C (secondary positive axis). The black arrows indicate the time of pore water sampling. The dark blue bars indicate the modelled groundwater flow in the fallow plot (only cash crop growth, no cover crops planted).

Rainfall data were obtained from a weather station of Wageningen University (the Veenkampen; 51°58'53.4"N 5°37'13.7"E); reference evapotranspiration data were obtained from the KNMI (Royal Dutch Meteorological Institute). The percentage ground cover by cover crops (Table S2.3, appendix) and the root length density at depth were used as crop input in the model. We obtained the data of the soil hydraulic properties, according to the soil type, from the handbook of SWAP (Kroes et al., 2017). The initial soil water status was assumed at field capacity in January of each year. Simulation of water infiltration, redistribution, and percolation in the soil is based on the Richards equation. Root water extraction is based on the potential transpiration, root density distribution and the drought reduction function of (Feddes et al., 1978). Groundwater levels were estimated based on data from a groundwater well in the vicinity (51°59'54.6"N 5°39'47.7"E, ~400 m distance from field experiment). The amount of N leached in each plot at each date was calculated by multiplying the mineral N concentration in the pore water and the simulated volume of water leached on that day (24 hours).

The amount of N leached on each day was calculated for the 17 sampling days when downward water flow occurred. We summed the N leached measured (in kg N/ha/day) on those days, thus the amounts of N leached shown are the sums of the 17 days N measured. We divided the amount of N leached in two periods: during the cover crop season (September to March) and during the cash crop season (April to August).

2.2.6 Bulk density and soil mineral N

Bulk density of the top soil (0-20 cm) was measured in each plot at the start of the field experiment in August 2016. An intact soil core (from 10 cm depth) was taken by inserting a metal ring of 100 cm^3 volume. Subsequently the soil was dried at 105 ºC to determine the dry weight. To determine soil mineral N, samples were collected on the day of cover crop sowing (August), cover crop subsampling (November) and cover crop incorporation (February or March) from each plot. We pooled 7 random samples, to a depth of 20 cm, of each plot together. Soil samples were dried at 40 °C for 48 hours, sieved over 2 mm and subsequently extracted with a 0.01 M $CaCl_2$ extraction (Houba et al., 2000a). The samples were analysed on an AutoAnalyzer (SEAL QuAAtro SFA system, Beun- de Ronde B.V. Abcoude, The Netherlands) for $NO_3^- + NO_2^-$ and NH_4^+.

2.2.7 Data analysis

All statistics were carried out in R version 3.5.1 (R core team, 2016). Figures were made using the packages ggplot2 (Wickham, 2016) and cowplot (Wilke, 2019). We tested the effect of cover crops (factor, 8 levels) on N leaching using linear mixed effects models with the function lme from the package nlme (Pinheiro et al. 2016) with 'block' as a random factor. Significance was determined with analysis of variance (ANOVA), anova (base) (R Core Team, 2018). Normality and homogeneity of model residual variances were assessed by plotting model residuals. Heterogeneous residual variances were addressed using a variance structure in the model with the function varIdent (nlme) (Pinheiro et al., 2019). The model with the variance structure was retained when it showed a significantly better fit than the model without, as determined by comparing the model's akaike information criterion (AIC) using the function anova (R Core Team, 2018). To determine significant differences between cover crops, a Tukey's honestly significant difference posthoc test (HSD) was used, with the function emmeans (Lenth et al., 2019) and CLD from package multcomp (Hothorn et al., 2008). For the correlation between N leached and cover crop parameters, we fitted non-linear (nls) models and used ANOVA (anova), as above.

2.3 Results

2.3.1 N leached

Leaching measured during the cover crop season was higher than during the cash crop season. Across the years leaching was highest in 2017, both during the cover crop and cash crop season. During the cover crop growing season leaching under vetch and fallow was similarly high in all three years and generally lower in the other cover crop treatments (Fig 2.3, Cover crop: $F_{7,28} = 6.09$, $p < 0.001$).

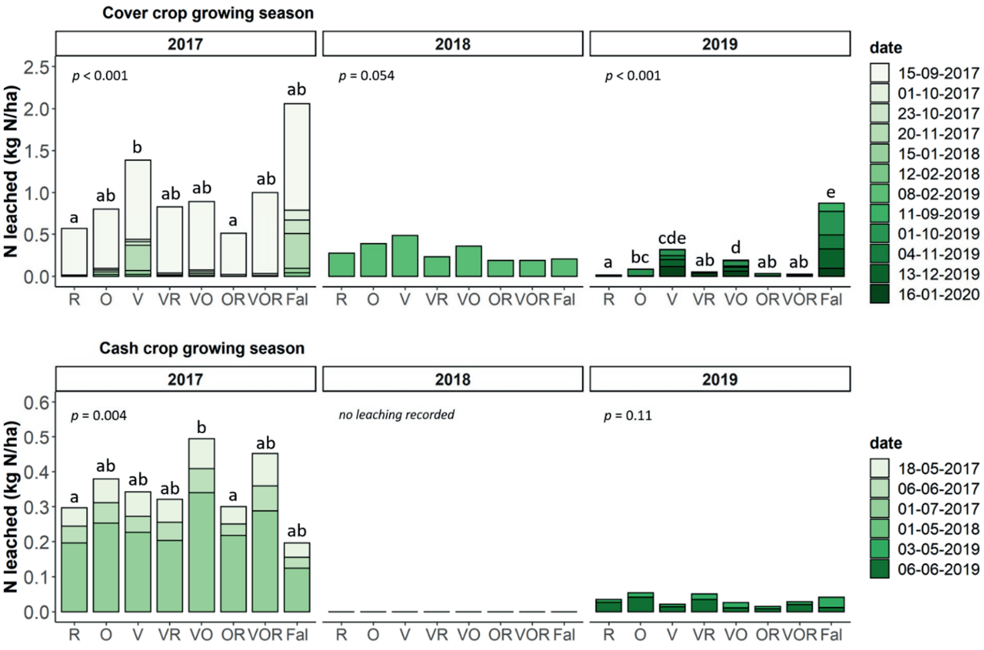

Figure 2.3 N leached during the cover crop growing season (between September and February) and during the cash crop growing season (between March and August). The different shades of green represent the measurement days. Different letters indicate significant differences between the sum of the amount of N leached of that time period. The cover crops are: R (radish), O (oat), V (vetch), all their combinations and fallow (Fal, no cover crop grown) (n = 5).

Leaching under radish was consistently lowest. Yet, leaching under the 2- and 3-species mixtures was not higher than leaching under radish monocultures in any of the years, with the exception of the vetch + oat mixture in 2019. During the cash crop season, cover crop treatments did not cause higher leaching compared to the fallow. Comparing the cover crop treatment effects during cash crop growth we only found higher leaching losses in crops grown in 2017 after the vetch + oat mixture compared to crops grown after the radish monocrop (Fig 2.3, Cover crop: $F_{7,28} = 3.94$, $p = 0.004$). The sum of N leached over the three years varied between cover crops (Fig S2.1, appendix, Cover crop: $F_{7,28} = 8.29$, $p < 0.001$). N leached in the vetch treatment was not different from the fallow soil. The amount of N leached was twice as high under soil where vetch was grown compared to radish. Mixtures containing radish had similar leaching losses to radish monocultures.

In 2018 we did not record leaching during the cash crop season due to drought and in 2019 cover crops did not cause higher leaching in spring compared to the fallow. Pore water collection in 2018 was only possible twice, and during summer and autumn no downward water flow occurred due to a dry spell (Fig 2.2). Throughout the year, the modelled groundwater flow for the fallow treatment was substantially higher than for treatments with cover crops (Table S2.2, appendix). Radish and radish-containing mixtures generally reached full groundcover quickest. In 2017, 50% groundcover was reached approximately 20 days later than in 2019. Differences in flow between cover crops were only present in September and October, generally radish (containing mixtures) reached full groundcover quickest and thus had the lowest downward waterflow. This difference was biggest on October 1st in 2019, on this day the difference was largest between the radish + vetch mixture and oat (2.5 m^3 ha^{-1} day^{-1}, i.e.0.25 mm day^{-1}).

2.3.2 Cover crop biomass and N yield

Cover crop biomass measured in November varied among the years, biomass was lowest (~3 Mg/ha) in 2017 and was approximately double that (~6 Mg/ha) in 2018 and 2019 (Fig 2.4A; $F_{2,80} = 12.9$; $p < 0.001$). Cover crop biomass varied among cover crops ($F_{6,80} = 6.4$; $p < 0.001$), on average across the years, the mixture of vetch + radish produced the highest (~6.1 Mg/ha) biomass whereas the monoculture vetch produced the lowest biomass (~1.9 Mg/ha). There was a significant interaction between year and cover crop on biomass ($F_{12,80} = 3.6$; $p < 0.001$).

On average over the years the monoculture species (radish, vetch and oat) produced 3.6, 1.3 and 4.7 Mg/ha shoot biomass (dry weight) respectively. Shoot biomass of the mixtures was similar to the biomass of radish. Root biomass also varied among the years ($F_{2,80} = 7.36$; $p = 0.001$) and among cover crops ($F_{6,80} = 26.28$; $p < 0.001$). Root biomass was lowest in vetch (0.5

Mg/ha) and highest in radish (1.6 Mg/ha). Cover crop mixtures containing radish had root biomass similar to radish monocultures.

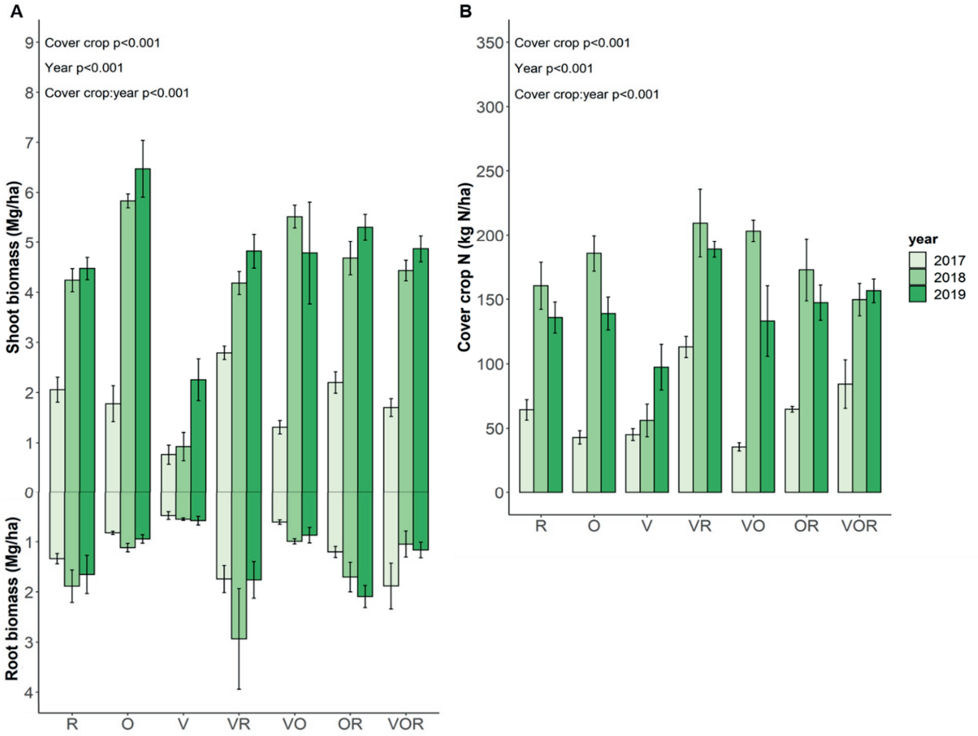

Figure 2.4 Cover crop A) shoot and root biomass and B) total N measured at maximum standing biomass in November each year. The different colours represent the year of measurement. The cover crops are: R (radish), O (oat), V (vetch), all their combinations. Statistics are on total cover crop biomass (shoot + root). Bars are means, error bars denote the standard error (n = 5).

Cover crop N yield varied among the years (Fig 2.4B; $F_{6,77}$ = 25.7; p < 0.001), between the cover crops ($F_{6,80}$ = 10.85; p < 0.001) and there was a significant interaction between cover crop and year ($F_{12,77}$ = 5.1; p < 0.001). N yield was considerably lower in 2017 (~ 50 kg N/ha) compared to 2018 and 2019 (~150 kg N/ha). On average over the years, N yield was lowest for vetch (66 kg N/ha) and highest for the vetch + radish mixture (170 kg N/ha). The C:N ratio (averaged over the years) of radish, vetch and oat were 18, 12 and 23 respectively.

2.3.3 Root length density

The average root length density (RLD) over depth measured in November 2019 varied between the cover crop species (Fig 2.5; $F_{6,80}$ = 2.57; p = 0.03). The average RLD over depth was lowest in vetch (6 cm/cm^3) and highest in the oat + radish mixture (10 cm/cm^3). The RLD in the deeper layer (20-30 cm) of the mixtures was similar to radish and oat. The RLD of vetch was significantly lower than the 3-species mixture in this layer ($F_{6,59} = 3.07$; $p = 0.01$) whereas the RLD of all cover crop (mixtures) were similar in the top two layers.

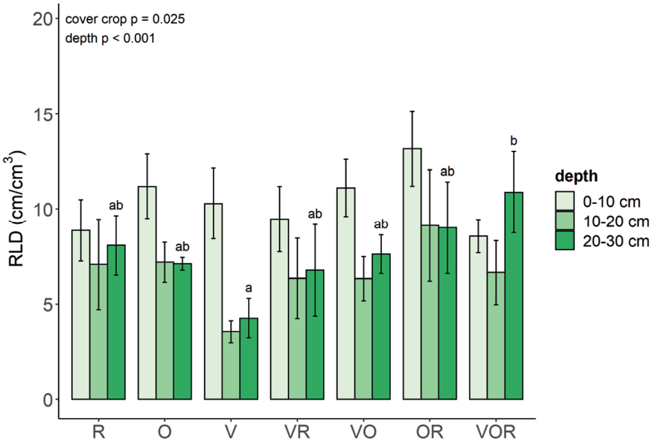

Figure 2.5 Root length density (RLD: cm/cm^3 soil) of cover crops at maximum standing biomass, November 2019, divided into 3 depth layers: 0-10 cm, 10-20 cm and 20-30 cm depth. Bars are means, error bars denote the standard error (n = 5). Bars with different letters indicate significant differences between the cover crops, differences between cover crops were only significant in the 20-30 cm depth layer ($p < 0.05$).

2.3.4 Soil N

Soil mineral N measured in March, August and November varied per cover crop treatment (Table 2.1; March $F_{7,92} = 13.48$, $p < 0.001$; August $F_{7,92} = 7.47$, $p < 0.001$; November $F_{7,124} = 7.52$, $p < 0.001$) and year (Table 2.1; $F_{2,92} = 31.67$, $p < 0.001$; $F_{2,92} = 9.35$, $p < 0.001$; $F_{3,124} = 125.92$, $p < 0.001$ respectively for 2017, 2018 and 2019). Soil mineral N was lower in November than in March and August. On average over the years, soil mineral N was highest under vetch at the end of the cover crop season in November. Yet, in March and August, vetch tended to have slightly lower soil mineral N compared to the other cover crop (mixtures). Soil mineral N of the fallow soil was generally lower than the cover crop treatments.

Table 2.1 Soil mineral N (kg $N\text{-}NO_3 + N\text{-}NO_2 + N\text{-}NH_4\ ha^{-1}$). Different lowercase letters indicate significant differences between cover crop treatments for each sample date (n = 5; * $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$). Radish (R), vetch (V), oat (O), their 2- and 3-species mixtures and a fallow (Fal).

	2017			2018			2019		
CC	March	August	November	March	August	November	March	August	November
R	32.1 ± 2.5 c	20.4 ± 3.0 ab	9.5 ± 0.8	24.5 ± 4.6 ab	40.8 ± 3.5	20.4 ± 2.7 abc	34.8 ± 2.4 bc	33.7 ± 3.0	17.1 ± 1.1 b
O	19.0 ± 0.8 ab	11.4 ± 2.7 a	8.4 ± 1.1	16.9 ± 2.2 a	41.3 ± 5.7	17.7 ± 0.8 bc	24.2 ± 2.2 ab	27.5 ± 1.6	17.1 ± 2.7 ab
V	24.8 ± 1.6 c	15.8 ± 4.4 ab	10.1 ± 1.4	17.4 ± 2.2 ab	36.4 ± 2.2	20.9 ± 2.7 abc	26.9 ± 2.2 ab	28.6 ± 1.4	24.2 ± 3.0 b
VR	35.1 ± 3.3 c	32.9 ± 6.0 b	10.3 ± 1.6	25.0 ± 5.7 ab	43.2 ± 2.4	20.9 ± 1.9 c	38.1 ± 5.4 abc	29.6 ± 2.4	20.9 ± 2.4 b
VO	27.7 ± 6.0 abc	15.2 ± 1.4 ab	10.1 ± 0.8	26.9 ± 4.4 ab	46.0 ± 3.0	20.1 ± 1.1 abc	30.5 ± 2.2 bc	30.5 ± 3.0	18.0 ± 1.4 b
OR	29.9 ± 2.2 c	23.9 ± 6.3 ab	9.8 ± 1.9	22.8 ± 3.8 ab	50.9 ± 5.2	17.7 ± 1.4 abc	46.0 ± 7.6 bc	28.3 ± 1.1	15.2 ± 0.5 ab
VOR	26.1 ± 1.9 bc	26.4 ± 5.2 ab	10.3 ± 1.4	20.4 ± 1.9 b	38.4 ± 3.5	15.5 ± 1.1 a	40.8 ± 3.3 c	27.0 ± 1.4	16.6 ± 1.1 ab
Fal	18.0 ± 1.1 a	13.9 ± 1.1 a	6.8 ± 0.5	13.3 ± 2.7 ab	40.0 ± 3.3	15.8 ± 1.4 ab	19.9 ± 2.2 a	28.0 ± 1.6	12.5 ± 0.5 a
	***	*	ns	**	ns	***	***	ns	**

2.3.5 Relationship between cover crop and N leaching

In 2019, the total cover crop biomass and the cover crop root biomass were related to the amount of N leached in November by a negative exponential relationship (Fig 2.6A, $y = 81e^{-0.37x}$, $p < 0.001$; Fig 2.6B, $y = 68.8e^{-1.86x}$, $p = 0.01$, respectively). A similar relationship was found in 2017 (Fig S2.2, appendix). The RLD (only measured in 2019) and the total N yield were not related to the amount of N leached in November 2019 (Fig 2.6 B and D).

2.4 Discussion

2.4.1 N leaching during the cover crop growing season

The objective of this study was to test if cover crop mixtures reduce N leaching more than sole cover crops. Contrary to what we expected, cover crop mixtures did not reduce N leaching more than the most productive cover crops: radish and oats. Radish consistently reduced N leaching the most. Yet, over the years, mixtures containing radish were as effective as radish grown alone at reducing N leaching. This suggests that growing radish alone, or in mixtures with oat or vetch can be a safe choice if reduction of N leaching is the aim. This corroborates findings by Creissen et al. (2016) and Couëdel et al. (2018) who found that crucifer-legume mixtures take up equally as much N as crucifers grown alone and thus have a good potential for N leaching reduction. Mixtures, on average over the years, had comparable biomass and N yield to radish and oat in our study, which were the most productive monoculture cover crops. This confirms findings by Elhakeem et al. (2019) that using mixtures can mitigate the risk of crop failure because the companion species can take over if the other species fails. This explains why leaching under mixtures was similarly reduced, even when vetch was in the mixture. This can be partly explained by the competitive strength of radish and oat compared to vetch. Generally radish and oat were dominant in the mixture in terms of their biomass, similar results were previously reported by Elhakeem et al. (2019). This can in part be explained because vetch fixes its own N and thus more N remains in the soil for uptake by oat or radish. Additionally, radish is a strong competitor for water, nutrients and light due to its fast initial root and shoot growth (Creissen et al., 2016). Thus, species that have a high demand for nutrients and light can be more competitive under low light and nutrient conditions. In fact, Wendling et al. (2019) suggested that the ratio between leguminous-non leguminous species changes according to soil conditions where leguminous species are stronger competitors on soil poor in N compared to on soil rich in N. Since our cover crops were fertilised with 30kg N/ha this would likely make vetch an especially poor competitor in our field conditions.

2.4.2 RLD of mixtures

Contrary to what we expected, the RLD in cover crop mixtures in 2019 was similar compared to the monoculture species. However, for the 20-30 cm soil depth layer we found a larger RLD in the three-species mixture compared to vetch monoculture. This is most likely because vetch generally has a thicker and less dense rooting system. Yet here we see that, in deeper layers, the mixtures had a similar RLD as the oat and radish monocultures. Previous studies in intercropping systems have often alluded to deeper rooting in mixtures as to explore more

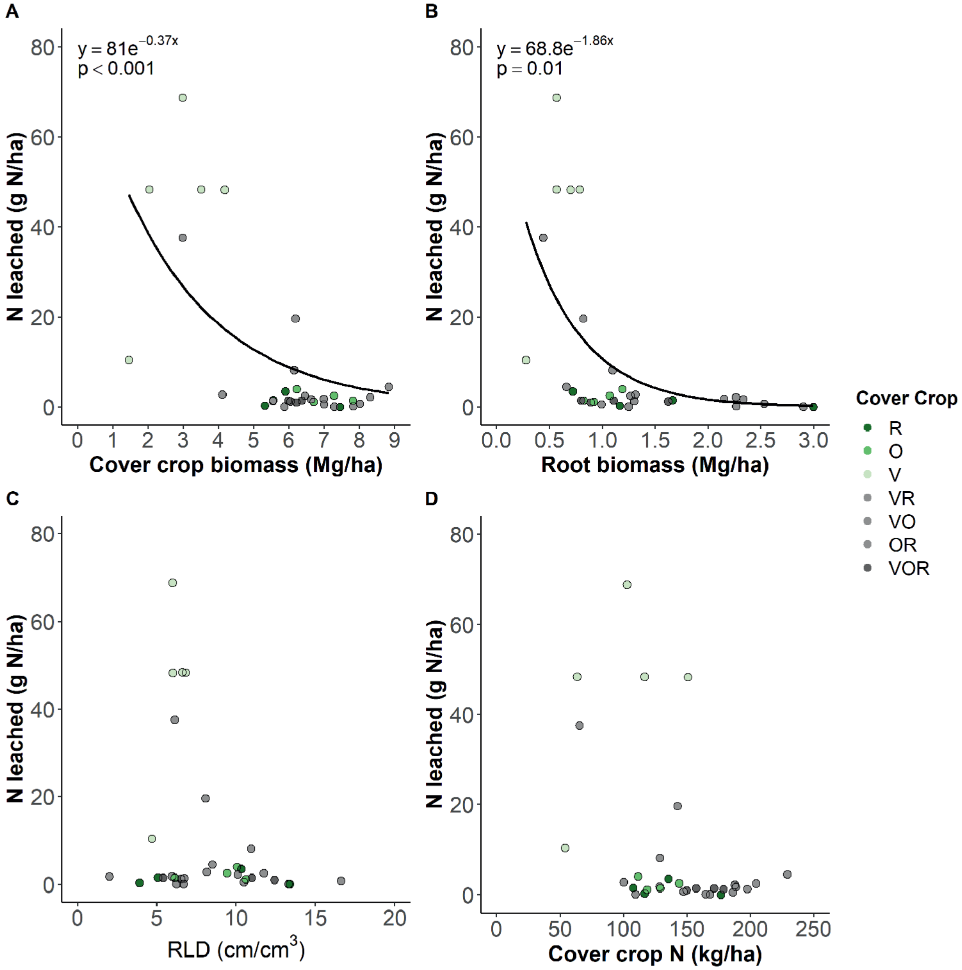

Figure 2.6 The relationship between the amount of N leached in November of 2019 and A) total cover crop biomass, B) cover crop root biomass, C) root length density (RLD) and D) cover crop N uptake measured in November of 2019. The colours indicate the different cover crops: R (radish), O (oat), V (vetch) and their combinations. Lines indicate significant negative exponential relationships $y = ae^{(-bx)}$.

surface area for nutrient uptake as an explanation for reduced N leaching (Andersen et al., 2014; Li et al., 2006). As mentioned above, the cover crops in our field were fertilised to kickstart growth, perhaps this meant that there was enough N present for uptake thus plant species mixtures may have experienced less N limitation and thereby invested less in deeper rooting as compared to other studies. Another explanation could be that cover crops are grown during less favourable climatic conditions in autumn (low light and temperature), whereas intercropping studies are mostly during spring and summer. Thus, it can be hypothesised that competition for light is more dominant than competition for soil nutrient uptake during cover crop growth in autumn.

2.4.3 Shoot and root biomass are related to N leaching, RLD and N yield are not

As expected, cover crop total biomass and root biomass negatively correlated to the amount N leached. Yet, cover crop N yield and root length density (RLD) were not related to the amount of N leached. In line with our hypothesis, during cover crop growth radish consistently reduced N leaching the most, followed by oat, whereas leaching under vetch was largest and comparable to that from fallow soil. High N leaching in vetch as compared to the other cover crop treatments can be primarily explained by its low biomass. N yield was probably not significantly related to N leaching because vetch, which has a relatively high N concentration, gets part of its N from biological N fixation.

In both 2017 and 2019 there seems to be a threshold at which N leaching reaches close to zero, at a total cover crop biomass of ~4 Mg/ha in 2019 and ~3 Mg/ha in 2017 or a root biomass of ~1 Mg/ha in both 2017 and 2019. Cover crop N yield ranged between 40-100 kg N/ha in 2017 and between 100-180 kg N/ha in 2019. Yet, there was only 20-35 (2017) and 27-46 (2019) kg N/ha mineral N in the soil at cover crop sowing, before fertilisation. Thus, cover crop N yield was high compared to the N already present in soil or added by fertilisation. Part of the N available in the soil for crop uptake came from the fertiliser (30 kg N/ha), possibly more N would have come available through decomposition of residues remaining from the cash crop. The amount of N leached in 2017 was a lot higher compared to 2018 and 2019. This was most likely caused by the later sowing date of cover crops of that year, two weeks later than the other years. It has been shown previously that later sowing of cover crops can significantly reduce their biomass due to the lower sunlight hours and temperatures of the fall (Thomsen and Hansen, 2014). We saw this expressed in the soil cover data, where the 50% soil cover was reached over 2 weeks later in 2017 compared to 2019. This, combined with the larger amount of rainfall that year and lower temperatures caused a very low cover crop biomass and increased leaching.

2.4.4 N leaching during the cash crop season

We had expected large leaching losses after cover crop incorporation from cover crop treatments with a high N yield and a relatively low C:N ratio (i.e. radish or vetch). Yet we did not see any consistent differences between cover crop treatments and leaching during cash crop growth. However, leaching could have occurred between cover crop incorporation and cash crop planting (at a time when leaching was not measured). If the cover crop C:N ratio is negatively correlated to decomposition rates, then comparing the monoculture cover crops, you would thus expect lowest leaching just after incorporation of oat (C:N of 23) and highest leaching after (a mixture consisting) radish (C:N of 19) or vetch (C:N of 12). Indeed the soil mineral N content in March reflects this expectation where soil mineral N is lowest after incorporation of oat and highest after incorporation of radish or vetch monocrops and the mixtures. Since soil mineral N is positively related to N leaching, the risk of leaching was probably higher in treatments with a high N content. Especially in march 2019 the soil mineral N in mixtures tended to be higher in mixtures containing radish.

2.5 Conclusion

This study showed that (i) cover crop mixtures reduce N leaching similarly as the most productive monoculture cover crop, and mixtures including a legume can be as effective as a non-legume cover crop in N leaching reduction; (ii) the effectiveness of cover crops in reducing N leaching is positively related to (root) biomass of the cover crop across the monocultures and mixtures. Thus, considering the stability in terms of yield and N uptake mixtures provided, we find these results promising for using cover crop mixtures in terms of N leaching reduction on sandy soils. In spring, leaching losses after incorporating cover crop mixtures were not higher than after a fallow field. Yet, increased soil mineral N after incorporation of high N yielding cover crop (mixtures) indicate that leaching could have occurred shortly after cover crop incorporation. Thus there might be a trade-off between N retention in autumn and the N supply in spring. We suggest further research is needed by to optimise this trade-off by using different species mixtures and to quantify the actual N leaching losses throughout the entire year.

2.6 Acknowledgements

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Research), the centre for soil ecology (CSE) and the Netherlands Institute of Ecology (NIOO-KNAW). This study was supported by a grant from the Netherlands Organization for Scientific Research (NWO green, grant number 870.15.071) with co-financing from seed producers (Agrifirm, Vandinter Semo, P.H. Petersen Saatzucht, and Joordens Zaden). We are grateful to Ye Tian, Peter Garamszegi, Tom Klein-Obbink, Yanina Willet, Willeke van Tintelen, Tamas Salanki, Hennie Halm and the staff at Unifarm and NIOO for help with the experimental work.

2.7 Supporting information

Table S2.1 Measured concentration of mineral N (in mg/L) in the collected leachates.

mineral N in leachate (mg L^{-1})										
	2017							2018		
	May 18	June 6	July 1	Sept 15	Oct 1	Oct 23	Nov 20	Jan 15	Feb 12	May 1
Radish	18.8±2.1 ab	20.9±2.4 abc	13.7±3.1	11.7±1.7 ab	15.1±3.4 a	2.9±2.8 a	0.1±0.03 a	0.02±0.01	0.02±0.01 a	20.8±8.9 ab
Vetch	24.9±3.7 ab	20.0±1.8 ab	15.8±3.1	20.1±4.8 ab	28.5±5.1 ab	34.6±2.9 d	30.0±2.1 b	2.8±1.0 ab	2.2±1.3 ab	12.1±3.2 a
Oat	23.9±2.6 b	25.8±5.8 abc	17.6±2.8	14.8±2.6 ab	23.5±2.4 ab	17.0±1.5 b	3.0±1.4 a	0.2±0.1 a	2.0±1.9 ab	18.4±5.9 ab
Radish + Vetch	23.2±3.4 b	22.8±4.5 abc	14.2±3.1	17.0±1.6 b	26.0±5.1 ab	4.6±2.0 a	0.2±0.1 a	0.2±0.2 a	0.7±0.7 ab	12.1±2.8 ab
Radish + Oat	17.4±2.8 ab	14.4±1.5 a	15.2±3.1	10.6±1.6 a	22.1±4.2 ab	0.6±0.4 a	0.1±0.1 a	0.02±0.02	0.1±0.1 a	25.0±13.8
Vetch + Oat	30.3±3.0 b	30.4±2.3 c	23.6±3.1	17.3±2.0 ab	21.0±1.6 ab	21.4±2.2 bc	2.2±1.5 a	0.1±0.05 a	0.8±0.5 a	39.0±16.2
Radish + Vetch + Oat	32.8±9.9 ab	31.5±4.7 bc	20.0±3.1	20.4±3.6 ab	32.1±5.6 b	5.8±2.5 a	0.04±0.02 a	0.02±0.02	0.03±0.02 a	27.8±15.6
Fallow (No cover	12.5±1.6 a	13.8±1.0 a	8.7±2.8	20.3±11.0	19.9±4.5 ab	28.0±2.5 cd	30.4±3.1 b	3.0±0.2 b	3.2±0.4 b	21.0±4.2 b
	***	***	ns	*	*	***	***	***	***	**

mineral N in leachate (mg L^{-1})								
	2019							2020
	Feb 8	May 3	June 6	Sep 11	Oct 1	Nov 4	Dec 13	Jan 16
Radish	13.0±3.5 ab	3.6±0.5 a	8.0±3.0 ab	12.0±5.3	1.2±1.0 a	0.2±0.1 a	0.1±0.04 a	0.3±0.1 a
Vetch	22.9±6.4 ab	3.7±0.7 a	4.1±1.8 ab	2.7±0.4	8.4±3.7 ab	6.9±1.4 b	3.0±0.4 b	9.0±2.9 ab
Oat	18.4±3.6 ab	6.1±3.0 ab	12.4±2.7 b	6.2±2.4	7.5±1.5 b	0.3±0.1 a	0.2±0.03 a	0.1±0.04 a
Radish + Vetch	10.9±3.8 ab	7.4±3.8 ab	10.5±4.0 ab	7.7±4.2	1.1±0.8 a	0.2±0.1 a	0.2±0.04 a	2.9±1.2 ab
Radish + Oat	8.9±3.1 ab	2.8±0.3 a	2.7±0.6 a	2.8±0.6	3.5±3.4 ab	0.1±0.1 a	0.1±0.05 a	0.09±0.03 a
Vetch + Oat	16.9±1.2 b	7.1±3.6 ab	3.3±0.9 ab	7.9±2.1	6.7±1.3 b	2.2±1.0 ab	1.6±0.7 ab	4.9±1.7 ab
Radish + Vetch + Oat	9.0±3.2 ab	3.8±0.5 a	6.1±2.2 ab	4.3±1.1	1.0±0.4 a	0.2±0.01 a	0.1±0.02 a	0.9±0.7 a
Fallow (No cover	8.1±1.3 a	14.3±1.9 b	3.5±1.2 ab	7.1±3.2	8.4±3.1 b	15.0±4.7 ab	7.5±1.3 c	1.7±0.6 b
	***	**	*	ns	***	***	***	***

Table S2.2 Modelled groundwater flow calculated with SWAP in m^3 ha^{-1} day^{-1}. The groundwater flow is calculated for each day for each cover crop throughout the 3 measurement years. Here we only show the data for days at which we also measured concentration of N in the groundwater.

Groundwater flow (m^3 ha^{-1} day^{-1})	2017							2018		
	May 18	June 6	July 1	Sept 15	Oct 1	Oct 23	Nov 20	Jan 15	Feb 12	May 1
Radish	2.82	2.26	14.38	47.28	0.72	1.17	10.05	15.94	10.61	0.00
Vetch	2.82	2.26	14.38	47.30	0.97	1.23	10.08	15.94	10.66	0.00
Oat	2.82	2.26	14.38	47.31	0.89	1.20	10.12	15.94	10.62	0.00
Radish + Vetch	2.82	2.26	14.38	47.30	0.81	1.19	10.06	15.94	10.62	0.00
Radish + Oat	2.82	2.26	14.38	47.31	0.81	1.19	10.03	15.94	10.62	0.00
Vetch + Oat	2.82	2.26	14.38	47.31	0.90	1.20	10.20	15.95	10.63	0.00
Radish + Vetch +	2.82	2.26	14.38	47.28	0.79	1.16	10.10	15.94	10.59	0.00
Fallow (No cover	2.82	2.26	14.38	61.25	6.24	5.68	13.57	17.73	13.03	0.00

Groundwater flow (m^3 ha^{-1} day^{-1})	2019							2020
	Feb 8	May 3	June 6	Sep 11	Oct 1	Nov 4	Dec 13	Jan 16
Radish	21.17	2.10	3.37	0.00	7.24	6.56	27.58	13.02
Vetch	21.15	2.10	3.37	0.58	8.53	6.54	27.58	13.03
Oat	21.16	2.10	3.37	0.83	9.97	6.57	27.58	13.03
Radish + Vetch	21.16	2.10	3.37	0.00	7.17	6.55	27.58	13.03
Radish + Oat	21.16	2.10	3.37	0.10	7.22	6.55	27.60	13.05
Vetch + Oat	21.20	2.10	3.37	0.74	9.47	6.56	27.58	13.03
Radish + Vetch +	21.18	2.10	3.37	0.15	7.74	6.59	27.59	13.02
Fallow (No cover	25.47	2.10	3.37	13.45	33.89	11.09	30.91	16.54

Table S2.3 Percentage soil cover measured between 2 and 8 weeks after sowing (WAS) and the number of days after sowing (DAS) until 50% ground cover was reached. Data on 2018 are not included since this was not needed for leachate prediction (no leachate measurement possible).

Year	Species	Percentage of soil covered by cover crops							$T_{50\%}$
		2WAS	3WAS	4WAS	5WAS	6WAS	7WAS	8WAS	DAS
2017	Radish	1	4	11	31	61	85	96	40
	Vetch	0	1	5	14	36	66	87	46
	Oat	1	2	6	16	37	65	85	46
	Radish + Vetch	1	2	8	24	53	81	94	42
	Radish + Oat	1	2	8	26	58	85	97	41
	Vetch + Oat	1	2	5	13	30	54	76	48
	Radish + Vetch + Oat	0	2	6	18	45	75	92	43
2019	Radish	19	51	82	95	98	98	99	21
	Vetch	5	16	43	75	92	97	99	30
	Oat	5	13	30	53	75	89	95	34
	Radish + Vetch	14	40	73	91	97	99	99	23
	Radish + Oat	12	34	65	86	95	97	98	25
	Vetch + Oat	5	14	34	62	83	94	98	32
	Radish + Vetch + Oat	10	29	59	83	94	97	98	26

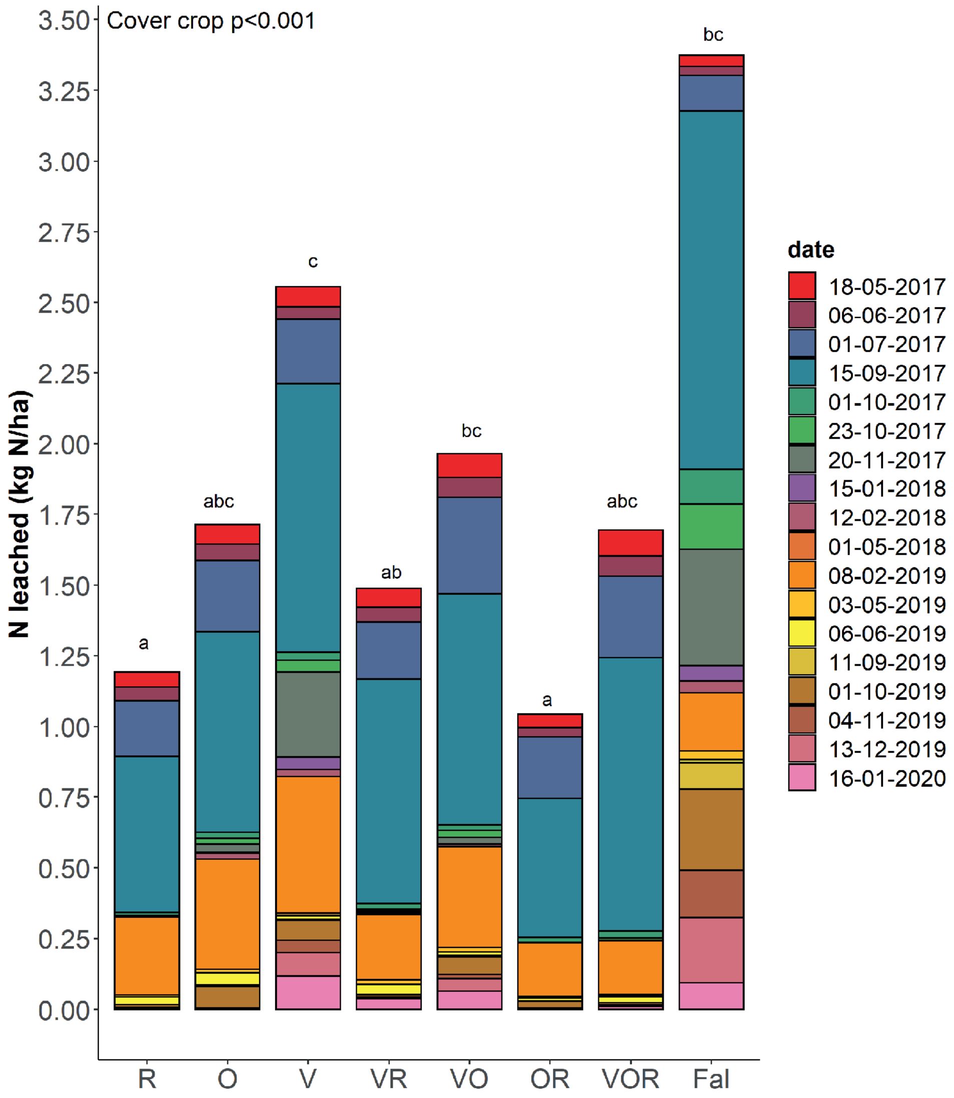

Figure S2.1 Mineral N leached between 2017 and 2020. The different colours represent the dates of measurement. Model results showed no flow to the groundwater on the 01-05-2018, thus leaching was zero at this date. Different letters indicate significant differences between the sum of the amount of N leached of the dates measured. The cover crops are: R (radish), O (oat), V (vetch), all their combinations and fallow (Fal, no cover crop grown) (n = 5).

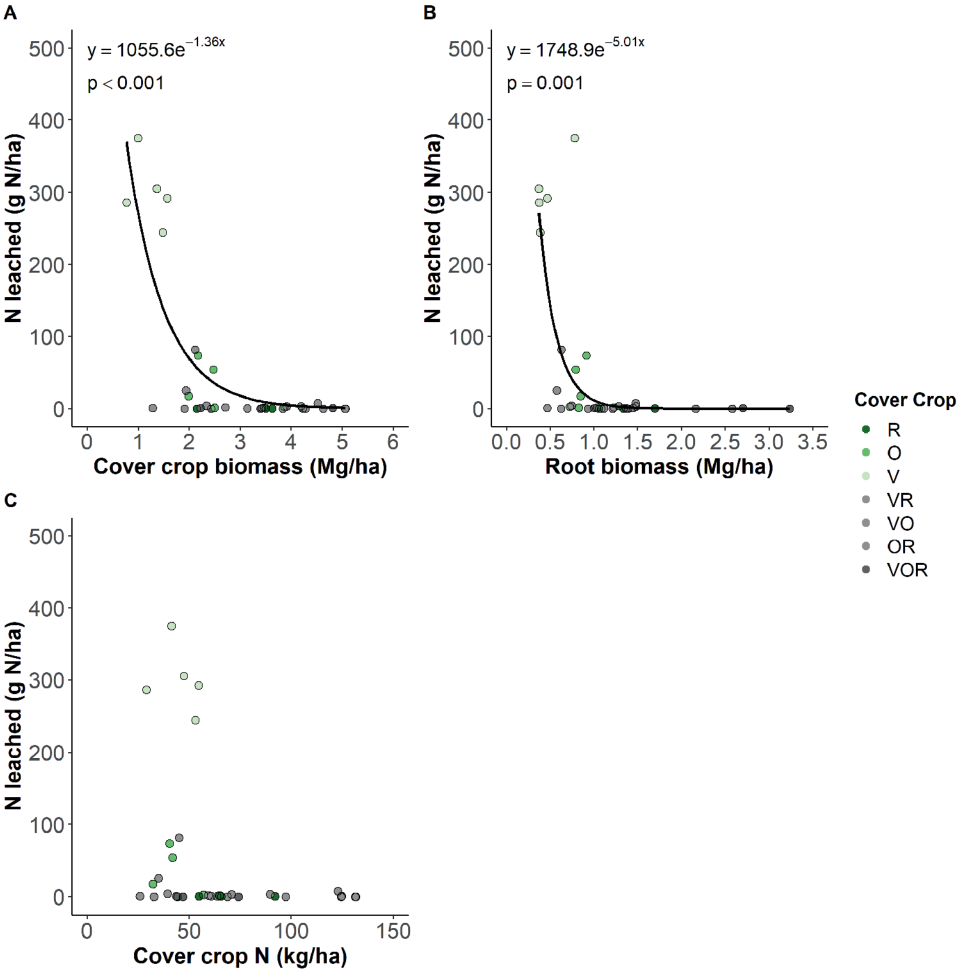

Figure S2.2 The relationship between the amount of N leached in November of 2017 and A) total cover crop biomass, B) cover crop root biomass and C) cover crop N uptake measured in November of 2017. The colours indicate the different cover crops: R (radish), O (oat), V (vetch) and their combinations. Lines indicate significant negative exponential relationships $y = ae^{(-bx)}$.

Chapter 3

Can N fertilisation of winter cover crops promote N uptake and reduce N-leaching from soil?

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Submitted

Abstract

Winter cover crops are often grown after a main crop to capture residual nutrients and accumulate biomass that is later incorporated into the soil to increase soil organic matter and to provide nutrients to the following main crop. The growing season of winter cover crops is short; therefore, a starting gift of N could kick-start their initial growth which could potentially increase their productivity and reduce N leaching. We hypothesised that 1) for non-leguminous cover crop species, a N gift improves biomass and N uptake, and lowers N leaching, 2) these effects are absent with leguminous species, and 3) mixing species improves biomass and N uptake and reduces leaching due to complementary N uptake. In a 2-year field study, 2018 and 2019, we grew oilseed radish (*Raphanus sativus*), black oat (*Avena strigosa*) and common vetch (*Vicia sativa*), as pure stands and as a 3-species mixture. Five levels of N fertiliser were applied ranging from 0 to 90 kg N/ha. All crops were sown in August and harvested 12 weeks later. Shoot and root biomass as well as N uptake were determined. Mineral N concentration in the soil pore water below the rooting zone was measured once a month if sufficient rainfall allowed using porous suction cups. Mineral N leached at these dates modelled based on the estimated volume of water leached and its mineral N concentration. Among pure stands, the effect of starting N gift on biomass was minor and only observed for oilseed radish in 2018. In this year, N uptake of oilseed radish and black oat increased with N gift. However, the recovered amounts of N were less than the applied amounts. Oilseed radish and black oat were the most productive species. Averaged over N gifts, their biomass ranged between 5.2 and 5.3 t ha^{-1} for oilseed radish and between 4.3 to 5.3 t ha^{-1} for black oat. Whereas, biomass of vetch ranged between 1.5 and 2.9 t ha^{-1}. Due to the low productivity of vetch, higher amounts of mineral N leached under vetch in 2019, compared to the other species. N yield of vetch did not respond to N gifts, risking more N loss with higher N additions. Biomass of the 3-species mixture was similar to or higher than the best performing species. For mixtures, in both years, there was a linear increase in N yield as a response to N application. Unlike pure stands, nett N capture of the 3-species mixture was not reduced at high N application rates. We conclude that application of N fertiliser to winter cover crops growing in sandy soils does not improve N capture service.

3.1 Introduction

Agricultural intensification has helped to match the increasing global demand for food products, but at the cost of nitrogen (N) losses. During the last decades, increased food production was accompanied by the doubling in global application of agrochemicals (N, phosphorus and pesticides) to our terrestrial ecosystems (Tilman et al., 2001). Ecological intensification was proposed to minimise the use of agrochemicals and replace it by natural mechanisms that provide nutrients, and control pests, diseases and weeds (Bommarco et al., 2013). These services can be obtained from growing cover crops, and therefore cover crops are an important component of ecological intensification (Wittwer et al., 2017; Wittwer and van der Heijden, 2020).

Cover crops are planted during the fallow period between two cash crops. They are not aimed to produce food, feed, fuel or fibre but to provide a range of ecosystem services (Blanco-Canqui et al., 2015). Cover crops capture nutrients and accumulate biomass that is later incorporated into the soil to increase soil organic matter. The incorporated biomass releases nutrients that may increase yields of the main crop (Chu et al., 2017; Sharma et al., 2018). Additionally, cover crops can prevent weed growth (Brennan and Smith, 2018; Brust et al., 2011b; Kruidhof et al., 2009) suppress pests e.g., plant parasitic nematodes (Kruger et al., 2013), prevent erosion (De Baets et al., 2011) and they have been shown to enhance soil physical properties (Steele et al., 2012).

Cover crops also play a role in the N cycle and the N use efficiency of the agroecosystem. During growth, they scavenge surplus soil nutrients and incorporate them into their biomass (Kaspar and Singer, 2011; Thorup-Kristensen et al., 2003). As a result of N capture, cover crops can decrease denitrification and leaching of mineral N (NO_3^- and NH_4^+) in autumn (Behnke and Villamil, 2019). Because cover crops also take up water, not only the concentration of nutrients but also the total volume of water leached is lower (Blanco-Canqui, 2018; Kaspar and Singer, 2011). In the study of Kasper and Singer (2011) all cover crops managed to reduce leaching, but a major difference was observed between the legume in this study (reduction 6%) and all other crops (average reduction 94%). A meta-analysis by Thapa et al. (2018) showed that non-legume cover crops reduced leaching on average by 56% while leguminous cover crops did not significantly reduce leaching losses compared to fallow soil.

The magnitude of ecosystem services provided by cover crops depends largely on the amount of biomass they produce (Blanco-Canqui et al., 2015). Vigorous growth early on, given the short growing season in late summer/early autumn, is an important feature of a successful cover crop. A starting gift of N could kick-start the initial growth of the cover crop and this could potentially reduce N leaching from soil throughout the growth period. High biomass producing species, that take up more water and nutrients, are generally most efficient in preventing N leaching (Blanco-Canqui, 2018; Thapa et al., 2018) and could possibly benefit from an initial N gift. Fertilising leguminous cover crops, which fix N from the atmosphere, would probably lead to even more N leaching.

In temperate regions, cover crops usually receive a starting N gift to ensure quick establishment and to boost growth in autumn. The amount of N applied differs among countries, regions and even farmers (Smit et al., 2019). For instance, in the Netherlands, the recommended amount of N fertilisation on a sandy soil is 0 kg N ha^{-1} for vetch and 50 kg N ha^{-1} for oilseed radish and black oat (van Leeuwen-Haagsma et al., 2019). In a case study by the European commission farmers from Spain, France, the Netherlands and Romania were asked about cover crop practices. From these countries, between 1% and 38% of farmers apply nitrogenous fertilisers to cover crops with the amount of N added ranging between 2 and 43 kg N ha^{-1} (Smit et al., 2019). Either way, the application of a starter N will only be useful if the extra N-captured by cover crops exceeds the amount of N applied. There are no clear guidelines on how and when to fertilise cover crops. We are not aware of any scientific studies on this topic. The current available information is limited to general manuals for farmers (van Leeuwen-Haagsma et al., 2019).

It is known from natural and agroecosystems that increased species biodiversity can increase resource exploitation, which leads to an increased plant biomass and thus C and N accumulation in the soil (Cong et al., 2014; De Deyn et al., 2009; Fornara and Tilman, 2008; Tilman et al., 1996). Rooting depth varies widely among species (Bukovsky-Reyes et al., 2019) and could lead to spatial complementarity in N uptake, *i.e.* exploitation of a larger soil volume, uptake of more nutrients and more reduction in N leaching compared to sole crops (Brooker et al., 2015; Hauggaard-Nielsen and Jensen, 2005; Heuermann et al., 2019; Miyazawa et al., 2010). In a study by (Burr-Hersey et al., 2017), they showed, by x-ray tomography, that *Vicia sativa* has more shallow roots, *Raphanus sativus* has a thick taproot and that *Avena strigosa* has fibrous roots that reach deeper into the soil. Moreover, leguminous species can fix N from the atmosphere, through biological N fixation (Blesh, 2018; Blesh et al., 2019; Bukovsky-Reyes et al., 2019). In a mixture with leguminous species, the growth of neighbouring plants can be increased because of the higher N availability in the soil. Because of this complementary resource use, cover crops grown in mixtures could thus have a higher residue quality (low C:N) as well as a higher biomass than expected from pure stands. This yield advantage, subsequently, can reduce the amounts of N that will be leached and return more nutrients to the soil in spring.

In this study we investigate the effect of different levels of mineral N fertilisation on the performance of cover crops and mineral N leaching during cover crop growth. We compared pure stands of vetch, black oat and oilseed radish and its corresponding 3-species mixture, which were given a starter N gift of 0, 15, 30, 60 and 90 kg N/ha. We hypothesised that: 1) for both oilseed radish and black oat, a moderate N gift improves nett N capture and biomass, and lowers N leaching, 2) N gift for vetch, does not affect nett N capture and biomass, and increases N leaching, and 3) mixture of cover crops is more efficient than pure stands in taking up N and reducing leaching due to complementary N uptake.

3.2 Materials and methods

3.2.1 Site description

The field experiments were conducted in the autumn of 2018 and 2019 at the experimental facility of Wageningen University, Wageningen, The Netherlands. Each year, cover crops were sown in August and harvested at maximum standing biomass, in November. Non-destructive measurements were made of soil cover and leachates over time. Cover crop biomass, nutrient content and mineral N in the soil were determined in November at the harvest. Both experimental fields are located on a sandy soil with a low (< 3%) soil organic matter content. Residual soil mineral N (in August) in 2018 and 2019 was 19 and 36 kg N ha^{-1}, respectively. The details of the two fields are summarised in Table 3.1.

3.2.2 Experimental design

The field experiment consisted of four cover crop treatments: *Raphanus sativus* c.v. Valencia (oilseed radish), *Vicia sativa* c.v. Amelia (vetch), *Avena strigosa* c.v. Pratex (black oat), and their mixture (oilseed radish + vetch + black oat) and five N application rates: 0, 15, 30, 60 and 90 kg N/ha as calcium ammonium nitrate (27% N - 6% CaO - 4% MgO). The experimental design was full factorial with three replicates (4 cover crop x 5 fertilisation x 3 replicates = 60 plots) and was planted in a completely randomised block design. Each plot was 3 x 5 m. Cover crops were sown at a row distance of 12.5 cm resulting in 24 rows per plot. Seeding rates were 30 kg ha^{-1} (oilseed radish), 110 kg ha^{-1} (vetch) and 90 kg ha^{-1} (black oat) according to farmer practice. The thousand seed weights of oilseed radish, vetch and black oat were 10.0 g, 51.7 g and 19.2 g, respectively. Species were mixed according to a replacement design, using, for each of the species, 33% of the seed rate in the pure stand. Seeds were sown using a Hege 80 belt cone planter. The plots were fertilised shortly after sowing.

Table 3.1 Site description and conducted field operations in 2018–2019. Soil properties were analysed in the topsoil (0-25cm).

Site characteristics	Born 4 (2018)	Hoge Hoef 4 (2019)
Location	Wageningen,	Wageningen
Texture	Sand 83%, Silt 12%, Clay	Sand 78%, Silt 16%, Clay
Soil organic matter %	1.7	2.7
C:N	13	14
Soil pH	5.3	5.8
Bulk density g cm^{-3}	1.3	1.5
Mineral N in August (kg N / ha)	19.2	35.6
Total N (kg N / ha)	4460	3500
Total P (kg P / ha)	770	905
Total K (kg K / ha)	335	255
Preceding cash crop	Winter barley	Fallow
Sowing date	24th August	23rd August
Harvesting date	21st November	25th November
Accumulated growth degree	725	750

3.2.3 Pore water collection below the rooting zone

Three ceramic cup samplers (rhizosphere research products, Wageningen) were placed in each plot on an angle of 45° at a depth of 70 cm below the soil surface. Pore water samples were stored in the freezer and subsequently analysed for NO_3^- and NH_4^+ using Segmented Flow Analysis (Houba et al., 2000a). The frequency of collecting leachates was once per month, when sufficient rainfall (> 5 mm) was predicted (Fig 3.1). Due to a drought in 2018 there was a large water deficit in the soil and pore water collection was not possible in October even if sufficient rainfall was predicted.

3.2.4 Cover crop soil cover

Every week, starting two weeks after sowing, photos were taken in each plot to determine the percentage of soil cover. Photos were taken at a fixed position (1 m between canopy and camera) with a camera pointed vertically downwards, mounted on a metal frame. The frame (1 × 0.75 m2) was positioned near the top of the cover crop canopy to standardise the area of the picture. This same area used for making the photos was harvested at 12 WAS. Green cover percentage was established by analysing the photos using the DIPimage toolbox for image analysis in MATLAB (MathWorks, 2013). A logistic model was fit to the observed ground cover data. Fraction ground cover for each day was estimated as:

$$GC_T = \frac{GC_{max}}{1+\exp^{-s(T-T\_50\%)}},$$

where GC_T is a fraction of ground cover at day T, GC_{max} is the maximum ground cover fraction, $T_{50\%}$ is the time when 50% of maximum predicted soil cover was reached, while s is a parameter that affect how steeply the function rises as it passes through $T_{50\%}$.

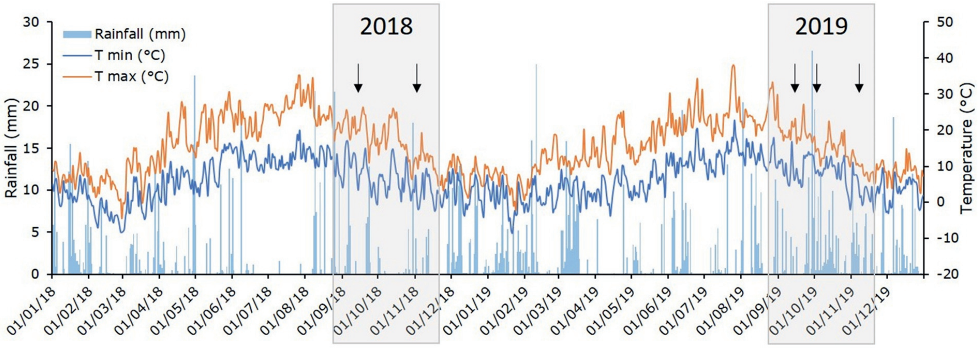

Figure 3.1 Weather data collected from weather station the Veenkampen (Wageningen university). The grey squares indicate the time period when the field experiments were conducted. Black arrows indicate collection times of pore water. Due to a water deficit in the soil, pore water collection in October 2018 did not result in sufficient pore water for nutrient analysis (<1 ml).

3.2.5 Cover crop biomass

Above- and belowground cover crop biomass was harvested in November of each year, at 12 WAS. Aboveground biomass was cut at ground level in an area consisting of eight rows with a length of 1 m (1 × 1 m^2) at the centre of each plot. In the mixture, cover crop species were separated and analysed separately. Per plot four root cores with an 8 cm diameter and 30 cm depth, were extracted. Two cores were sampled in the row and two cores between the rows. Each core was divided in four depth layers, 0-5 cm, 5-10 cm, 10-20 cm and 20-30 cm. Samples from each plot were pooled together per depth layer. Harvested samples were washed to remove adhering sand and organic matter particles, oven-dried for 48 h at 70 °C to measure total dry weight. The dried plant materials were ground and analysed for the concentration of C and N in shoot and root separately using a CN elemental analyser. The amount of N in shoot and root was calculated as product of the multiplication of biomass and N concentration.

3.2.6 Nett N-capture and N-recovery

Nett N- capture was calculated to understand the effect of different levels of N application on N capture service provided by the cover crops. Nett N-capture (kg N ha^{-1}) was calculated as: $N_{yield} - N_{app}$; where N_{yield} is the total N in plants, in shoot and root (kg N ha^{-1}) and N_{app} is N applied as a fertiliser (kg N ha^{-1}). Any N application is advantageous if the nett N-capture exceeds that at no N application. In this case, as compared to no application, plants have taken up more mineral N from the soil in addition to the amount added as a fertiliser. The optimum N application rate is, subsequently, the application rate that corresponds to the highest nett N-capture value. N-recovery in percent was based on the regression between nett N-capture and N application. If the slope of the regression is positive, then N-recovery will exceed the amount of applied N and extra amount of N is recovered by plants from the soil.

3.2.7 Soil mineral N

Soil samples were collected each year at two-time points namely at cover crop sowing and at cover crop harvest (12 WAS). Soil samples were collected from each plot to a depth of 20 cm from five random locations within each plot. We pooled the five samples of each plot together. Soil samples were dried at 40°C for 48 hours and sieved over a 2 mm mesh sieve. The soil mineral N was determined by extracting dry soil in a 1:10 (w/v) suspension with 0.01 M $CaCl_2$ (Houba et al., 2000a). The extracts were analysed with a segmented flow analyser for NO_3^- + NO_2^- and NH_4^+ (SFA; Skalar 6 channel SFA analyser, Skalar, Netherlands, 2003).

3.2.8 Leachate estimates

We used the Soil Water Atmosphere Plant (SWAP) model to estimate leaching losses at the plot scale (Kroes et al., 2017; van Dam et al., 2008). SWAP simulates the water movement and root water extraction in the soil, which we used to estimate the total volume of water percolation to the subsoil for the entire cover crop growing season, and thus the total amount of N leached by accounting for the concentrations of mineral N in the leachates. Rainfall data were obtained from a weather station located on the Wageningen University experimental farm (the Veenkampen); reference evapotranspiration data were obtained from the KNMI (Royal Dutch Meteorological Institute). Crop input data were soil cover and rooting depth (measured in the experiment). We obtained the data of the soil hydraulic properties, according to the soil type, from the handbook of SWAP (Kroes et al., 2009; Kroes et al., 2017; van Dam et al., 2008). Simulation of water infiltration, redistribution, and percolation in the soil is based on the Richards equation. Root water extraction is based on the potential transpiration, root density distribution and the drought reduction function of (Feddes et al., 1978). We assumed a uniform root density distribution. Groundwater levels were estimated based on data from a groundwater well in the vicinity (51°59'54.6"N 5°39'47.7"E). The model was initialised with a soil water status assumed at field capacity in January of the modeled year. The amount of N leached in each plot at each date was calculated by multiplying the mineral N concentration in the pore water and the simulated volume of water leached on that day (24 hours).

3.2.9 Data analysis

All statistics were carried out in R version 3.5.1 (R Core Team). Figures were made using the packages ggplot2 (Wickham, 2016) and cowplot (Wilke, 2019). We tested the effect of cover crop type (factor, 4 levels) and fertilisation (factor, 5 levels) on soil mineral N, cover crop biomass, cover crop N uptake and the amount of mineral N leached using linear mixed effects models with the function lme from the package nlme (Pinheiro et al. 2016) with 'block' as a random factor. Significance was determined with analysis of variance (ANOVA), anova (base) in R. Type III sum of squares was used when significant interaction between cover crop and fertilisation was present. Normality and homogeneity of model residual variances were assessed by plotting model residuals. Data on N leached in September 2018 and November 2019 was not normally distributed and log-transformed. Heterogeneous residual variances were addressed using a variance structure in the model with the function varIdent (nlme) (Pinheiro et al., 2019). The model with the variance structure was retained when it was a significantly better fit than the model without, determined by comparing the model's akaike information criterion (AIC) using the function anova in R. To determine significant differences between levels of a factor (i.e. between cover crop residues), a Tukey's honestly significant difference posthoc test (HSD) was used, with the function emmeans (Lenth et al., 2019) and CLD from package multcomp (Hothorn et al., 2008). Regression analysis was conducted to test the response of nett N capture to N application, linear and second degree polynomial functions were tested. After testing the linearity assumption, the linear function was selected.

3.3 Results

3.3.1 Soil cover by cover crop

Soil cover by cover crops was not affected by N application, except for oilseed radish in 2018. Oilseed radish with no N applied covered 80% of the soil, whereas in the treatments with N applied full soil cover was reached. Except for radish with no N applied in 2018, all species and the mixture completely covered the soil by the end of their growing period. Vetch was the slowest species to cover the soil. Averaged over the two years and N applications, vetch covered 50% of the soil after the fifth week from the sowing time whereas, black oat, oilseed radish and the mixture covered 50% of soil around the third week after sowing (Table 3.2).

3.3.2 Cover crop biomass

Cover crop total biomass varied from 1.5-6.0 t ha^{-1} and varied significantly between the cover crop species (2018: $F_{3,38} = 91.3$; $p < 0.001$; 2019: $F_{3,38} = 66.0$; $p < 0.001$). In both years vetch had the lowest biomass; vetch produced 1.5 and 2.9 t ha^{-1} of biomass in 2018 and 2019, respectively (Fig 3.2). Among the pure stands, the highest biomass was produced by black oat (5.3 t ha^{-1}) and oilseed radish (5.2 t ha^{-1}) in 2018 and by oilseed radish (5.3 t ha^{-1}) in 2019. Compared to the most productive species, the 3-species mixture produced a similar amount of biomass in 2018 and 0.66 t ha^{-1} higher biomass in 2019. We did not observe any effect of fertilisation on biomass except for oilseed radish in 2018 ($F_{4,38} = 4.50$; $p = 0.005$). In this year, oilseed radish biomass was 2.1 t ha^{-1} higher at 15 kg N ha^{-1} than at no N application.

Table 3.2 percentage of soil covered by cover crop species; black oat, oilseed radish, vetch and mixture of the 3-species at 2,3,4,6 and 8 weeks after sowing. $T_{50\%}$ represents time when 50% of maximum predicted soil cover was reached, represented in days after sowing.

Year	Species	Percentage of soil covered by cover crops					$T_{50\%}$
		2 WAS	3 WAS	4 WAS	6 WAS	8 WAS	DAS
2018	Black oat	8%	39%	81%	98%	98%	23
	Oilseed	11%	42%	78%	92%	93%	22
	Vetch	4%	8%	16%	48%	82%	43
	Mixture	9%	33%	69%	96%	98%	24
2019	Black oat	12%	56%	92%	100%	100%	20
	Oilseed	39%	87%	98%	100%	100%	15
	Vetch	5%	15%	34%	83%	98%	32
	Mixture	19%	62%	92%	100%	100%	19

3.3.3 Cover crop N yield

The concentration of N in plant tissues varied among species (2018: $F_{3,38} = 70.3$; $p < 0.001$; 2019: $F_{3,38} = 50.9$; $p < 0.001$). Averaged over the 2 years, vetch had the highest N concentration with an average of 3.6%, followed by oilseed radish 2.3%, black oat 2.0% and the 3-species mixture 2.1% (Fig S3.1 in the supporting information). Following from the biomass and N concentration, N yield was significantly different among the cover crop species (2018: $F_{3,38} = 10.1$; $p < 0.001$; 2019: $F_{3,38} = 21.9$; $p < 0.001$). All species captured more N from the soil in

2019 than in 2018 (Fig 3.3). Vetch, though having the highest N concentration, produced the lowest N yields as compared to the other species (56 and 105 kg N ha^{-1} in 2018 and 2019, respectively). In both years, oilseed radish and the mixture captured the highest amounts of N (oilseed radish = 102 and 140 kg N ha^{-1}; mixture = 91 and 151 kg N ha^{-1} in 2018 and 2019, respectively). Black oat captured a similar amount of N as oilseed radish and the mixture in 2018 and a similar amount as vetch in 2019 (87 and 102 kg N ha^{-1} in 2018 and 2019, respectively). The effect of N application on N yield was significant in both years. In 2018, except for vetch, there was a linear increase in N yield as a response to N application in oilseed radish, black oat and the mixture ($F_{4,38} = 11.1$; $p < 0.001$). In 2019, this linear response was only visible in the mixture ($F_{4.38} = 4.8$; $p = 0.003$).

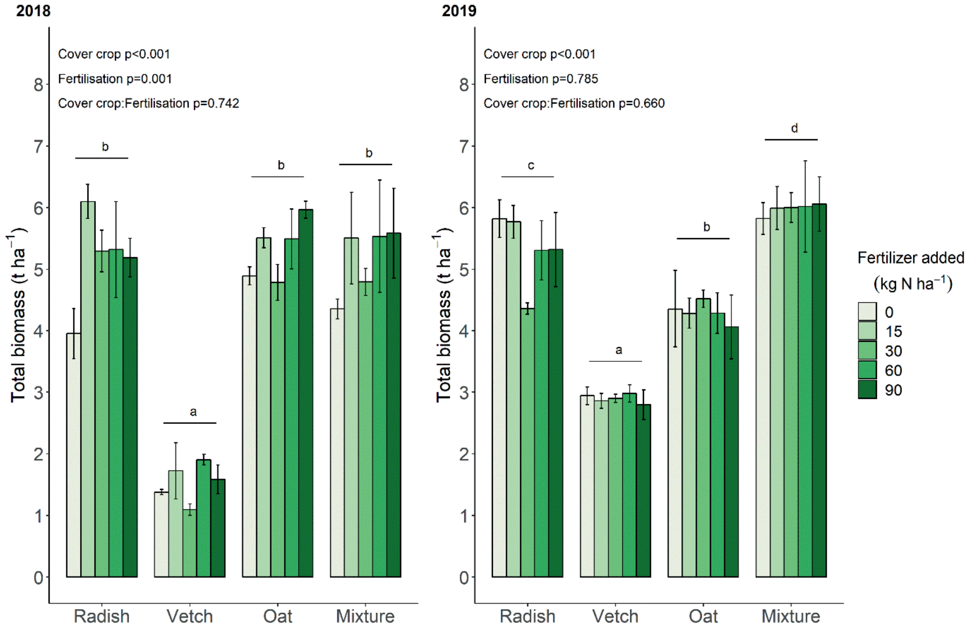

Figure 3.2 Total (aboveground and belowground) dry weight of three species of cover crops: oilseed radish, vetch and black oat and their mixture in 2018 and 2019. Error bars denote the standard error (n = 3), different letters indicate significant differences between the cover crops in total biomass (p < 0.05).

3.3.4 Nett N capture

Nett N capture either decreased or was not affected by N application (Fig 3.4). In 2018, only the nett N capture of vetch showed a negative response to N application ($R^2 = 0.88$; $p = 0.019$), the other crop treatments were not affected. The slope of the regression suggests that, approximately, only 12% of the applied N to vetch was recovered in 2018. In 2019, when the soil organic matter and soil mineral N content was higher, nett N capture of all pure stands was negatively affected by N application. N-recovery differed among species. From the applied amounts of N, oilseed radish recovered 44% ($R^2 = 0.89$; $p = 0.017$), black oat recovered 16% ($R^2 = 0.99$; $p < 0.001$) and vetch recovered 0% ($R^2 = 0.99$; $p < 0.001$). In both years, nett N capture of the mixture was not affected by N application.

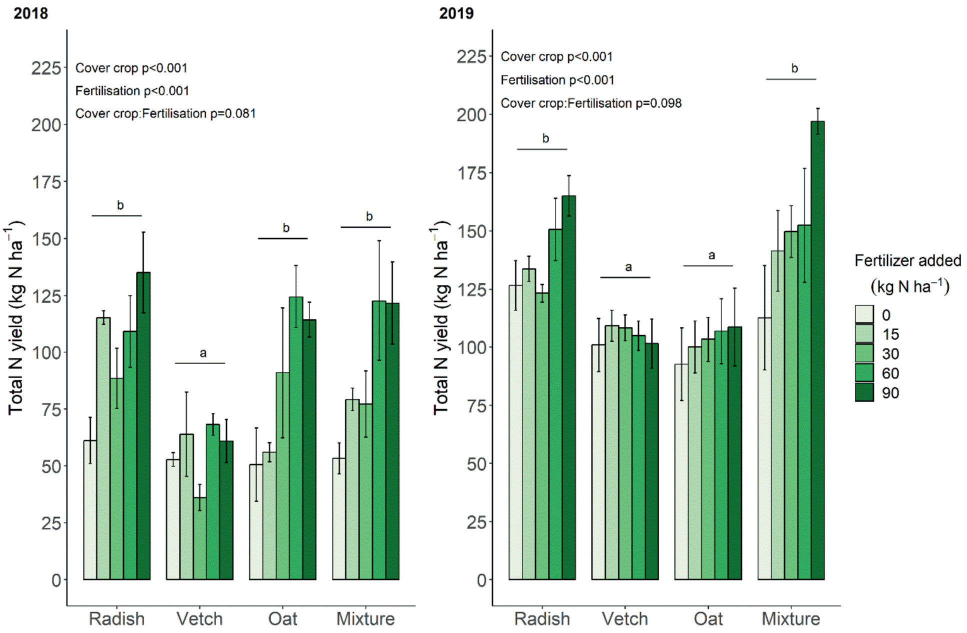

Figure 3.3 Total (aboveground and belowground) N yield of three species of cover crops: oilseed radish, vetch and black oat and their mixture in 2018 and 2019. Error bars denote the standard error (n = 3), different letters indicate significant differences between the cover crops in total N yield ($p < 0.05$).

3.3.5 Mineral N leaching

Pore water collection, and thus analysis of the concentration of mineral N in the pore water, was done on five different dates (Fig S3.2 and S3.3 in the supporting information). The modeled downward flow of water per cover crop and fertilisation treatment (in m^3 ha^{-1} day^{-1}) differed over time (Table S3.1). In early November 2018 no downward water flow occurred due to a dry spell. Overall the modeled groundwater flow did not differ much among treatments. Only on October 1st, 2019 there was a difference of approximately 2 m^3 ha^{-1} day^{-1} (= 0.2 mm day^{-1}) per treatment. This difference occurred because the soil cover at this time was different between the vetch crop and the other crop treatments (Table 3.2). N leaching could be calculated for the 4 sampling days when downward water flow occurred. The estimated amount of mineral N leached (in kg N ha^{-1} day^{-1}) was, for all cover crops, highest on the 12th of September 2018 (between 0.13 and 0.48 kg N ha^{-1}, Fig 3.5). On this day N leaching on average increased with fertiliser rate ($F_{4,36} = 3.92$, $p = 0.003$). Mineral N leached below radish was ~ 0.3 kg N ha^{-1} day^{-1} higher when 90 kg N ha^{-1} was applied compared to no N fertilisation. In 2019 there was no effect of fertilisation on any of the measured dates. The amount of N leached was significantly higher below vetch compared to all other cover crop treatments on sampling days in October and November in 2019 (Oct: $F_{3,33} = 39.29$, $p < 0.001$, Nov: $F_{3,38} = 18.84$, $p < 0.001$).

3.3.6 Soil mineral N

N fertilisation significantly increased soil mineral N in November of 2018 ($F_{4,38} = 1.89$, $p < 0.001$, Fig 3.6). In November of 2019 soil mineral N was significantly higher when vetch or black oat was grown compared to oilseed radish and the mixture in 2019 ($F_{3,38} = 15.32$, $p < 0.001$, Fig 3.6). In 2018 there were no significant differences between cover crops.

3.4 Discussion

Our results demonstrate that in most instances, N application did not significantly improve cover crop biomass. Contrary to our hypothesis, N application had either no effect or a negative effect on N-capture services by the cover crops and no consistent effect on N leaching. The identity of crop species has a significant effect on productivity and N-capture service of winter cover crops. Vetch, as a legume growing in autumn, has a slow soil cover and low productivity. Whereas black oat and oilseed radish are more productive and have the ability to capture the residual soil mineral N. It was expected that a starting gift of N fertiliser would further improve their growth and subsequently higher amounts of N would be captured and less would be prone to leaching to ground water. We showed that, in some instances, only the highest amounts of N application (60 and 90 kg N ha^{-1}) can significantly increase N capture by cover crops as compared to no N application (Fig 3.3). These extra captured amounts of N ranged between 64 to 84 kg N ha^{-1}, which translates into 75% to 146% as compared to the amounts captured at no N application. However, the extra captured amounts of N by plants were either the same or less than the applied amounts, resulting in a linear negative trend in nett N-capture for vetch in 2018 and for vetch, black oat and oilseed radish in 2019. Based on these results, we refute the first hypothesis stating that N application can improve biomass and nett N capture and reduce leaching under black oat and oilseed radish.

Our results support, in part, the second hypothesis that a N gift to vetch neither improves biomass accumulation nor nett N capture. This was as expected since legume cover crops can fix N from the atmosphere (Moller and Reents, 2009). Possibly, vetch fixed more N when 0-15 kg N ha^{-1} was applied which could explain the similar N yields in vetch at the different fertiliser applications. As expected, leaching was much higher under the leguminous species (vetch) compared to the non-leguminous species (black oat and oilseed radish), which was previously also shown in literature (Gabriel et al., 2012; Quemada et al., 2013; Tonitto et al., 2006).

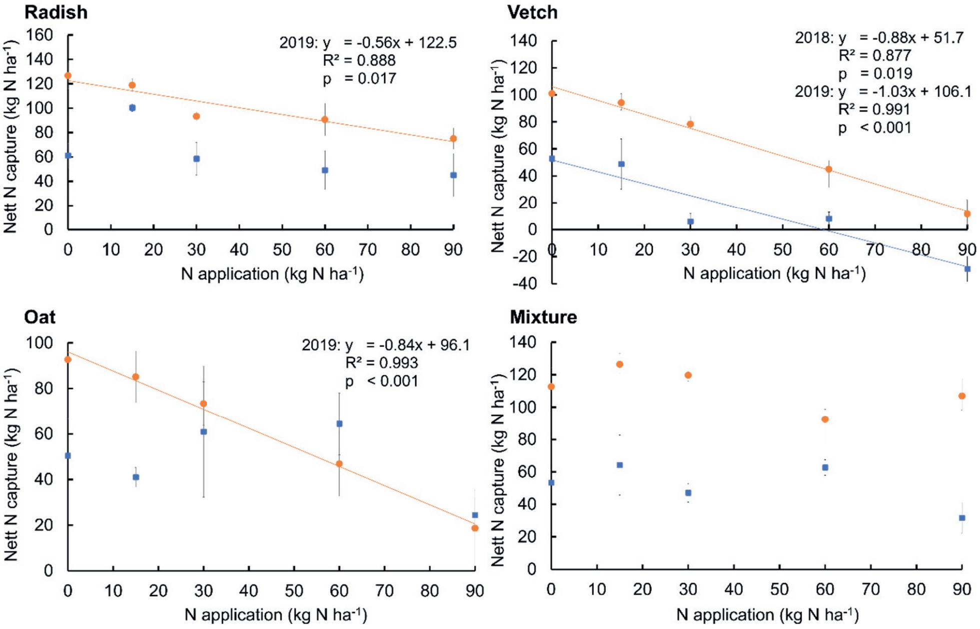

Figure 3.4 Average nett N-capture as a response to five levels of N applications (0, 15, 30, 60 and 90 kg N ha-1) of three crop species (winter vetch, black oat and oilseed radish) and a mixture of the three species. Blue closed squares represent data from 2018 and orange closed circles represents data from 2019. Nett N-capture was calculated as N yield – N application in kg N ha^{-1}.

This highlights that vetch is not a good crop when you try to reduce leaching losses. Surprisingly higher application of N fertiliser did not consistently increase N leaching losses under vetch, rejecting in part hypothesis two. Under black oat and oilseed radish a similar trend existed in 2019, where N leaching did not increase at a higher N fertilisation rate. For black oat and oilseed radish, applying 90 kg N ha^{-1} resulted in a N yield increase of 50-70 kg N ha^{-1} in 2018 and 20-60 kg N ha^{-1} in 2019 suggesting that 20 to 70 kg N ha^{-1} was lost. The difference between the years is most likely attributed to the lower amount of mineral N in the soil in 2018 compared to 2019 and potentially the lower precipitation in 2018 (Table 3.1). Possibly the dynamics of N leaching were not constant over time and thus more measurements are needed to capture all N loss via leaching. Or, N was lost through another pathway, namely N_2O or N_2 emissions. Nitrous oxide emissions are directly related to the soil N content and fertiliser application (Butterbach-Bahl et al., 2013; Syakila and Kroeze, 2011).

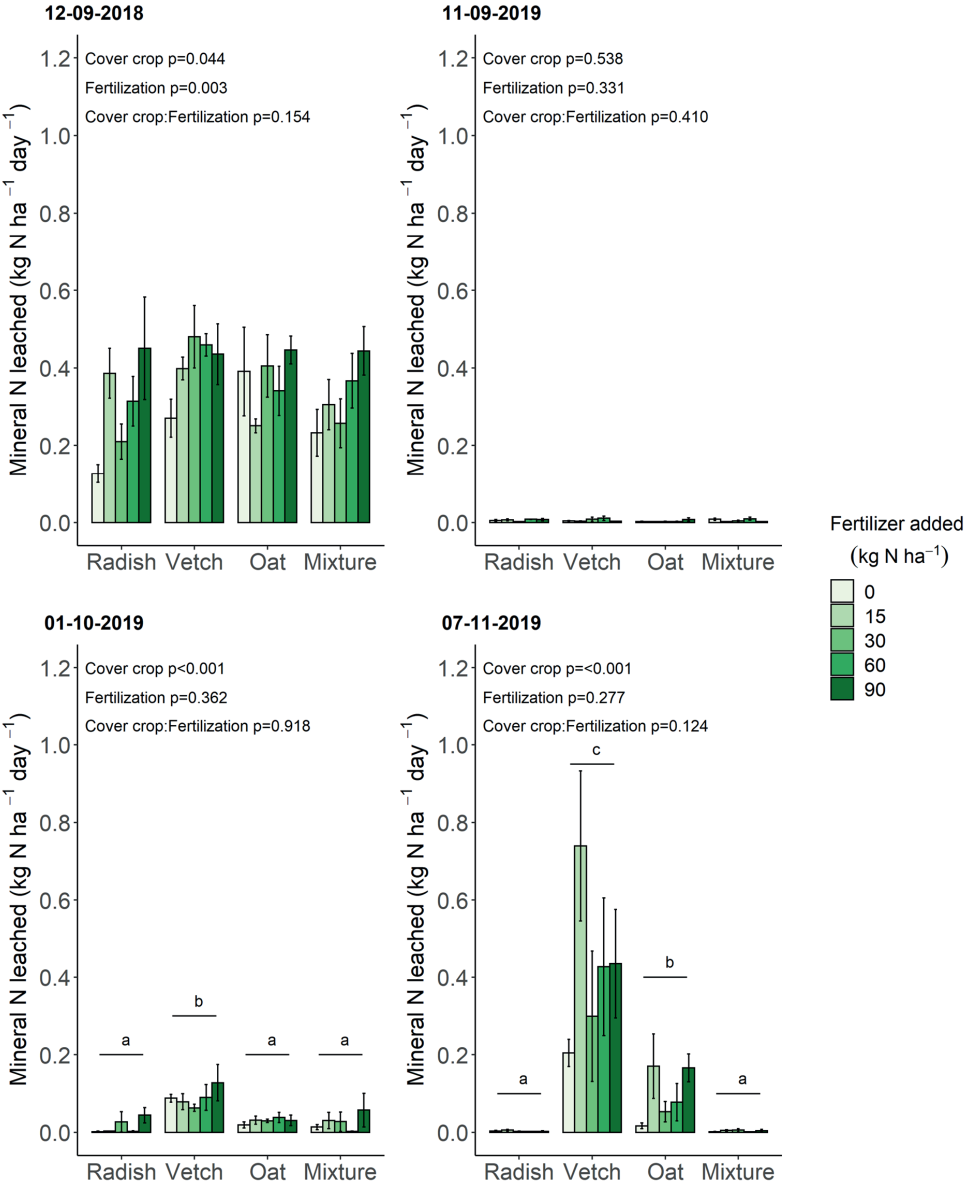

Figure 3.5 Amount of N leached calculated for the 12th of September 2018, the 11th of September 2019, the 1st of October 2019 and the 7th of November 2019. Error bars denote the standard error (n = 3), different letters indicate significant differences between the cover crops ($p < 0.05$).

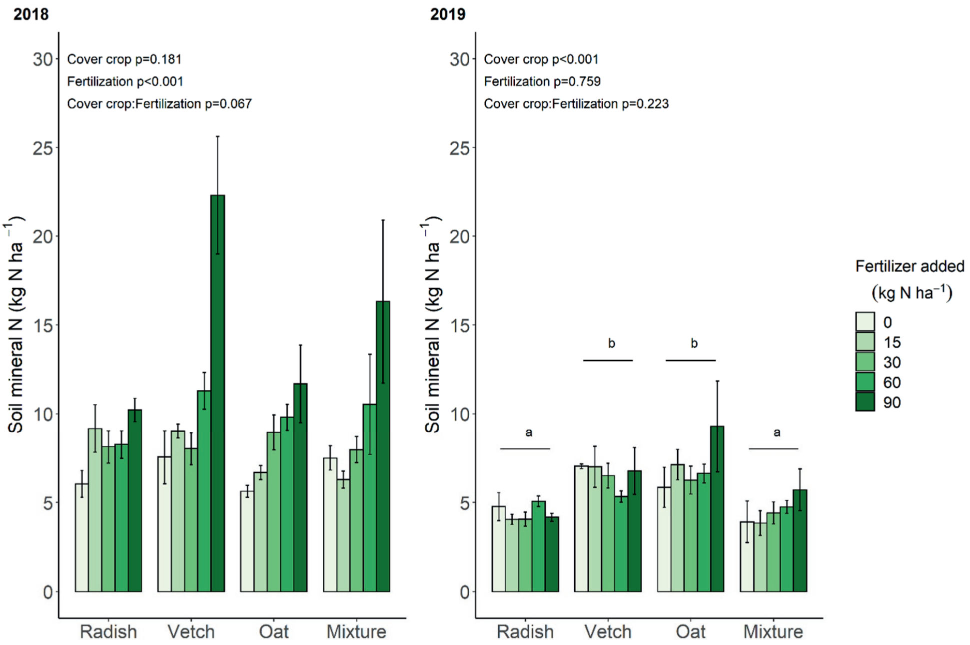

Figure 3.6 Soil mineral N measured at the time of harvest in 2018 and 2019. Error bars denote the standard error (n = 3), different letters indicate significant differences between the cover crops ($p < 0.05$).

Biomass of the 3-species mixture was either the same (2018), or higher (2019) compared to best performing monoculture. Beneficial interactive effects for biomass production resulting from mixing species of winter cover crops was also reported in earlier studies (Blesh, 2018; Elhakeem et al., 2019; Finney et al., 2016; Florence et al., 2019; Murrell et al., 2017; Wendling et al., 2017). In the current study, in 2018, black oat was the most productive species and it contributed approximately 66% to the total biomass in the mixture. While in 2019, oilseed radish was the most productive species and contributed approximately 72% to the total biomass in the mixture (data not shown). These compensations by the most productive species played a role in stabilising the yield of the mixture as shown earlier by (Elhakeem et al., 2019). Unlike the pure stands, the 3-species mixture always captured extra amount of N with N application, resulting in no extra losses in both experimental years. Considering the high biomass and the low N losses of the 3-species mixture at the different levels of N applications over the 2 years, we can therefore accept the third hypothesis stating that the mixture outperforms pure stands. It was earlier shown that root systems as well as N capture patterns differ greatly among cover crops species (Wendling et al., 2016). Subsequently, mixing different species resulted in complementarity for nutrient capture and consequently yield advantage (Wendling et al., 2017).

For the 3-species mixture, the nett N capture is not related to N application rates. N fertilisation can be beneficial for an increased N-content of the biomass produced; yet risking losses and reducing the N use efficiency of the system.

3.5 Conclusion

Based on this study we conclude that application of mineral N to winter cover crops does not enhance nett N-capture from soil and does not reduce leaching losses. Black oat and oilseed radish are suitable species as winter cover crops due to their high productivity and relatively low leaching losses. Vetch growing in pure stands, should not be used because of relatively high leaching losses. Mixing species of black oat, oilseed radish and vetch showed its potential to enhance biomass while it has similarly low N losses as oilseed radish and black oat. Finally, policies on cover crop fertilisation are not based on clear scientific evidence. Our results suggest that the benefits of N fertilisation are not strong enough to promote fertilisation of winter cover crops.

3.6 Acknowledgements

This study is a part of the Clever Cover Cropping project which is a collaboration between the Soil Biology Group and the Centre for Crop Systems Analysis (Wageningen University & Research), the Centre for Soil Ecology (CSE) and the Netherlands Institute of Ecology (NIOO-KNAW). This study was supported by a grant from the Netherlands Organization for Scientific Research (NWO green, grant number 870.15.071) with co-financing from seed producers (Agrifirm, Vandinter Semo, P.H. Petersen Saatzucht, and Joordens Zaden). We are grateful to Betty Rutendo Masamba, Shengfang Yang and Péter Garamszegi for help with the experimental work.

3.7 Supporting information

Table S3.1 Modelled downward water flow at 70 cm depth calculated with SWAP in m^3 ha^{-1} day^{-1}. Only results for days at which we also measured concentration of N in the groundwater are presented.

		Groundwater flow (m^3 ha^{-1} day^{-1})				
		12-09-2018	01-11-2018	11-09-2019	01-10-2019	07-11-2019
Oilseed radish	N0	13.05	0.00	0.70	6.91	22.46
	N15	13.07	0.00	0.74	6.91	22.46
	N30	13.02	0.00	0.72	6.91	22.46
	N60	12.97	0.00	0.72	6.91	22.46
	N90	12.96	0.00	0.72	6.91	22.46
Vetch	N0	13.73	0.00	1.36	9.00	22.39
	N15	13.63	0.00	1.39	9.32	22.39
	N30	13.75	0.00	1.37	9.33	22.39
	N60	13.69	0.00	1.37	9.14	22.39
	N90	13.66	0.00	1.37	9.38	22.39
Black oat	N0	13.10	0.00	1.04	7.10	22.38
	N15	13.14	0.00	1.06	7.16	22.38
	N30	13.05	0.00	1.06	7.16	22.38
	N60	13.12	0.00	1.04	7.10	22.40
	N90	13.26	0.00	1.01	6.98	22.38
Mixture	N0	12.96	0.00	0.72	6.91	22.46
	N15	13.09	0.00	0.93	6.84	22.38
	N30	13.14	0.00	0.91	6.83	22.38
	N60	13.26	0.00	0.93	6.83	22.38
	N90	13.09	0.00	0.97	6.89	22.38

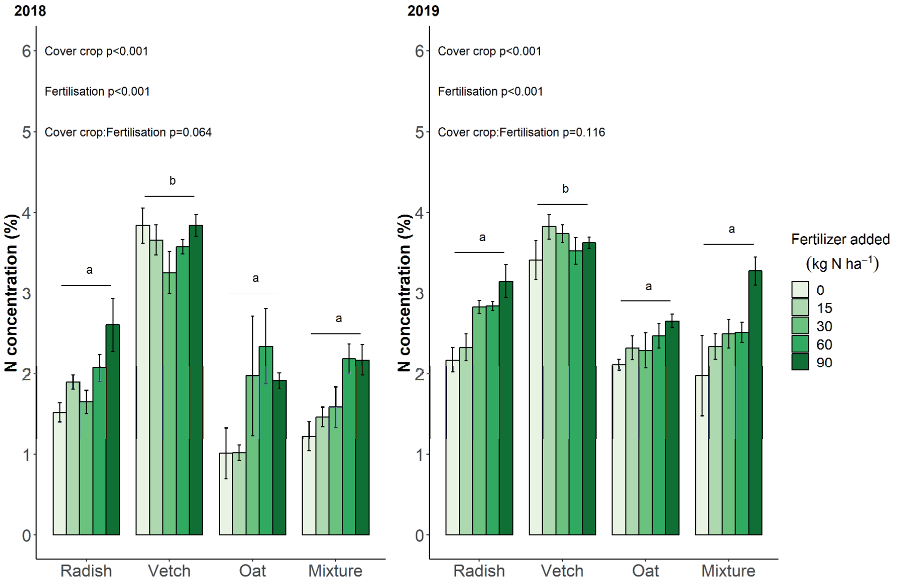

Figure S3.1 Concentration of N in total biomass (aboveground and belowground) of three species of cover crops: oilseed radish, vetch and black oat and their mixture in 2018 and 2019. Error bars denote the standard error (n = 3), different letters indicate significant differences between the cover crops in total N yield ($p < 0.05$).

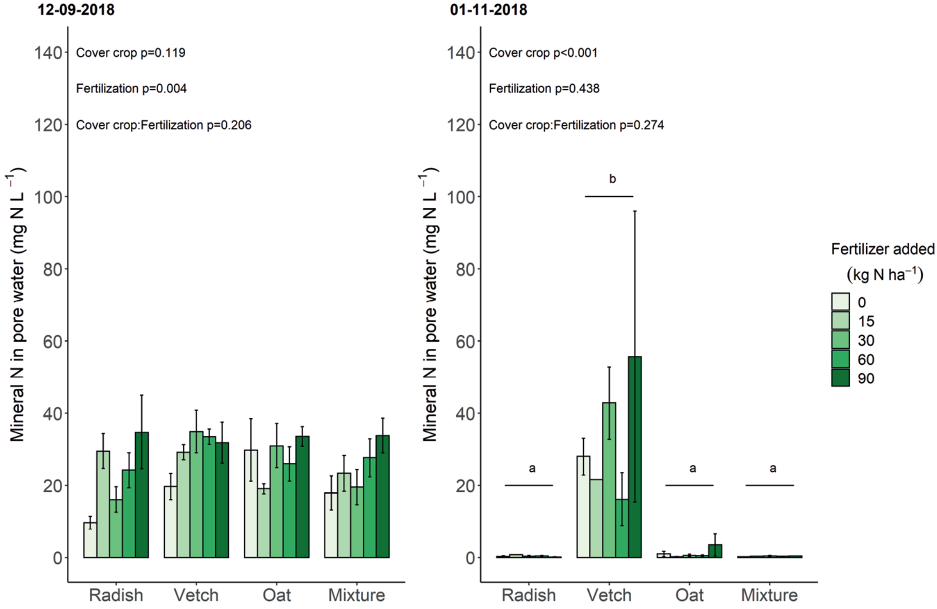

Figure S3.2 Concentration of mineral N in pore water measured on the 12th of September and the 1st November during cover crop growth of 2018. Error bars denote the standard error (n = 3), different letters indicate significant differences between the cover crops ($p < 0.05$).

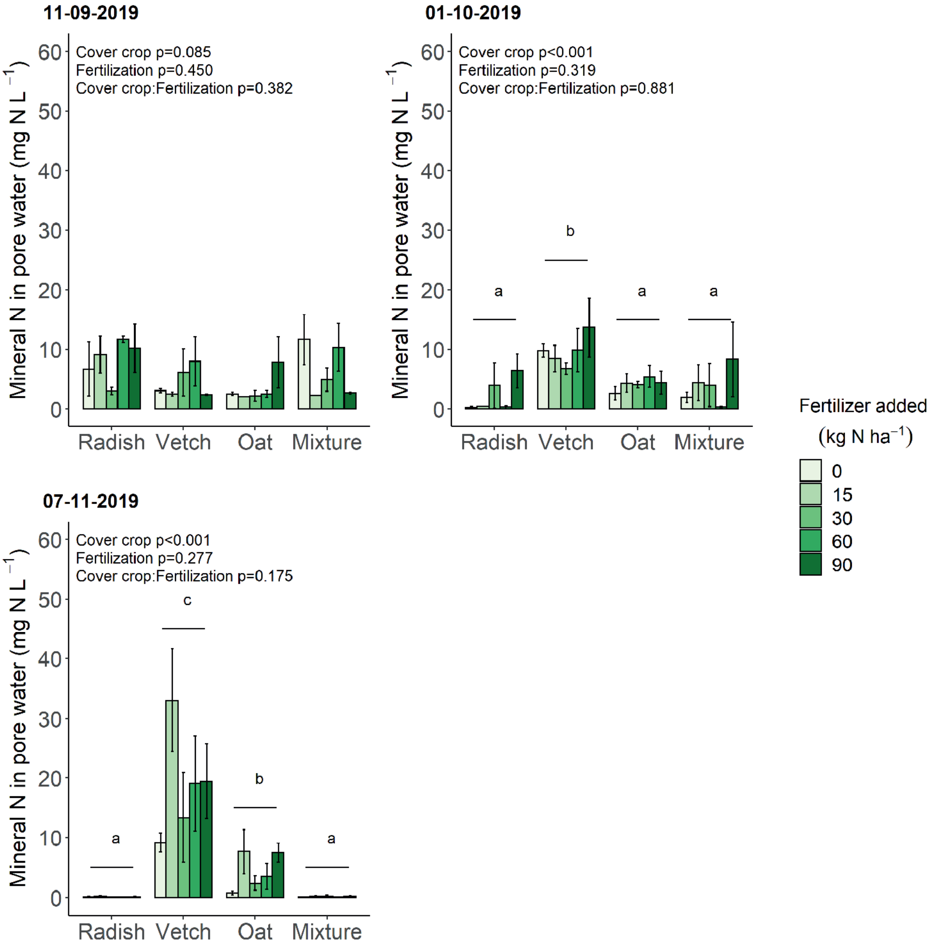

Figure S3.3 Concentration of mineral N in pore water measured on the 11th of September, the 1st of October and the 7th of November during cover crop growth of 2019. Error bars denote the standard error (n = 3), different letters indicate significant differences between the cover crops ($p < 0.05$).

Chapter 4

Is litter decomposition enhanced in species mixtures? A meta-analysis

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Abstract

Litter decomposition is a key process in the C balance of soils. Commonly, plant litters occur in mixtures where the species differ in quality traits such as the nutrient concentration and organic C quality. Many studies explored if mixing litters retards or speeds up litter decomposition compared to species decomposing alone, with varying results. To identify consistent trends with an overarching quantitative synthesis, we test in a meta-analysis whether on average across studies, the mass loss of mixed litters of two plant species is faster than the average mass loss of single litters. We hypothesise that larger trait divergence of the litter quality of the species in a mixture results in a faster mass loss of the mixture than expected based on the single species. Furthermore we hypothesise that part of the variation in litter mixture mass loss can be explained by experimental design and environmental factors. Explanatory variables used were chemical litter trait dissimilarity in the C, N, P, lignin, cellulose, phenolics concentration as well as soil properties, ecosystem, climate, the duration of litter decomposition and the experimental design. Interactions were studied if supported by mechanistic hypotheses. In the majority of studies and on average, we found that the mass loss of mixed litters is equal to the weighted average of the mass loss of the constituent single litters. None of the hypothesized explanatory variables was consistently associated with litter mixture effects on the mass loss and explained variation in mass loss of significant models was invariably only a few percent of all variation. While further data exploration might elucidate further, interactive, patterns, many of these could not be explored due to lacking data. This meta-analysis therefore refutes the notion that mixing litters in general enhances rates of decomposition. We conclude that the effects of litter mixing are in many cases predictable from the decomposition rates of the individual species. According to our results, any interactive effects (positive or negative) between litter species are contextual, and cannot be generalized and predicted beyond the context in which the results were obtained.

4.1 Introduction

Plant litter input and its decomposition rate are the two primary controls on C storage in soil (von Lützow et al., 2006). Thus, in order to estimate the amount of C returned to the soil and its potential residence time, litter decomposition rates need to be predicted (Aerts, 1997; Gessner et al., 2010). Decomposition rates are influenced by many litter quality parameters as well as the micro-climate (mainly temperature and soil moisture), soil chemistry and community of decomposer organisms (Aerts, 1997; Cornwell et al., 2008; Preston et al., 2009). The decomposition of single litters is relatively well understood. However, in nature, plants rarely grow in monocultures thus litters customarily decompose as species mixtures. Conversely, in agricultural systems, many food crops have been grown in monoculture, yet even then weeds contribute to the biomass in the field. Furthermore, crops are usually grown in a rotation of species such that litters with different quality are mixed in the soil over time. Moreover, in order to promote biodiversity and yields, intercropping, agroforestry and cover cropping are advocated, which results in mixed species litters in those systems too (Isbell et al., 2017). It is thus essential to get a better understanding of litter mixture decomposition mechanisms in order to be able to predict the consequences of plant species diversification in plant communities for C dynamics in soils.

As a null model, the decomposition rate of litter consisting of mixtures from different plant species can be expected to be equal to the average decomposition rate of the species in the mixture. However, when litter species with divergent qualities are mixed there could be interactions between the litters during the decomposition process, resulting in non-additive effects of species mixtures on decomposition (Handa et al., 2014). Litter traits of plant species vary widely both in their chemical and physical characteristics. Litter chemical quality is most often described as the N or lignin concentration, the C:N ratio or the lignin:N ratio and therefore these parameters are also frequently measured (Aerts, 1997; Campbell et al., 2016). However, multiple studies have shown that the C:N ratio alone cannot explain variation in decomposition rates (García-Palacios et al., 2016; Hättenschwiler and Jørgensen, 2010; Lin and Zeng, 2018; Wardle et al., 2003). A comprehensive study with data from 110 research sites, globally distributed, showed that the total amount of nutrients (N, P, K, Ca and Mg combined) and the C:N ratio accounted for 70.2% of the variability measured in litter decomposition (Zhang et al., 2008). Other litter chemical parameters such as cellulose, hemicellulose, sugar, starch and phenols may also influence litter decomposition rates (Delgado-Baquerizo et al., 2015; García-Palacios et al., 2013; Hättenschwiler and Jørgensen, 2010; Hoorens et al., 2003; Sariyildiz and

Anderson, 2003). Polyphenols can sometimes have a larger (antagonistic) effect on decomposition rates than the more frequently measured litter quality parameters (N, P and lignin), since polyphenol-protein complexes are resistant to most decomposing organisms (Hättenschwiler et al., 2005; Hättenschwiler and Vitousek, 2000). Generally, single litters with a higher nutrient concentration and a lower concentration of complex C molecules are expected to decompose faster.

Non-additive effects on litter mixture mass loss have been frequently described. A review by Gartner and Cardon (2004) of all litter mixture studies available up to the year 2000 showed that by vote counting, 67% of mixtures exhibited non-additive mass loss. They found that synergistic interactions (higher mass loss in mixture than expected based on single species) were more common than antagonistic interactions (lower mass loss than expected). Since then many additional studies have been published on litter mixing. It would be valuable to integrate quantitatively how differences in litter qualities or other environmental factors can explain the variation in non-additive mass loss.

The mechanisms causing non-additive effects in litter mixture decomposition are still not fully understood (Pérez Harguindeguy et al., 2008). To explain non-additive mass loss, the nutrient transfer hypothesis is most frequently mentioned. This hypothesis states that decomposers preferentially feed on high N litters. Subsequently N is released that could then be transferred to the low N litter and thus facilitate the decomposition of the more recalcitrant litter (Hättenschwiler et al., 2005), causing non-additive mass loss in mixtures by accelerating the decomposition rate of the more recalcitrant litter (Handa et al., 2014; Hättenschwiler et al., 2005). Some studies support the hypothesis (Bonanomi et al., 2014), while others do not (Thomas 1968, Klemmedson 1992). Hoorens et al. (2003) studied litter mixtures with a range of litter quality trait differentiation and concluded that the difference in initial single litter chemistry parameters of the components did not predict non-additive mass loss. In contrast, a recent study that looked at the environmental, decomposer and litter trait differentiation effects on litter mass loss showed that litter trait differentiation was the most important variable explaining non-additive mass loss (García-Palacios et al., 2017). Other mechanisms that could cause non-additive mass loss are improved water retention due to one of the component litters in a mixture (Wardle et al., 2003), transfer of toxic compounds and/or phenolics between litter components causing non-additive negative effect (Freschet et al., 2012), and enhanced chemical diversity fostering a richer microbial and fungal decomposer community and thus promoting litter decay rates (Hättenschwiler et al., 2005; Otsing et al., 2018). Yet, except the total litter phenolics concentration, these parameters are not often measured.

Litterbag studies generally have a standard set-up; litter of two or more species is mixed in a litterbag which is placed on or in the soil, usually in its natural decomposition environment. However, there are still methodological differences between studies. The ratio of species in the mixture is not always 50% -50% (Montané et al., 2013; Wu et al., 2014). Litterbag placement is customarily in the litter layer, yet, occasionally litterbags are buried in the soil (Li et al., 2018; Poffenbarger et al., 2015; Prieto et al., 2017). A study comparing litterbags placed at the litter-mineral soil interface to litterbags placed on top of the litter layer showed additive mass loss at the litter-soil interface whereas the same mixtures showed non-additive mass loss on top of the litter layer (Conn and Dighton, 2000). The litterbag mesh size varies greatly between studies, where mesh sizes of <100 μm only allow microfauna to reach the litter, whereas larger mesh sizes allow mesofauna (0.1-2 mm mesh) or macrofauna (>2 mm mesh) inside the litterbags (Gartner and Cardon, 2004; Kampichler and Bruckner, 2009). A diverse litter mixture in terms of the C:N:P ratio could more easily satisfy a diverse decomposer community because more food sources of different qualities are present (Lecerf et al., 2011). Interactions between litter nutrient concentrations and faunal inclusion could be expected. For example, mass loss of *Quercus petraea* litter increased with increasing litter diversity in the presence of millipedes, yet this effect was not found when earthworms were present (Hättenschwiler and Gasser, 2005).

Next to variations in experimental set-up, litterbags are placed in a wide range of ecosystems (natural or arable), soil types and climates. Soils with a low N availability are expected to result in larger non-additive litter mixing effects since the microbial community, decomposing a N poor litter, could potentially benefit more from a high N litter present since there is not enough N in the soil that could be mined (Bonanomi et al., 2014; Bonanomi et al., 2017; Knorr et al., 2005; Lummer et al., 2012). Further, a wetter climate could influence litter mixing by promoting soil moisture, thus making nutrient transfer between litters easier (Makkonen et al., 2013; Wardle et al., 2003). It would not be surprising if litter mixing in arable systems gave different results than litter mixing in natural systems, as, in contrast to natural systems, arable systems have fertiliser inputs, often mechanical disruption of soil by ploughing and (in general) a lower plant diversity. Moreover, in crop systems, the majority of the plant materials are taken away from the field at harvest.

It is essential to develop a mechanistic understanding of litter mixture decomposition in order to predict soil organic matter dynamics in natural and agricultural systems with higher plant species diversity. To date, the generality of a mechanistic understanding of litter mixing on mass loss across studies is lacking. The absence of a quantitative synthesis of previous studies constitutes an important gap in the state of knowledge on the effect of litter mixing on decomposition. The overarching aim of this paper is to find out if, on average, there are non-additive litter mixing effects and, more importantly, to quantify what parameters control the size and direction of non-additive litter mixing effects by doing a meta-analysis on all peer reviewed published litter mixture litterbag studies to date. We hypothesise that 1) greater chemical litter dissimilarity in leaf litter mixtures will cause larger non-additive mass loss in litter mixtures, and 2) larger non-additive litter mixing effects will be found in soils with a low soil N content. We further explore if other experimental design or environmental factors (such as; mesh size, exposure time, ecosystem, climate and soil quality) can explain part of the variation in litter mixture mass loss. Interactions between explanatory factors are explore in as far as these interactions can be motivated mechanistically.

4.2 Materials and methods

4.2.1 Data collection and extraction from the literature

A literature search was conducted on 29 October 2018 in the ISI-Web of Science core collection (ISI SCI) with the search terms: “Litter AND Mixture AND Decomposition NOT Stream” as well as “Decomposition AND Mixture AND Soil AND (Litter OR Residue)”. This resulted in 523 and 525 publications, respectively, for each search term. After removing the duplicates we had a total of 677 publications. These publications were screened and a publication was included in our dataset if 1) It concerned a litter decomposition experiment which was conducted with the litterbag method, 2) mass loss was reported of both single species litters as well as a 2-species mixture, 3) The ratio of the two litters in the mixture was reported, 4) the time of exposure of litterbags to the environment was stated. If the litter mass loss in a publication was not reported in such a way that the data could be extracted for analysis, we reached out to the author in order to include the data in our database. After screening, 78 publications met the criteria and were used for further analysis. We chose to only include litter mixtures of 2 species because we expected larger effects of litter trait divergence when only 2 distinct litter species were included in the mixture. With more species included divergence would be smaller overall than between the extremes. An experiment was defined as a unique combination of a two-species litter mixture and incubation site, but could include multiple time points at which the litterbags were collected. If the same litter combination was used at a different site or in a different season or year, this was considered as a different experiment. Different litter combinations at the same site and time of burial also constituted different experiments. We coded each experiment within each publication in order to account for random publication and experiment effects in the data analysis. The 78 publications yielded in total 126 sites, 529 experiments and 1359 observations. The information on each publication as well as the response and explanatory variables extracted are reported in Table 4.1.

4.2.2 Response variable

To be able to compare the decomposition rate of the litter mixtures with the expected decomposition rate based on the single species litters present in the mixture we determined the observed mass loss (Obs, in %) as reported in the publications and the expected mass loss (Exp, in %). The expected mass loss was calculated as the weighted mean mass loss of the two single species litters as follows:

$$M_{E\mathrm{xp}} = \frac{\sum_i f_i M_i}{\sum_i f_i}$$

Where M_i is the mass loss in % of a single species, and f is the mass fraction of each litter in the mixture. The response ratio of litter mixing was then calculated as follows:

$$\mathrm{Ln}(R) = \ln\left(\frac{M_{\mathrm{Obs}}}{M_{\mathrm{Exp}}}\right)$$

Where M_{Obs} is the observed mass loss (in %) of the mixture and M_{Exp} is the expected mass loss (in %) of the mixture. A positive value for ln(R) indicates that the observed mass loss was greater than expected based on the mass loss of the two single species.

4.2.3 Explanatory variables

Litter quality variables quantified at the start of the litter incubation were used as explanatory variables for litter mass loss: the percent dry weight in the litter of C, N, phosphorus, lignin, cellulose, hemicellulose and phenolics as well as the litter C:N, C:P, N:P and Lignin:N mass ratio. All chemical litter quality components were expressed as the absolute difference (in % dry weight) between the two litters used in the mixture. Furthermore we described litters based on the types of plant species in our database (e.g. coniferous tree leaf litter, deciduous tree leaf litter, shrub/heath, annual plant shoot residue, annual plant root residue or peat moss) as well as being a woody/non-woody plant (Table 4.1), we differentiated mixtures that had identical plant types (i.e. both coniferous tree leaves) from mixtures that had two different plant types in the mixture (e.g. coniferous + deciduous tree leaves), similarly we did this with woody/non-woody plant mixtures. We examined if the soil organic matter content (SOM), the total soil C and N content, the soil C:N ratio and the soil pH had an effect on litter mixture effects. Regarding the experimental design we examined if the litterbag exposure time or the litterbag placement (in or on the soil) had an effect on litter mixing effects.

Table 4.1 Variables extracted from publications and their corresponding units.

Variable	Definition	Data type/Unit
Title	Title of publication	Text
Authors	Authors of publication	Text
Continent	Continent where study was carried out	Text
Country	Country where study was carried out	Text
Latitude/Longitude	Latitude and longitude of study site	Decimal degrees
Ecosystem	Ecosystem in which study was carried out	Categorical
Precipitation	Annual average precipitation of study site	mm/year
Temperature	Annual average temperature of study site	°C (average/year)
Climate	According to Köppen classification	Categorical
SOM	The soil organic matter content at the study site	g/kg
Soil C	The total soil C content at the study site	%
Soil N	The total soil N content at the study site	%
Soil C:N ratio	The soil C:N at the study site	ratio
Soil pH	The soil pH at the study site	pH unit
Size litterbags	The total surface area of the litterbag	cm^2
Mesh size	The mesh size of the litterbag	mm^2
Burial location	Was the litterbag buried in or placed on top of the soil?	Categorical
Time	Exposure time of the litterbag to the decomposing environment	Days
Species	The species name of both single litters	Text
Litter type	Coniferous leaf litter, deciduous leaf litter, shrub/heath, annual plant shoot residue, annual plant root residue or peat moss	Categorical
Woody plant	Did the litter originate from a woody plant?	Categorical
Dried	Was the litter (oven) dried before placing it in a litterbag?	Categorical
Size of litter	Was the litter cut in pieces, if yes what size?	cm
Ratio litter added	The ratio of the two litters in the mixture	ratio
Litter Quality	C, N, P, Lignin, Total Phenolics, Hemicellulose, Cellulose concentration of the litters	All in %
Litter stoichiometry	The C:N, C:P, N:P and lignin:N ratio of both litters	ratio
Mass loss	The amount of mass loss reported after burial	%

Additionally, we grouped studies according to litterbag mesh size, with the cut-off at different faunal inclusions: only micro fauna (<0.01 mm), micro- and meso fauna (between >0.01 and <2.0 mm) and micro-, meso-, and macro fauna (>2.0 mm). In terms of the environmental conditions we tested if annual average rainfall and temperature affected the response ratio. Further we explored if litterbag placement in different continents, climates according to Geiger (1954); Köppen (1900), ecosystems, natural habitats or arable fields had an effect on the response ratio. Additionally, we checked if the size of the litter fragments or drying the litters had an effect on mass loss.

4.2.4 Statistical analysis

In order to test if litter mixtures show non-additive litter mass loss overall, we tested if ln(R) was significantly different from zero (zero=additive mass loss, > 0 is positive non-additive mass loss, < 0 is negative non-additive mass loss) by performing a one-sample t-test. A one-way analysis of variance (ANOVA) was used to test if categorical variables such as continent, ecosystem, natural/arable, faunal inclusion, could explain patterns in non-additive mass loss. We used mixed models to test if initial litter quality or soil properties had a significant effect on the non-additive mass loss (Ln(R)). Random effects were included to account for the possibility of correlation between data originating from the same publication and experiment (Zuur et al., 2009). Additionally, we tested if interactions between co-variables had an effect on ln(R). These interactions were based on ecological relevance where we expected interactions between the litter nutrient concentration, the C compounds and/or interactions between mesh size and environmental parameters.

All statistical analyses were carried out in R, version 3.5.0 (R Development Core Team, 2013). The package nlme (Pinheiro et al., 2019) was used to fit linear mixed effects models. Model selection was conducted using the R functions AIC and ANOVA (R package stats; (R Core Team, 2018). The package function r.squaredGLMM from the MuMIn package was used to extract R^2 values of the fixed effects from the mixed effects models (Barton, 2019). Fifty-nine mixed effects models were fitted to the data (Table S4.1, supporting information). Observations with missing values of a variable were excluded from all analyses which required that variable. This restriction led to several non-identical subsets of the data and not all interactions between litter quality parameters could be tested.

All statistical analyses were performed on three different subsets of the data: 1) all observations extracted from literature (1359 observations), 2) observations that reported the standard error and sample size (860 observations) and 3) observations that reported significant non-additive mass loss (125 observations).

In the two subsets of data which included reported standard errors we gave weights to each observation according to the variance of ln(R). This variance was calculated as:

$$\mathrm{Var}_{\ln(R)} = \frac{SE_{obs}^2}{\mathrm{X}_{obs}^2} + \frac{a^{\,2}{SE_{X1}}^2 + (1-a)^{\,2}{SE_{X2}}^2}{(a\,X_1 + (1-a)\,X_2)^2}$$

Where SE is the standard error of the mass loss of litter 1, litter 2, and of the mixture, *X* is the mass loss of litter 1, 2 and of the mixture and *a* is the fraction of litter 1 in the mixture.

A funnel plot of standard error against ln(R) of each observation was made to assess publication bias.

4.3 Results

4.3.1 Publication bias

There was no publication bias in our database as the funnel plot was symmetrical (Fig S4.1, Supporting information). There were a few missing values in the bottom right corner which represent studies with a high standard error and a positive non-additive litter mixing effect, however, the number of data points with large SE was too small to conclude that bias exists.

4.3.2 Descriptive analysis

A total of 1359 observations were extracted from the literature. Over 90% of the data originated from studies in Asia (almost solely China), North America (mostly the USA) and Europe (Fig 4.1A). The majority of studies were done in natural systems (72%, Fig 4.1B), mostly in either temperate or continental climates (Fig 4.1C). The number of studies per ecosystem varied greatly. The dominant ecosystem was deciduous forest (47%,) followed by arable crop fields (17%) (Fig 4.1D).

4.3.3 Litter mixing effects

The response ratio ln(R) varied between a minimum value of -2.17 to a maximum value of 2.42, indicating that litter mixtures at times decompose approximately 10 times faster ($e^{2.42}$) or slower ($e^{-2.17}$) than expected. Fifty percent of all the observations were close to zero with a ln(R) between -0.06 and 0.10 (Fig S4.2A, supporting information). Overall, 768 observations showed positive mixing effects and 591 observations showed negative litter mixing effects on litter decomposition rate. The average response ratio of mass loss in litter mixtures was not significantly different from zero (-0.003 ± 0.007 ; $p = 0.62$), indicating that on average litter mixtures decomposed at rates similar to what was expected.

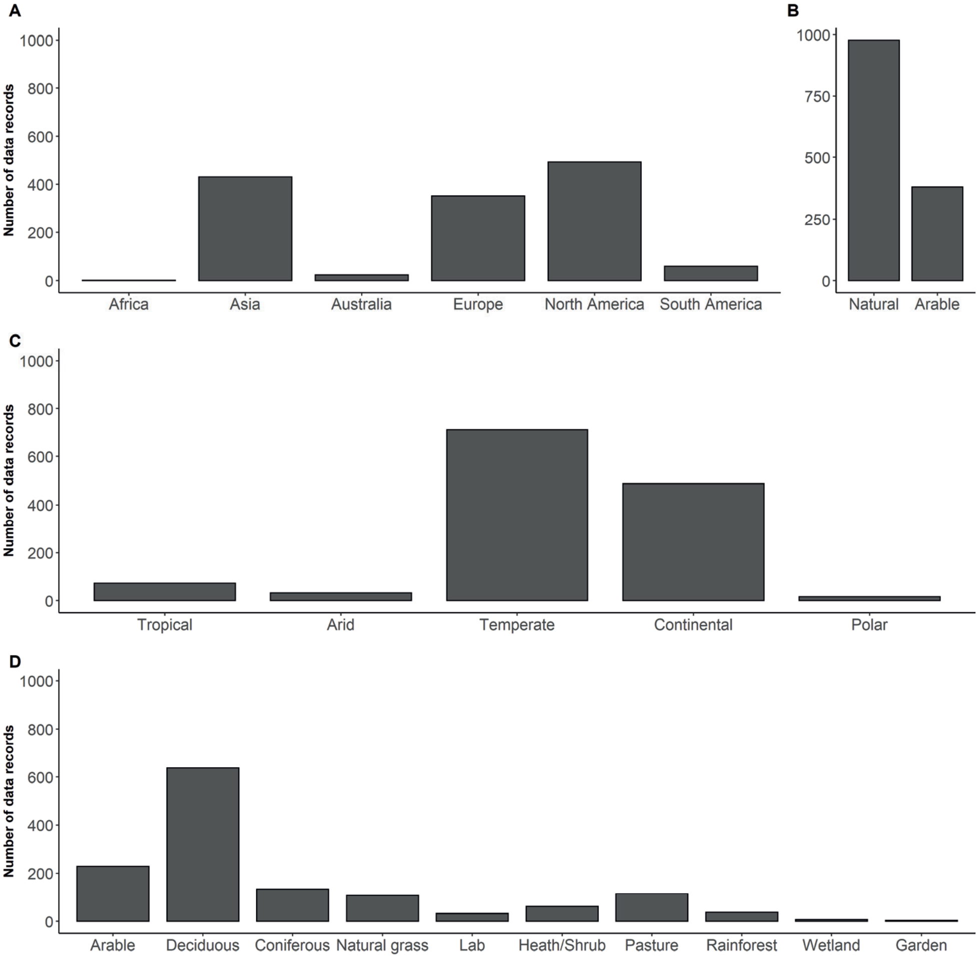

Figure 4.1 Number of observations extracted from the literature separated by A) continent, B) natural and arable systems, C) climate, according to Köppen-Geiger, and D) ecosystem.

A subset of 860 observations reported the standard error and sample size. From this subset the average response ratio of mass loss in litter mixtures was slightly above zero (0.019 ± 0.008, $p = 0.02$, Fig S4.2B, supporting information). Within this subset, 125 observations showed significant non-additive mass loss ($p < 0.05$), which is 15% of the litter mixtures. Fifty five percent of these observations showed significant positive non-additive mass loss and 45% showed significant negative non additive mass loss.

On average, litter mixtures did not decompose at different rates than expected, nevertheless given the variation in the response, we further explored potential litter quality effects in explaining variation in the litter decomposition response ratios.

4.3.4 Chemical litter trait dissimilarity

In contrast to what we hypothesised, differentiation of litter N concentration in the constituent litters in a mixture was unrelated to ln(R). Neither did the differentiation in the C, phosphorus, C:N ratio, N:P ratio, lignin, phenolics or (hemi-) cellulose concentration of the two litter species in the mixture (Fig 4.2 and 4.3). Only the C:P ratio and the lignin:N ratio showed a significant yet weak effect on non-additive mass loss ($\beta_1 = -5.6\ e^{-5}$, $p < 0.01$, $R^2 = 0.033$ and β_1 -1.6 e^{-3}, $p < 0.01$, $R^2 = 0.039$ respectively).

These analyses were repeated on the subset of the data that included standard errors and sample sizes. Here an increase in litter ΔC:N (β_1 -0.0007, $p < 0.01$) and Δ lignin:N (β_1 -0.001, $p < 0.01$) resulted in a lower ln(R) (Fig 4.7A and B). While significant, these regressions explained only 0.05% and 0.07% of the variation in ln(R) respectively.

4.3.5 Experimental set-up, environment and ecosystem

The time of litterbag exposure did not significantly affect litter mixture effects on mass loss (Fig 4.4A). This conclusion is drawn under the limitation of the data that 84% of the studies had litter incubation times shorter than one year. The average annual rainfall and temperature did not have a significant effect on ln(R) (Fig 4.4B and C). None of the soil quality parameters significantly explained the variation in litter mixture mass loss (Fig 4.5A-E). These analyses were repeated on the subset of the data that included standard errors and sample sizes. Here an increase in total soil N (β_1 -0.11, $p < 0.05$) and soil C:N ratio (β_1 -0.0006, $p < 0.05$) resulted in a lower ln(R) (Fig 4.7C and D). While significant, these regressions only explained 0.03% and 0.04% respectively of the variation in ln(R).

Faunal inclusion, litterbag placement, ecosystems, continents, climates, systems (natural vs arable) and the difference between woody:woody, woody:non-woody and non-woody:non-woody species mixtures did not explain variation in litter mixture effects on decomposition (Fig 4.6A-F). Non-additive effects on mass loss were not different when we differentiated mixtures that had identical plant types (i.e. both coniferous tree leaves) to mixtures that had two different plant types in the mixture (e.g. coniferous + deciduous tree leaves). Drying litters and the size to which the litters were cut before the incubation period also did not have an effect on ln(R).

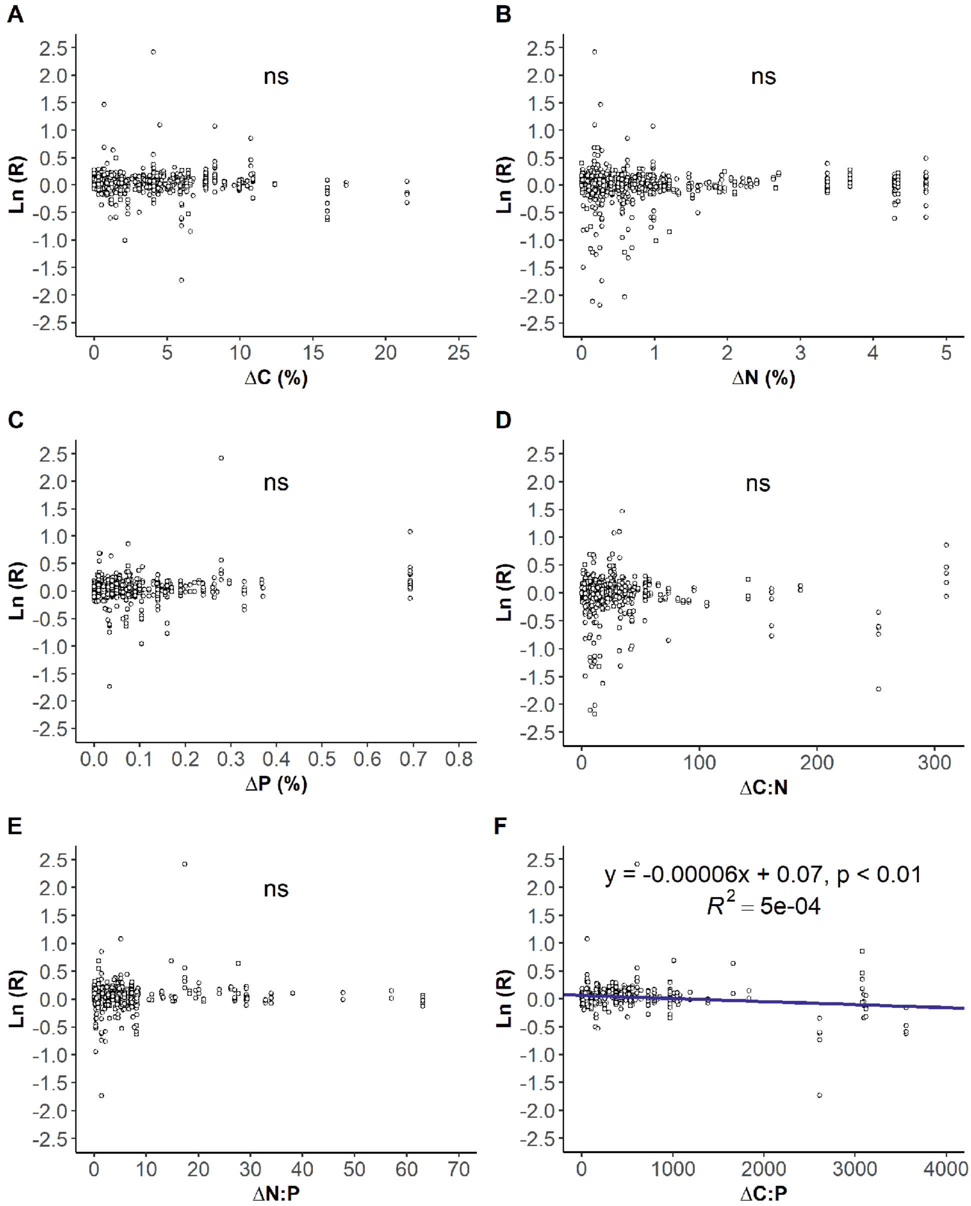

Figure 4.2 Relationship between ln(R) and A) ΔC, B) ΔN, C) ΔPhosphorus, D) ΔC:N, E) ΔN:P and F) ΔC:P, estimated with mixed effects models. Model 2 to 7 (Table S4.1, supporting information), i.e. $\ln(R)_{ijk} = \beta_0 + \beta_1 * \Delta\text{Quality} + a_i + b_{ij} + \epsilon_{ijk}$, ns = not significant ($p>0.05$).

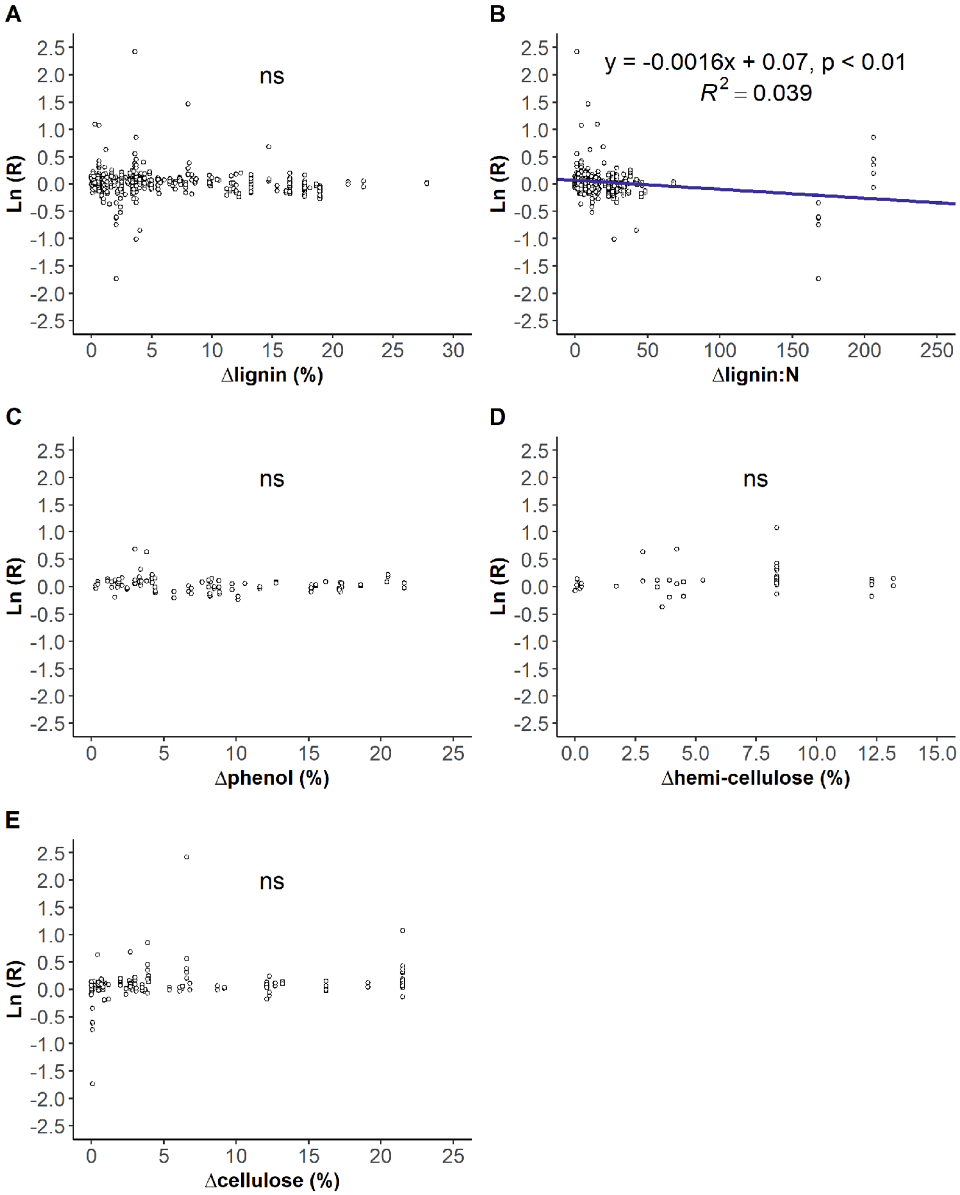

Figure 4.3 Relationship between ln(R) and A) Δlignin, B) Δlignin:N, C) Δphenolics, D) Δhemicellulose and E) Δcellulose, estimated with mixed effects models. Model 8 to 12 (Table S4.1, supporting information), i.e. $\ln(R)_{ijk} = \beta_0 + \beta_1 * \Delta\text{Quality} + a_i + b_{ij} + \epsilon_{ijk}$, ns = not significant ($p>0.05$).

4.3.6 Interactions

We tested interactions between the different litter quality parameters, as well as interactions between litter quality and climate, time, mesh size and soil N (models 29 to 59, Table S4.1, supporting information). From the tested models the two-way interaction between litter ΔN * time ($p < 0.05$), litter ΔP * time ($p < 0.05$), litter ΔC:P * rainfall ($p < 0.01$), litter ΔC:P * temperature ($p < 0.05$) and the 4-way interaction between ΔN * ΔP * Δlignin * time ($p < 0.05$) were significant (Table S4.2, supporting information). These analyses were repeated on the subset of the data that included errors and sample size. Here the interaction between and litter ΔP * time was no longer significant. Yet the two- way interaction of litter ΔC:P * rainfall ($p < 0.05$), litter ΔC:P * temperature ($p < 0.01$) and the three-way interaction between litter ΔN * ΔP * time were significant ($p < 0.05$). Additionally two-way interactions between ΔC:N * rainfall and ΔC:N * temperature as well as Δlignin:N * temperature were also significant. Yet these interactions explained only 0.06%, 0.08% and 0.08% respectively of the variation in litter mixing. Further data exploration included a repetition of above analysis executed for each ecosystem and climate separate (if the number of observations allowed). Although there were significant interactions between litter quality and experimental design and ln(R) these interactions were weak with generally a very low R2 (Table S4.3 and S4.4 in supporting information).

4.4 Discussion

The decomposition rate of litter mixtures composed of two species is on average not faster or slower than expected based on the decomposition rate of the single species litters and their proportions in the mixture. Many studies report non-additive mass loss in litter mixtures. In the data set assembled for this meta-analysis, 15% of the records showed a significantly higher or lower mass loss than expected, which is considerably lower than the 67% of significant non-additive mass loss reported previously in a vote-counting review by Gartner and Cardon (2004). In our analysis, none of the selected parameters explained a pattern in this data. For the majority of litter mixtures (85%) the mass loss can be predicted based on component single litter mass losses.

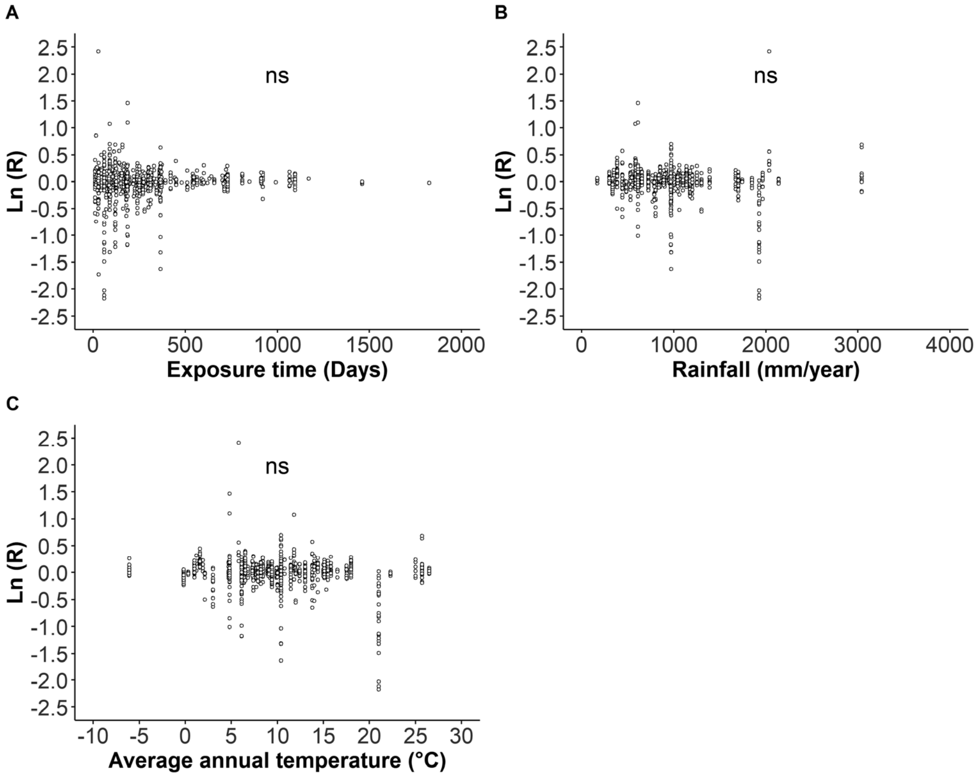

Figure 4.4 Relationship between ln(R) and A) exposure time of the litterbag, B) the average rainfall per year (mm/year) and C) the average annual temperature. Estimated with model 20, 23 and 24 respectively (Table S4.1, supporting information), i.e. $\ln(R)_{ijk} = \beta_0 + \beta_1 * X + a_i + b_{ij} + \epsilon_{ijk}$, ns = not significant ($p$>0.05).

4.4.1 Chemical litter trait dissimilarity

Contrary to what many studies suggest, a larger difference in litter N concentration did not result in greater non-additive litter mixture effects, even though the range of ΔN and ΔC:N included in our dataset was sufficiently large (ΔN between 0.0 – 4.72 %; ΔC:N between 0.2 – 309.84) in order for nutrient transfer to have taken place. Therefore we conclude that the difference in litter N concentration does not as single factor control the size and direction of non-additive mass loss. This conclusion is consistent with two other studies. Lummer et al. (2012) found that N transfer from a N rich species to a N poor species did occur, yet mixture mass loss was still additive in these mixtures. Schimel and Hättenschwiler (2007) showed in a microcosm experiment with N-labelled litter that N transfer in litter mixtures was not determined by the difference in the N concentration between the two litters but by the mass fraction of N in the leaf litter with the higher mass fraction of N. Many factors apart from the difference in N concentration could affect the rate of decomposition of mixed litters. Thus, not showing an effect of different N concentration in litters on the rate of decomposition of the mixture, as we do here, does not necessarily mean that the effect does not exist, but it does mean that this effect, if it exists, is not strong enough to emerge consistently under the varying conditions represented by the data assembled for this meta-analysis. We cannot exclude that other factors, such as toxic compounds or water limitation that hamper nutrient flow, could interfere with or mask a potential effect of N transfer.

None of the other litter quality parameters could explain non-additive mass loss in mixtures. Surprisingly the presence of phenolic compounds in one of the litters did not show an antagonistic effect on litter mixture decomposition, even though the phenolics concentration ranged between 0 and 23% of dry mass (out of yields commonly reported of 1-25% of leaf dry mass (Hättenschwiler and Vitousek, 2000)). That phenolics did not cause antagonistic effects could also be explained by the fact that there is a range of phenolic compounds. The two types of phenolics, low molecular weight phenolics and condensed tannins, can either provides a substrate for microbial growth or inhibit microbial growth (Hättenschwiler et al., 2005) and thus could potentially result in opposite effects on litter mass loss (Hättenschwiler and Vitousek, 2000).

Even when we examined the subset of studies showing significant non-additive mass loss, litter chemical trait dissimilarity could not convincingly explain patterns in non-additive mass loss. This is in agreement with Tardif and Shipley (2015), who reported non-additive mass loss in a subset of 42 different litter mixtures studied yet could not generalise litter chemical diversity effects on non-additive mass loss. We further explored the data by repeating the analysis of litter quality and or the experimental set-up effects on non-additive mass loss in different ecosystems and climate zones (if enough data was available in each subset). This did not result in any clear patterns in subsets of the data with a specific ecosystem or climate (Table S4.3 and S4.4, supporting information). It has to be noted that the majority of litterbag studies were executed in forest ecosystems; therefore additional studies in the grasslands, peatlands and arable lands are necessary in order to make a more robust analysis for these ecosystems.

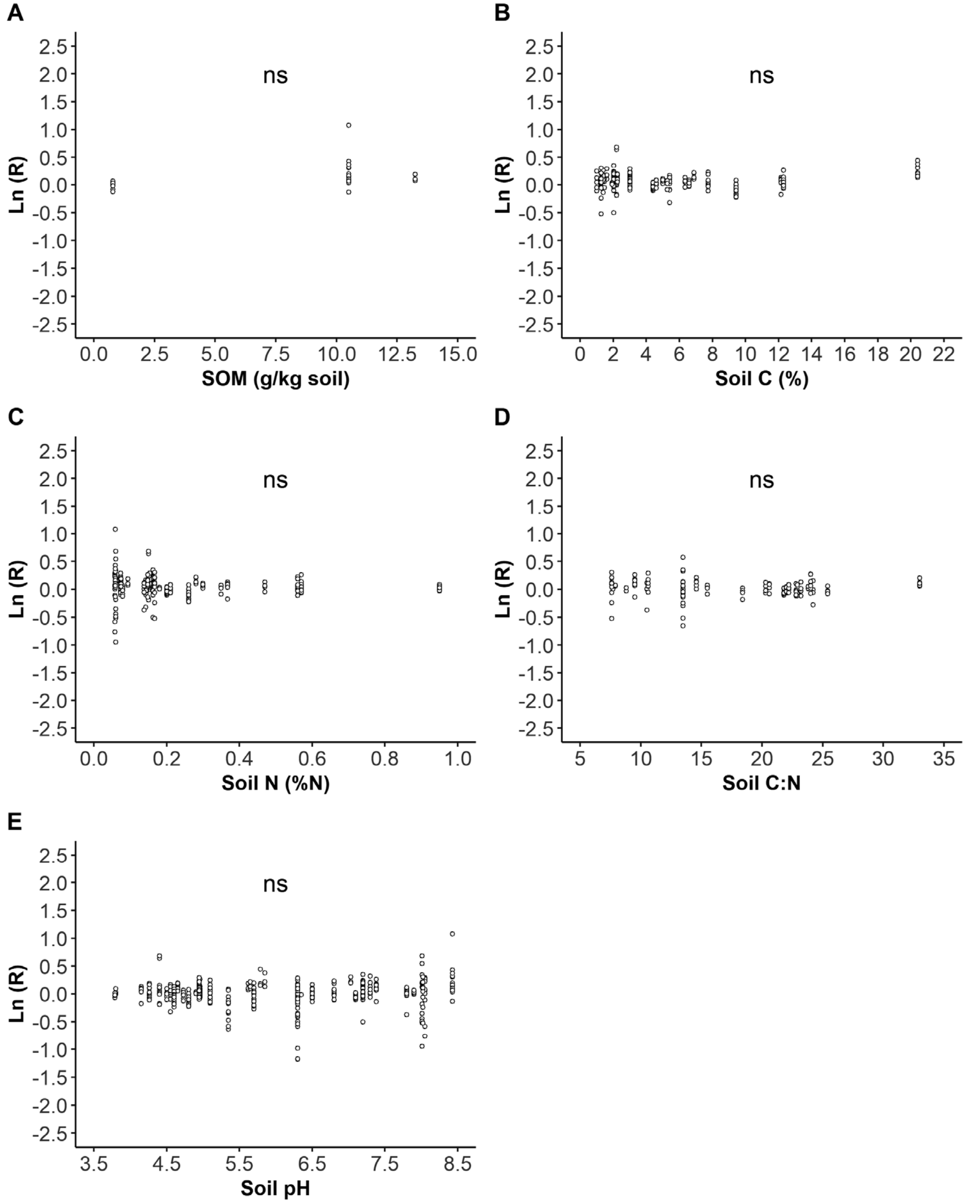

Figure 4.5 Relationship between ln(R) and A) Soil organic matter, B) Soil total C content, C) Soil total N content, D) Soil C:N ratio , E) Soil pH, estimated with mixed effects models. Model 13 to 17 (Table S4.1, supporting information), i.e. $\ln(R)_{ijk} = \beta_0 + \beta_1 * \text{Soil Quality} + a_i + b_{ij} + \epsilon_{ijk}$, ns = not significant ($p>0.05$).

4.4.2 Experimental design

We did not find a difference between the different faunal inclusions on the size of non-additive mass loss in litter mixtures. Previously, Barantal et al. (2014) showed that soil fauna played a key role as a driver of litter mixture effects. As expected they found that synergistic non-additive mass loss increased with larger trait dissimilarity when meso- and macrofauna were included. Yet, in our meta-analysis, there was no significant interaction between ΔN and faunal inclusion.

Another major factor which did not explain the size of non-additive mass loss was the exposure time of the litterbags to the environment. A longer exposure time has been shown to create larger non-additive effects (Srivastava et al., 2009). These authors hypothesised that the mechanisms of litter decomposition and thereby potential non-additive effects in litter mixtures change over time from rapid nutrient leaching to an increasing reliance on the soil fauna to breakdown more complex molecules. Perhaps we did not see this trend because the large majority of the studies (84%) had an exposure time of less than 1 year.

Additionally we also expected factors like the litterbag placement (in or on soil) to have an effect (Conn and Dighton, 2000). Litterbags buried in the soil are in close contact with soil microbes and mineral surfaces whereas litterbags placed on top of the soil are in contact with other litters, thus surrounding leaves may interact with the litter species in the litterbags, creating more diverse mixtures. However when we distinguished between studies in which the litters were buried in the soil (no contact with other litters) and studies with litterbags placed on the soil (contact with other litters) we did not find significant differences. The issue with this is that some studies are performed in sites with single litter species (litter bed), and others in a site with a wide variety of litters present (natural forest). It can therefore be argued that studies examining litter mixture decomposition when placing litter on a litter layer might not always be valid due to other interactions present with external litters.

4.4.3 Environment

We expected that poor soils in terms of the mineral N content would show larger non-additive mass loss effects. We did not find a significant effect of the soil N content on the size of non-additive mass loss. However this is perhaps not surprising since the soil nutrient status was not often reported, and when soil N was reported it was as the total N content. A large part of this total N content could be unavailable to the decomposer community, depending on the soil type, and this could thus influence the mass loss. Moreover, Vivanco and Austin (2011) found that additive litter mixture effects in soil without N addition turned into synergistic effects when N was added to the system. They suggested that it might be possible that once N limitation was removed, other limitations constrained mass loss, and synergistic effects were observed again indicating that the N availability in itself is not a good predictor of litter mixture effects. Contrary to what we expected we did not find a significant difference in litter mixture effects between natural and arable systems.

4.4.4 Lacking explanatory variables

Surprisingly, the large majority of litter mixtures had close to additive mass loss (Fig S4.2, supporting information). And only 15% of the data showed significant non-additive mass loss in mixtures. Moreover, no litter or soil quality parameter showed a (strong) significant relationship with the direction/size of the non-additive effect. Even so, non-additive effects are found with mass loss being sometimes ten times as fast or slow as expected. Thus litter mixing can sometimes have a substantial effect on the C balance, hence it is important to predict these occasionally strong litter mixing effects.

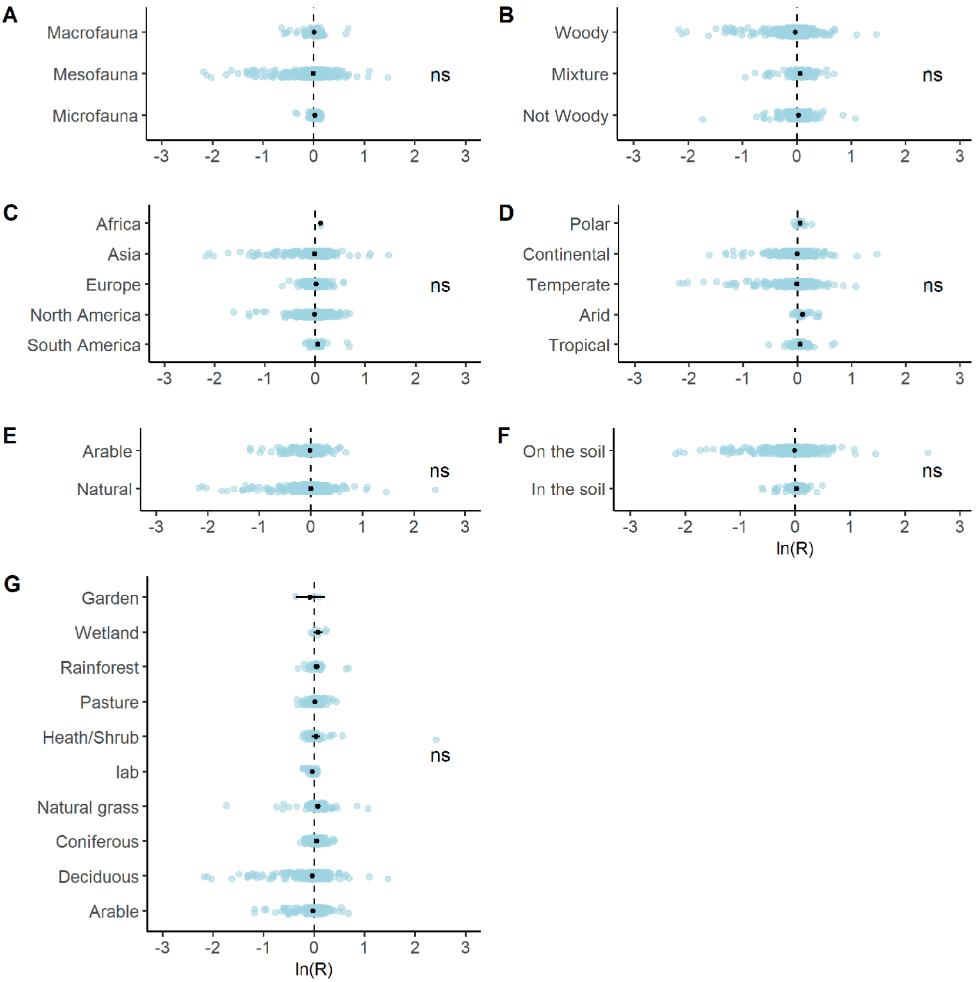

Figure 4.6 Response ratio of litter mixture decomposition, ln(R), plotted against A) faunal inclusion separated into: microfauna (mesh <0.01mm), mesofauna (mesh 0.01 - 2mm) and macrofauna (mesh > 2mm), B) Litter mixtures of: only woody plants, woody and non-woody plants, and only non woody plants, C) continent, D) climate, E) natural vs. arable sites, F) location of litterbag placement and G) the ecosystem. The black dots show the average and the light blue dots show all the data on which the average is based. Error bars (hardly reach outside average) show the standard error. None of these factors significantly influenced the size of the non-additive effect (*p*<0.05, Table S4.2 supporting information).

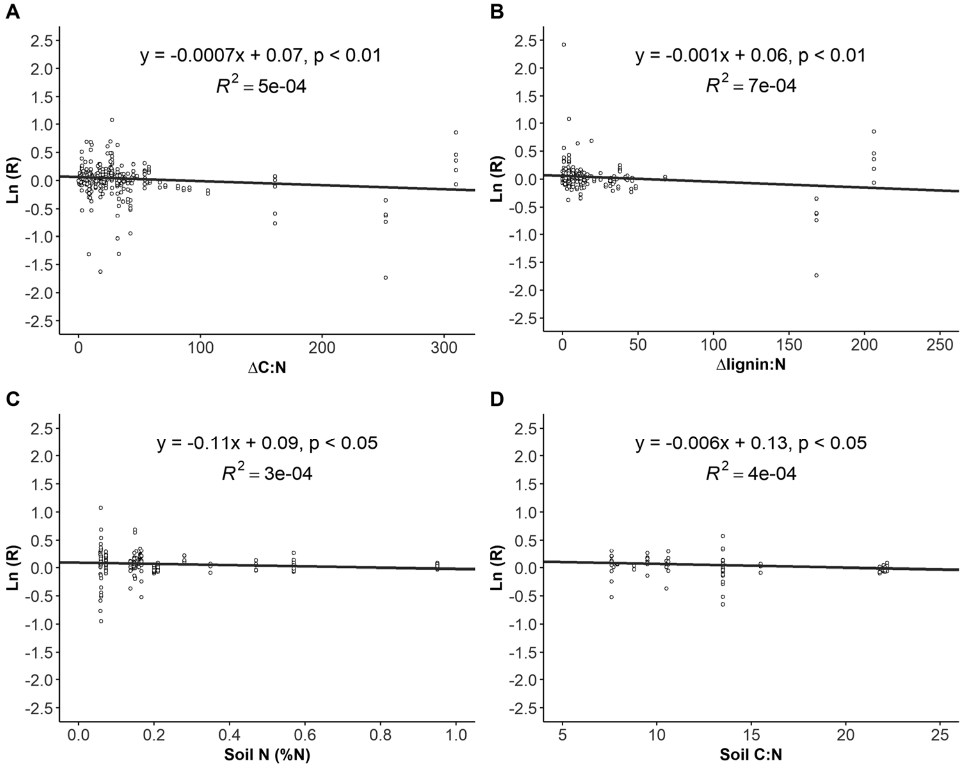

Figure 4.7 The relationship between ln(R) and A) ΔC:N (unitless), B) Δlignin:N (unitless), C) Soil N (% N) and D) Soil C:N (unitless) estimated with mixed effects models on the subset of studies that reported the error term (860 observations). Model 4, 9, 15 and 16 (Table S4.1, supporting information), i.e. $\ln(R)_{ijk} = \beta_0 + \beta_1 * \Delta\text{Quality} + a_i + b_{ij} + \epsilon_{ijk}$ The data in the model were weighted according to the reported variance

The majority of litterbag studies (78%) report litter N concentration, yet few publications report a more extensive range of litter quality parameters (such as; C, N, P, lignin, phenol) (Barantal et al., 2014; Makkonen et al., 2013). Additionally, other litter characteristics, such as litter physical traits (leaf thickness/elongation), are hardly ever measured and could be important in interaction with litter chemical trait dissimilarity. A mixture of small and large litter fragments could ameliorate the microclimate for decomposition, which has been proposed as a mechanism to explain non-additive effects of litter mixture decomposition (Hättenschwiler et al., 2005). Makkonen et al. (2013) showed indeed that micro-climatic conditions and litter physical traits can determine if non-additive effects were synergistic or antagonistic. Thus, they proved that litter physical and chemical trait dissimilarity alone cannot predict the direction of litter mixture interaction. In a study by Anderson and Hetherington (1999) the chemical composition of the two litters was very similar and still non-additive mass loss was found. They speculated that decomposition was enhanced by the synergistic interaction of different fungal species associated with the two litter types. Therefore it is an issue that most studies to date have focussed on either extensive litter chemical quality measurements or only on soil decomposer communities or on climate. Only few studies have included all relevant parameters, even though this could be necessary to predict non-additive litter mixture mass loss.

Overall, there are a myriad of factors that can influence the litter mixing effects on mass loss such as the initial litter quality, the experimental design and environmental conditions. Besides the parameters mentioned in this study, which were often measured, many other factors such as leaf thickness, decomposer community, the presence or absence of a certain decomposer and the soil structure could also play a (minor) role. This could make it nearly impossible to predict if mass loss will be non-additive and what the direction and size this non-additive mass loss in mixtures will have. Even minute differences in the starting situation could create a wide range of different outcomes in terms of litter mixture mass loss. Perhaps any small change in the litter quality, micro-climate, soil quality and decomposer community at the start of the experiment could overrule the effects of more commonly reported (and also in this study included) litter quality parameters. There are many interactions possible between many parameters that not a single study has reported on all variables. As an example, if we compare a litter mixture of a moss + deciduous leaf to a litter mixture of a coniferous + deciduous leaf, not only the litter nutrient concentration or C structure is important. Many other factors such as the litter thickness, hydrophobicity, wax layer, water holding capacity, size, etc. are likely to covary and play a role.

4.5 Conclusion

This meta-analysis shows that the majority of reported results of studies on litter mixture mass loss can be predicted based on single litter mass loss since 85% of all 2-species litter mixtures show additive mass loss. Even so, non-additive effects are found with litter mixture mass loss being sometimes ten times greater or smaller than expected. The number of cases with less than additive mass loss (49) was only somewhat lower than the number of cases with more than additive mass loss (76). We found no overriding dominant trends in litter traits driving additive, less than additive, or more than additive mass loss in litter mixtures. However, evidence exists that such effects do emerge under specific circumstances. None of the parameters that were tested stand out alone as a dominant driver that can explain a significant portion of the variability present among the studies included in our meta-analysis. Besides the most often observed chemical litter quality traits, other parameters such as other nutrients than N and P, leaf size, structure, soil quality, climate, soil fauna and their interactions could play a role. This raises the question if we need more studies that integrate all possible parameters that could influence litter mixing effects or if it is simply impossible to predict non-additive mass loss since minute differences in the starting situation could alter the size and direction of non-additive mass loss.

4.6 Acknowledgements

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4.8 Supporting information

Table S4.1 Specification of the models fitted to the data, where the effect of explanatory variables on the response ratio are modelled. Where β_0 and β_1 report the intercept and the slope, a_i is a random publication effect and b_{ij} is a random experiment effect nested within the *i*th publication. ϵ_{ijk} is a residual random error assumed normally distributed with constant variance. ϵ_{ijk} is constant in models without weights and variable in models with weights (on the subset of data with standard errors reported). The variance terms a_i, b_{ij} and ϵ_{ijk} were all assumed independent. The final columns indicate the number of publications, experiments and observations that could be included in each model (without weights).

Model	Equation	Publication	Experiment	Observation
1	$\ln(R)_{ijk} = \beta_0 + a_i + b_{ij} + \epsilon_{ijk}$	78	529	1359
2	$\ln(R)_{ijk} = \beta_0 + \beta_1\,\Delta C + a_i + b_{ij} + \epsilon_{ijk}$	47	299	768
3	$\ln(R)_{ijk} = \beta_0 + \beta_1\,\Delta N + a_i + b_{ij} + \epsilon_{ijk}$	63	406	1056
4	$\ln(R)_{ijk} = \beta_0 + \beta_1\,\Delta C/N + a_i + b_{ij} + \epsilon_{ijk}$	49	327	779
5	$\ln(R)_{ijk} = \beta_0 + \beta_1\,\Delta P + a_i + b_{ij} + \epsilon_{ijk}$	36	189	535
6	$\ln(R)_{ijk} = \beta_0 + \beta_1\,\Delta N/P + a_i + b_{ij} + \epsilon_{ijk}$	36	189	535
7	$\ln(R)_{ijk} = \beta_0 + \beta_1\,\Delta C/P + a_i + b_{ij} + \epsilon_{ijk}$	29	155	437
8	$\ln(R)_{ijk} = \beta_0 + \beta_1\,\Delta \mathrm{Lignin} + a_i + b_{ij} + \epsilon_{ijk}$	28	173	432
9	$\ln(R)_{ijk} = \beta_0 + \beta_1\,\Delta \mathrm{Lignin/N} + a_i + b_{ij} + \epsilon_{ijk}$	27	169	416
10	$\ln(R)_{ijk} = \beta_0 + \beta_1\,\Delta \mathrm{Phenol} + a_i + b_{ij} + \epsilon_{ijk}$	6	80	97
11	$\ln(R)_{ijk} = \beta_0 + \beta_1\,\Delta \mathrm{HemiCell} + a_i + b_{ij} + \epsilon_{ijk}$	5	29	46
12	$\ln(R)_{ijk} = \beta_0 + \beta_1\,\Delta \mathrm{Cellulose} + a_i + b_{ij} + \epsilon_{ijk}$	11	82	137
13	$\ln(R)_{ijk} = \beta_0 + \beta_1\,\mathrm{SOM} + a_i + b_{ij} + \epsilon_{ijk}$	3	8	26
14	$\ln(R)_{ijk} = \beta_0 + \beta_1\,\mathrm{SoilC} + a_i + b_{ij} + \epsilon_{ijk}$	24	100	303
15	$\ln(R)_{ijk} = \beta_0 + \beta_1\,\mathrm{SoilN} + a_i + b_{ij} + \epsilon_{ijk}$	27	108	341
16	$\ln(R)_{ijk} = \beta_0 + \beta_1\,\mathrm{SoilCN} + a_i + b_{ij} + \epsilon_{ijk}$	10	56	161
17	$\ln(R)_{ijk} = \beta_0 + \beta_1\,\mathrm{SoilpH} + a_i + b_{ij} + \epsilon_{ijk}$	36	155	503
18	$\ln(R)_{ijk} = \beta_0 + \beta_1\,\mathrm{Mesh} + a_i + b_{ij} + \epsilon_{ijk}$	77	525	1347
19	$\ln(R)_{ijk} = \beta_0 + \beta_1\,\mathrm{Burial\ location} + a_i + b_{ij} + \epsilon_{ijk}$	76	527	1350
20	$\ln(R)_{ijk} = \beta_0 + \beta_1\,\mathrm{Time} + a_i + b_{ij} + \epsilon_{ijk}$	78	529	1359
21	$\ln(R)_{ijk} = \beta_0 + \beta_1\,\mathrm{Ecosystem} + a_i + b_{ij} + \epsilon_{ijk}$	77	526	1356
22	$\ln(R)_{ijk} = \beta_0 + \beta_1\,\mathrm{Climate} + a_i + b_{ij} + \epsilon_{ijk}$	78	529	1359
23	$\ln(R)_{ijk} = \beta_0 + \beta_1\,\mathrm{Rainfall} + a_i + b_{ij} + \epsilon_{ijk}$	64	473	1121

24	$\ln(R)_{ijk} = \beta_0 + \beta_1\,\text{Temperature} + a_i + b_{ij} + \epsilon_{ijk}$	64	473	1121
25	$\ln(R)_{ijk} = \beta_0 + \beta_1\,\text{LitterType} + a_i + b_{ij} + \epsilon_{ijk}$	78	529	1359
26	$\ln(R)_{ijk} = \beta_0 + \beta_1\,\text{Woody} + a_i + b_{ij} + \epsilon_{ijk}$	78	529	1359
27	$\ln(R)_{ijk} = \beta_0 + \beta_1\,\text{Dried} + a_i + b_{ij} + \epsilon_{ijk}$	59	341	878
28	$\ln(R)_{ijk} = \beta_0 + \beta_1\,\text{Size litter} + a_i + b_{ij} + \epsilon_{ijk}$	8	42	182
29	$\ln(R)_{ijk} = \beta_0 + \beta_1\,\Delta C + \beta_2\,\Delta N + \beta_3\,\Delta C{*}\Delta N + a_i + b_{ij} + \epsilon_{ijk}$	47	299	768
30	$\ln(R)_{ijk} = \beta_0 + \beta_1\,\Delta C + \beta_2\,\Delta P + \beta_3\,\Delta C{*}\Delta P + a_i + b_{ij} + \epsilon_{ijk}$	29	155	437
31	$\ln(R)_{ijk} = \beta_0 + \beta_1\,\Delta N + \beta_2\,\Delta P + \beta_3\,\Delta N{*}\Delta P + a_i + b_{ij} + \epsilon_{ijk}$	36	189	535
32	$\ln(R)_{ijk} = \beta_0 + \beta_1\,\Delta N + \beta_2\,\Delta\text{Phenol} + \beta_3\,\Delta N{*}\Delta\text{Phenol} + a_i + b_{ij} + \epsilon_{ijk}$	6	80	97
33	$\ln(R)_{ijk} = \beta_0 + \beta_1\,\Delta C + \beta_2\,\Delta N + \beta_3\,\Delta P + \beta_4\,\Delta C{*}\Delta N{*}\Delta P + a_i + b_{ij} + \epsilon_{ijk}$	29	155	437
34	$\ln(R)_{ijk} = \beta_0 + \beta_1\,\Delta N + \beta_2\,\Delta P + \beta_3\,\Delta\text{Lignin} + \beta_4\,\Delta N{*}\Delta P{*}\Delta\text{Lignin} + a_i + b_{ij} + \epsilon_{ijk}$	14	82	212
35	$\ln(R)_{ijk} = \beta_0 + \beta_1\,\Delta N + \beta_2\,\Delta\text{Lignin} + \beta_3\,\Delta N{*}\Delta\text{Lignin} + a_i + b_{ij} + \epsilon_{ijk}$	27	169	416
36	$\ln(R)_{ijk} = \beta_0 + \beta_1\,\Delta N + \beta_2\,\Delta\text{Lignin} + \beta_3\,\Delta\text{Phenol} + \beta_4\,\Delta N{*}\Delta\text{Lignin} * \Delta\text{Phenol} + a_i + b_{ij} + \epsilon_{ijk}$	5	78	89
37	$\ln(R)_{ijk} = \beta_0 + \beta_1\,\Delta N + \beta_2\,\text{Time} + \beta_3\,\Delta N * \text{Time} + a_i + b_{ij} + \epsilon_{ijk}$	63	406	1056
38	$\ln(R)_{ijk} = \beta_0 + \beta_1\,\Delta P + \beta_2\,\text{Time} + \beta_3\,\Delta P * \text{Time} + a_i + b_{ij} + \epsilon_{ijk}$	36	189	535
39	$\ln(R)_{ijk} = \beta_0 + \beta_1\,\Delta\text{Lignin} + \beta_2\,\text{Time} + \beta_3\,\Delta\text{Lignin} * \text{Time} + a_i + b_{ij} + \epsilon_{ijk}$	28	173	432
40	$\ln(R)_{ijk} = \beta_0 + \beta_1\,\Delta N + \beta_2\,\Delta P + \beta_3\,\text{Time} + \beta_4\,\Delta N * \Delta P * \text{Time} + a_i + b_{ij} + \epsilon_{ijk}$	36	189	535
41	$\ln(R)_{ijk} = \beta_0 + \beta_1\,\Delta N + \beta_2\,\Delta P + \beta_3\,\Delta\text{Lignin} + \beta_4\,\text{Time} + \beta_5\,\Delta N * \Delta P * \Delta\text{Lignin} * \text{Time} + a_i + b_{ij} + \epsilon_{ijk}$	14	82	212
42	$\ln(R)_{ijk} = \beta_0 + \beta_1\,\text{Mesh} + \beta_2\,\text{Time} + \beta_3\,\text{Mesh} * \text{Time} + a_i + b_{ij} + \epsilon_{ijk}$	77	525	1347
43	$\ln(R)_{ijk} = \beta_0 + \beta_1\,\text{Mesh} + \beta_2\,\Delta N + \beta_3\,\text{Mesh} * \Delta N + a_i + b_{ij} + \epsilon_{ijk}$	62	402	1044
44	$\ln(R)_{ijk} = \beta_0 + \beta_1\,\text{Temperature} + \beta_2\,\Delta C + \beta_3\,\text{Temperature} * \Delta C + a_i + b_{ij} + \epsilon_{ijk}$	38	256	580
45	$\ln(R)_{ijk} = \beta_0 + \beta_1\,\text{Rainfall} + \beta_2\,\Delta C + \beta_3\,\text{Rainfall} * \Delta C + a_i + b_{ij} + \epsilon_{ijk}\,\epsilon_{ijk}$	38	256	580
46	$\ln(R)_{ijk} = \beta_0 + \beta_1\,\text{Temperature} + \beta_2\,\Delta N + \beta_3\,\text{Temperature} * \Delta N + a_i + b_{ij} + \epsilon_{ijk}$	51	355	837
47	$\ln(R)_{ijk} = \beta_0 + \beta_1\,\text{Rainfall} + \beta_2\,\Delta N + \beta_3\,\text{Rainfall} * \Delta N + a_i + b_{ij} + \epsilon_{ijk}$	51	355	837
48	$\ln(R)_{ijk} = \beta_0 + \beta_1\,\text{Temperature} + \beta_2\,\Delta P + \beta_3\,\text{Temperature} * \Delta P + a_i + b_{ij} + \epsilon_{ijk}$	28	170	454
49	$\ln(R)_{ijk} = \beta_0 + \beta_1\,\text{Rainfall} + \beta_2\,\Delta P + \beta_3\,\text{Rainfall} * \Delta P + a_i + b_{ij} + \epsilon_{ijk}$	28	170	454
50	$\ln(R)_{ijk} = \beta_0 + \beta_1\,\text{Temperature} + \beta_2\,\Delta C/N + \beta_3\,\text{Temperature} * \Delta C/N + a_i + b_{ij} + \epsilon_{ijk}$	39	302	690
51	$\ln(R)_{ijk} = \beta_0 + \beta_1\,\text{Rainfall} + \beta_2\,\Delta C/N + \beta_3\,\text{Rainfall} * \Delta C/N + a_i + b_{ij} + \epsilon_{ijk}$	39	302	690
52	$\ln(R)_{ijk} = \beta_0 + \beta_1\,\text{Temperature} + \beta_2\,\Delta C/P + \beta_3\,\text{Temperature} * \Delta C/P + a_i + b_{ij} + \epsilon_{ijk}$	24	144	387
53	$\ln(R)_{ijk} = \beta_0 + \beta_1\,\text{Rainfall} + \beta_2\,\Delta C/P + \beta_3\,\text{Rainfall} * \Delta C/P + a_i + b_{ij} + \epsilon_{ijk}$	24	144	387
54	$\ln(R)_{ijk} = \beta_0 + \beta_1\,\text{Temperature} + \beta_2\,\Delta N/P + \beta_3\,\text{Temperature} * \Delta N/P + a_i + b_{ij} + \epsilon_{ijk}$	28	170	454

55	$\ln(R)_{ijk} = \beta_0 + \beta_1\ \text{Rainfall} + \beta_2\ \Delta N/P + \beta_3\ \text{Rainfall} * \Delta N/P + a_i + b_{ij} + \epsilon_{ijk}$	28	170	454
56	$\ln(R)_{ijk} = \beta_0 + \beta_1\ \text{Temperature} + \beta_2\ \Delta\text{Lignin}/N + \beta_3\ \text{Temperature} * \Delta\text{Lignin}/N + a_i + b_{ij} + \epsilon_{ijk}$	22	155	364
57	$\ln(R)_{ijk} = \beta_0 + \beta_1\ \text{Rainfall} + \beta_2\ \Delta\text{Lignin}/N + \beta_3\ \text{Rainfall} * \Delta\text{Lignin}/N + a_i + b_{ij} + \epsilon_{ijk}$	22	155	364
58	$\ln(R)_{ijk} = \beta_0 + \beta_1\ \Delta N + \beta_2\ \text{Ecosystem} + \beta_3\ \Delta N * \text{Ecosystem} + a_i + b_{ij} + \epsilon_{ijk}$	63	406	1056
59	$\ln(R)_{ijk} = \beta_0 + \beta_1\ \Delta N + \beta_2\ \text{Soil N} + \beta_3\ \Delta N * \text{Soil N} + a_i + b_{ij} + \epsilon_{ijk}$	25	100	313

Table S4.2 Model results of single variables, two- and three-way interactions on ln(R). The p-values are given for each model (Table S4.1) as well as the R^2 value in case of a significant linear model result for the complete dataset, the dataset that reported standard errors and the dataset that reported significant non-additive mass loss. The number of observations in each model is between brackets. NA = not enough observations for model testing

Variable	Analysis without weights, all data	Analysis with weights, subset for data having SEs	Analysis with weights, for observations with significant non-additive mass loss
ΔC	0.0811 (768)	0.2237 (480)	0.6665 (75)
ΔN	0.4500 (1056)	0.2847 (660)	0.4907 (101)
ΔC:N	0.1113 (779)	**0.0028, R^2=5e^{-4} (384)**	**0.0316, R^2=5e^{-4} (74)**
ΔP	0.1438 (535)	0.9695 (324)	0.5243 (56)
ΔN:P	0.4739 (535)	0.1115 (324)	0.2877 (56)
ΔC:P	**0.0064, R^2=0.03 (437)**	0.0883 (237)	**0.0369, R^2=9e^{-4} (49)**
ΔLignin	0.3900 (432)	0.7070 (220)	0.2846 (50)
ΔLignin:N	**0.0051, R^2=0.04 (416)**	**0.0074, R^2=7e^{-4} (220)**	0.0990 (50)
ΔPhenol	0.4386 (97)	0.7849 (86)	0.7243 (20)
ΔHemicellulose	0.4437 (46)	0.7912 (40)	0.1784 (11)
ΔCellulose	0.1736 (137)	0.7270 (104)	0.5354 (25)
SOM	0.2997 (26)	NA (15)	NA (8)
Soil C	0.2851 (303)	0.7275 (205)	0.7994 (31)
Soil N	0.4475 (341)	**0.0415, R^2=3e^{-4} (235)**	**0.0001, R^2=1e^{-4} (39)**
Soil C:N	0.3250 (161)	**0.0126, R^2=4e^{-4} (89)**	0.2443 (20)
Soil pH	0.5927 (503)	0.8127 (308)	0.0761 (49)
Mesh size	0.9143 (1347)	0.5966 (848)	0.5981 (125)
Burial location	0.9213 (1350)	0.7474 (860)	0.7085 (125)
Time	0.0992 (1359)	0.5519 (860)	0.7874 (125)
Ecosystem	0.7783 (1356)	0.8397 (857)	**0.0001, R^2=4e^{-4} (124)**
Climate	0.9223 (1324)	0.4918 (842)	0.2650 (123)
Rainfall	0.4312 (1121)	**0.0489, R^2=2e^{-4} (676)**	0.0549 (105)
Temperature	0.1562 (1121)	0.4317 (676)	0.3202 (105)
Natural/arable system	0.6473 (1356)	0.1671 (857)	**0.0377, R^2=8e^{-4} (124)**
Litter type	0.4107 (1359)	0.9689 (860)	0.7292 (125)
Woody	0.5531 (1359)	0.4636 (860)	0.9948 (125)
Litter drying	0.5262 (878)	0.0811 (671)	0.2965 (111)
Litter size	0.5757 (182)	0.5713 (162)	NA (16)
ΔC * ΔN	0.8647 (768)	0.9818 (480)	0.9421 (75)
ΔC * ΔP	0.0898 (437)	0.4358 (237)	0.4181 (49)
ΔN * ΔP	0.8580 (535)	0.7966 (324)	0.1520 (56)
ΔN * ΔPhenol	0.2527 (97)	0.5641 (86)	0.8685 (20)
ΔC * ΔN * ΔP	0.1289 (437)	0.4080 (237)	**0.0211, R^2=2e^{-3} (49)**
ΔN * ΔP * ΔLignin	0.8751 (212)	0.3375 (136)	NA (26)
ΔN * ΔLignin	0.5970 (416)	0.5258 (220)	0.9446 (50)

ΔN * ΔLignin * ΔPhenol	0.2960 (89)	0.1645 (86)	0.5867 (20)
ΔN * Time	**0.0256, R^2=0.04 (1056)**	**0.0005, R^2=8e^{-4} (660)**	0.7223 (101)
ΔP * Time	**0.0311, R^2=0.02 (535)**	0.1501 (324)	0.7175 (56)
ΔLignin * Time	0.9911 (432)	0.7083 (220)	0.6174 (50)
ΔN * ΔP * Time	0.9507 (535)	**0.0280, R^2=2e^{-4} (324)**	0.5344 (56)
ΔN * ΔP * ΔLignin * Time	**0.0131, R^2=0.10 (212)**	**0.0167, R^2=4e^{-3} (136)**	NA (26)
Mesh size * Time	0.5734 (1347)	0.6108 (848)	0.9690 (125)
Mesh * ΔN	0.4305 (1044)	0.7558 (648)	0.8148 (101)
ΔN * Ecosystem	0.8721 (1056)	0.2396 (660)	**0.0006, R^2=5e^{-4} (101)**
ΔN * Soil N	0.4864 (313)	0.2677 (223)	0.1232 (37)
ΔC * Rainfall	0.7110 (580)	0.8411 (324)	0.8321 (56)
ΔC * Temperature	0.9909 (580)	0.9165 (324)	0.1972 (56)
ΔN * Rainfall	0.5060 (837)	0.8939 (479)	0.9142 (81)
ΔN * Temperature	0.9255 (837)	0.3000 (479)	0.0633 (81)
ΔP * Rainfall	0.6044 (454)	0.5683 (278)	0.9621 (47)
ΔP * Temperature	0.7115 (454)	0.6203 (278)	**0.0185, R^2= R^2=1e^{-3} (47)**
ΔC/N * Rainfall	0.0516 (690)	**0.0001, R^2=6e^{-4} (323)**	**0.0010, R^2=1e^{-3} (63)**
ΔC/N * Temperature	0.1182 (690)	**0.0001, R^2=8e^{-4} (323)**	**0.0087, R^2=1e^{-3} (63)**
ΔC/P * Rainfall	**0.0041, R^2=0.07 (387)**	**0.0281, R^2=1e^{-3} (216)**	**0.0397, R^2=7e^{-3} (41)**
ΔC/P * Temperature	**0.0137, R^2=0.05 (387)**	**0.0080, R^2=1e^{-3} (216)**	**0.0018, R^2=6e^{-3} (41)**
ΔN/P * Rainfall	0.8665 (454)	0.9192 (278)	0.6460 (47)
ΔN/P * Temperature	0.6811 (454)	0.9607 (278)	0.4298 (47)
ΔLignin/N * Rainfall	0.7651 (364)	0.2129 (205)	0.2860 (44)
ΔLignin/N * Temperature	0.2800 (364)	**0.0304, R^2=8e^{-4} (205)**	NA (44)

Table S4.3 Model results of single variables and their effect on ln(R) for each of the climates provided if enough data was available. The p-values are given for each model (Table S4.1) as well as the R^2 value in case of a significant linear model.

Variable	Tropical	Temperate	Continental
ΔC	0.0537 (66)	0.7787 (477)	__0.0015**, R^2=0.06 (231)__
ΔN	0.0990 (75)	0.7528 (628)	0.0880 (353)
ΔC:N	0.9737 (65)	0.1324 (385)	__0.0285, R^2=0.02 (311)__
ΔP	0.9860 (49)	0.2096 (271)	0.6546 (213)
ΔN:P	0.7109 (49)	0.2574 (271)	0.5848 (213)
ΔC:P	0.0566 (43)	0.1716 (241)	__0.0001***, R^2=0.27 (151)__
ΔLignin	0.1043 (52)	0.2284 (251)	0.7196 (151)
ΔLignin:N	__0.0385*, R^2=0.12 (36)__	0.0575 (235)	__0.0041**, R^2=0.07 (135)__
ΔPhenol	0.4065 (12)	0.4874 (15)	0.6909 (70)
ΔHemicellulose	0.6827 (12)	0.3678 (34)	NA (0)
ΔCellulose	0.7213 (18)	0.1821 (79)	0.5697 (40)
SOM	NA (8)	NA (15)	NA (3)
Soil C	__0.0180*, R^2=0.12 (81)__	0.1233 (163)	0.1307 (95)
Soil N	0.6821 (82)	0.3284 (208)	0.7216 (85)
Soil C:N	NA (12)	0.0966 (73)	0.7524 (70)
Soil pH	0.9478 (92)	0.4375 (279)	0.4777 (174)
Mesh size	0.1369 (97)	0.9571 (736)	0.4051 (510)
Burial location	0.6875 (108)	0.9930 (747)	NA (513)
Time	0.4143 (109)	__0.0058**, R^2=0.01 (748)__	0.8473 (522)
Ecosystem	0.1226 (109)	0.7669 (748)	__0.0067**, R^2=0.08 (522)__
Natural/arable system	0.1219 (109)	0.4788 (748)	0.5335 (522)
Litter type	__0.0246*, R^2=0.07 (109)__	0.2498 (748)	0.1029 (522)
Woody	0.7976 (109)	0.9716 (748)	__0.0286*, R^2=0.04 (522)__
Litter drying	NA (105)	0.5269 (739)	NA (522)
Litter size	NA (35)	0.5404 (164)	NA (41)

Table S4.4 Model results of single variables and their effect on ln(R) for each of the ecosystems provided if enough data was available. The p-values are given for each model (Table S4.1) as well as the R^2 value in case of a significant linear model.

Variable	Arable	Deciduous forest	Coniferous forest	Natural grass	Pasture
ΔC	0.6128 (136)	**0.0045**, R^2=0.03 (406)**	0.3645 (84)	0.2856 (53)	0.5240 (48)
ΔN	0.9669 (160)	0.5101 (539)	0.4811 (94)	0.6706 (104)	0.9784 (66)
ΔC:N	0.1933 (30)	0.9570 (420)	0.5698 (108)	0.3041 (71)	**0.0139*, R^2=0.08 (90)**
ΔP	0.0790 (34)	0.2235 (284)	0.0517 (68)	0.2339 (89)	**0.0274*, R^2=0.42 (19)**
ΔN:P	0.3134 (34)	0.5917 (284)	0.1161 (68)	0.5771 (89)	0.4390 (19)
ΔC:P	NA (10)	**0.0001***, R^2=0.17 (279)**	0.1383 (68)	0.2986 (53)	NA (1)
ΔLignin	NA (10)	0.0549 (264)	0.2981 (36)	0.8490 (45)	0.5943 (6)
ΔLignin:N	NA (10)	**0.0086**, R^2=0.04 (264)**	0.9632 (36)	0.3536 (45)	0.5943 (6)
ΔPhenol	NA (0)	0.5724 (55)	NA (8)	NA (0)	NA (0)
ΔCellulose	NA (0)	0.6811 (55)	0.3009 (18)	0.2693 (30)	0.5943 (6)
Soil C	**0.0419*, R^2=0.36 (16)**	0.6130 (140)	NA (24)	NA (44)	0.0716 (31)
Soil N	0.5912 (40)	0.7188 (151)	0.6402 (28)	0.4306 (59)	NA (12)
Soil C:N	0.5957 (26)	0.1654 (92)	0.1485 (40)	NA (0)	NA (0)
Soil pH	0.9552 (83)	0.5726 (233)	0.1810 (28)	0.3459 (77)	0.5968 (31)
Mesh size	0.9540 (229)	0.9902 (637)	NA (134)	NA (107)	NA (114)
Burial location	0.0818 (229)	NA (637)	NA (134)	NA (99)	0.6441 (113)
Time	**0.0304*, R^2=0.03 (229)**	**0.0312*, R^2=0.007 (637)**	0.7435 (134)	0.7510 (107)	0.1291 (114)
Litter type	0.4759 (229)	0.1797 (637)	**0.0128*, R2=0.11 (134)**	**0.0188*, R^2=0.12 (107)**	**0.0002**, R^2=0.40 (114)**
Woody	0.1120 (229)	0.7357 (637)	0.2676 (134)	0.5887 (107)	**0.0005**, R2=0.41 (114)**
Litter drying	NA (171)	NA (633)	0.1064 (110)	NA (107)	NA (114)
Litter size	NA (120)	NA (0)	NA (0)	0.7151 (18)	NA (13)

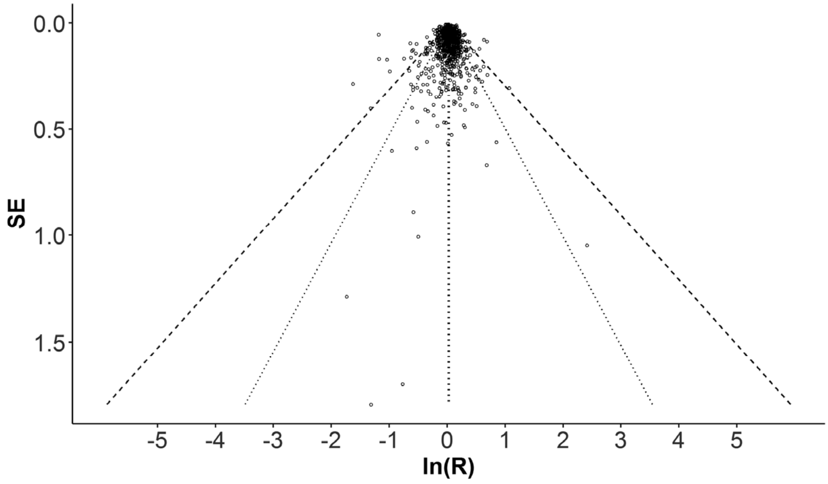

Figure S4.1 Funnel plot of standard error against the log of the response ratio (ln(R)). The vertical line represents the estimated mean of ln(R) via mixed effect model 1: $\ln(R)_{ijk} = \beta_0 + a_i + b_{ij} + \epsilon_{ijk}$. The dotted line indicates the 95% CI and the dashed line the 99% CI.

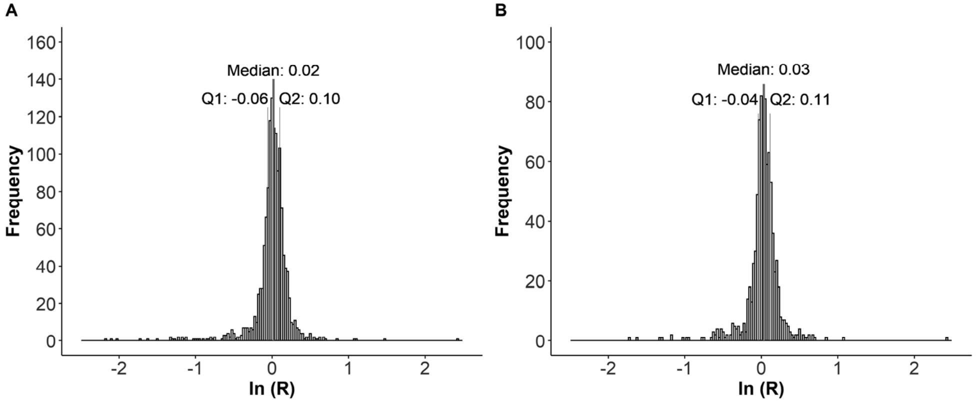

Figure S4.2 A histogram of the natural logarithm of the response rate. Where R is the expected mass loss divided by the observed mass loss. Thus a value of ln(R) of zero indicates additive mass loss, any value above zero indicates positive non-additive mass loss and below zero indicates negative non-additive mass loss. A) the complete data set and B) the subset of data that reported errors and samples sizes. Vertical lines in the panels of the frequency distribution indicate the first quartile (Q1), median and the third (Q2) quartile of ln(R).

Chapter 5

Cover crop quality affects the trade-off between nitrogen supply to the main crop and loss through N_2O emissions

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Abstract

Cover crops can improve soil quality and increase productivity of the following cash crop. Promoting nitrogen (N) mineralisation from incorporated cover crop residues for uptake by the cash crop, while minimising nitrous oxide (N_2O) emissions from decomposing residues remains challenging. Decomposing cover crop mixtures with different C/N ratios could provide N for crop growth while limiting N_2O emissions relative to monocultures. In a greenhouse experiment we incorporated monocultures and mixtures of cover crops of different qualities (*Avena strigosa*, *Raphanus sativus*, *Vicia sativa*) at equal quantity in a sandy loam soil. Soil mineral N and N_2O emissions were measured for three weeks after cover crop incorporation in unplanted pots. In a second set of pots, wheat (*Triticum aestivum*) was planted 7 and 14 days after incorporation of the cover crops, to test if wheat N capture from the decomposing residues depends on planting time. Cover crops with a low C/N ratio (*R. sativus*, *V. sativa*) increased soil mineral N soon after incorporation in the soil. This fast mineralisation concurred with high N_2O emissions for *R. sativus*, whereas N_2O emissions remained significantly lower for *V. sativa*. Low N_2O emissions also occurred for *A. strigosa*, yet soil mineral N was significantly lower compared to *V. sativa*. Wheat planted after *A. strigosa* had poor growth and low N uptake when planted early. Early planting after *R. sativus* increased wheat N uptake compared to later planting and may reduce the high N_2O emissions as observed in unplanted soil. Incorporation of cover crop mixtures did not result in higher wheat biomass or lower N_2O emissions than predicted from the single residue addition. Cover crop quality should be considered when determining the timing of cover crop incorporation and cash crop sowing to maximise cash crop N uptake and reduce N_2O emissions.

5.1 Introduction

In agriculture cover crops are grown between main crops for numerous reasons such as the control of erosion, nutrient leaching and weeds, as well as to increase C storage and nutrient cycling in the soil (Blanco-Canqui et al., 2015; Thorup-Kristensen et al., 2003). A few weeks before sowing the main crop, the cover crops are incorporated into the soil. After incorporation the cover crop residues the soil organisms start to decompose and mineralise nutrients from this fresh organic matter, which provides plant available nutrients such as nitrogen (N) (Dabney et al., 2001). However, as with any fertiliser, there is a trade-off between benefits of additional N input to the soil for the main crop and the risks of N losses such as N-leaching via the soil water (Quemada et al., 2013) and nitrous oxide (N_2O) emissions to the atmosphere (Snyder et al., 2009). Ideally the N supply from cover crops is synchronised to the N demand of the main crop in order to minimize the N losses and stimulate plant growth (Crews and Peoples, 2005).

In the soil, microbial communities decompose cover crop residues, mineralising part of the organic N in the cover crop residues into soil mineral N available for uptake by the main crop (Blanco-Canqui et al., 2015). Ideally, this mineralised N becomes available when the cash crop's demand for N is high. However, it is difficult to predict when the mineral N will be released from the decomposing residues because residue quality, abiotic factors such as temperature and soil moisture, and decomposer organisms all influence N mineralisation rate (Aerts, 1997; Bradford et al., 2016). A study by Nevins et al. (2020) found greatest release of cover crop residue C to the soil in the first 50-68 days after the incorporation of the cover crops in the soil indicating that most of the residues are decomposed early on. Under the same abiotic conditions cover crop residue quality, determined by the nutrient content and carbon (C) compounds (e.g. lignin and phenolics), determine the rate at which soil microbes decompose and mineralise these residues, and thus when nutrients become available for plant uptake (Aerts, 1997; Cornwell et al., 2008). Generally, residues with a higher N content (i.e. residues with low C/N ratio) are expected to decompose and release N faster than residues with a lower N content (Aerts, 1997; Manzoni et al., 2008). Previous studies have shown that incorporating cereal cover crops such as oats, that are generally low in N and have a high C/N ratio, can (temporarily) immobilise N in the soil making mineral N less available as compared to soil without residue addition, thereby hampering the growth of the main crop (Cúpina et al., 2017; Dabney et al., 2001; Gabriel et al., 2016; Quemada et al., 2013). In contrast incorporating legume cover crops such as vetch, typically rich in N and with a low C/N ratio, are expected to release N quickly, thereby promoting initial growth of the main crop (Miguez and Bollero, 2005; Stute and Posner, 1995; Thorup-Kristensen et al., 2003). Even though C/N ratio is a good indicator of residue quality from the perspective of its impact on decomposition and mineralisation rate, it is important to note that high lignin, cellulose, and phenolics in residues can slow the mineralisation of N from residues with high N content and low C/N ratio (Hättenschwiler and Jørgensen, 2010; Hättenschwiler and Vitousek, 2000). Thus, it is still difficult to predict how cover crop residue N mineralisation affects the subsequent crop growth (Abdalla et al., 2019). To avoid losses of N from soil, a close balance between the supply and crop demand of N is needed (Crews and Peoples, 2005).

When decomposing cover crop residues release mineral N when the main crop does not have a large N demand, there is a risk for N losses to the environment. One of the most concerning N loss pathways is via N_2O emissions (Cameron et al., 2013). Nitrous oxide is a potent greenhouse gas 265 times the global warming potential of CO_2 (IPCC, 2014; Stocker et al., 2013) and is produced in soil through microbial processes in the N cycle, e.g. denitrification and nitrification (Butterbach-Bahl et al., 2013). Previous field research has shown that the largest peak of N_2O emissions generally occurs in spring after cover crop incorporation (Li et al., 2015). More N_2O is produced when cover crop residues with a lower C/N ratio are added to the soil (Baggs et al., 2000; Muhammad et al., 2019). The main controls of N_2O emissions are soil moisture, temperature , pH and the amount of C and N in the soil (Butterbach-Bahl et al., 2013; Muhammad et al., 2011). Besides from affecting the amount of N in the soil, decomposing residues also affect the labile C pool, increasing the energy source for heterotrophic microbes that drive the pathways which produce N_2O (Basche et al., 2014). Therefore, the C/N ratio of the cover crop residue is relevant in predicting N losses via N_2O emissions (Huang et al., 2004).

Growing cover crop mixtures (cover crops composed of multiple plant species) is also an easy way to increase plant biodiversity in agriculture, and are now promoted in legislation, for example in the Netherlands (RVO, 2015). Cover crop mixtures produce biomass of plant species with different quality as compared to the individual monocultures. If cover crop biomass (residues) of different qualities vary in their decomposition rate, adding residues of multiple cover crop species (and qualities) to the soil could provide a more constant supply of nutrients over a longer period of time than the addition of a single-species cover crop. This could benefit the growth of the main crop by having a steady supply of N in the soil and thereby limiting the potential N losses such as via N_2O emissions. Synergistic or antagonistic interactions between residues of different quality could also occur, leading to faster or slower decomposition and N mineralization than expected, i.e. litter mixing effects, although additive effects may be the rule and interactive effects occurring under special conditions (Gartner and Cardon, 2004; Porre et al., 2020). It is thus still unclear if a mixture of cover crop residues will provide N at a time that the main crop needs it, or if interactions between species occur upon decomposition.

Timing cover crop N release to the soil, in synchrony with the main crop demand is essential to optimise the N uptake and minimise N losses to the environment. In this greenhouse study we determine how incorporation of cover crop residues with different qualities in monoculture and mixtures affects the soil mineral N content and N_2O emissions over time, as well as subsequent main crop growth. We hypothesise that: (1) the C/N ratio of the residues incorporated into the soil will be negatively related with soil mineral N levels and N_2O emissions in the following weeks; (2) cover crop residues with a high C/N suppress plant growth when plants are planted soon after residue incorporation, but this effect fades when planted later; (3) mixtures of cover crop residues of different qualities will simultaneously provide N for plant growth and will limit N_2O emissions from unplanted soil, relative to soil with residues of the individual species.

5.2 Materials and methods

5.2.1 Cover crop residue and soil collection

Cover crop residue and soil were collected from an existing 2-year cover crop field experiment. The field experiment was located at the Wageningen University & Research experimental farm in Wageningen, The Netherlands (51°59'44.9"N 5°39'37.8"E). The field experiment consisted of eight treatments: three single cover crop species (commonly grown in the Netherlands): *Raphanus sativus* (R), *Vicia sativa* (V) and *Avena strigosa* (O), all possible 2- and 3- species mixtures of these single cover crops and a fallow (no cover crop planted). The soil used for the greenhouse experiment (see below) was collected from the fallow treatments to standardise the soil conditions. The treatments were replicated five times and were sown on the 28th of August 2017 as a complete randomised block design in plots of 10 x 6 m. The seeding rates for the singles species R, V, and O were 30, 110 and 70 kg/ha respectively, according to farmer practice. Species were mixed according to a replacement design, using, for each of the species, 50% (2-species mixture) or 33% (3-species mixture) of the seed rate in pure stand. After sowing, the cover crops were fertilised with calcium ammonium nitrate (CAN) at a rate of 30 kg N/ha on the 20th September 2017 in order to ensure establishment.

Aboveground cover crop residues were harvested from the field, by cutting just above the soil surface, on the 5th of March 2018, just before they would be incorporated into the soil in the field. At the time of collection, the cover crops residues were partly senesced. The residues where washed with water to remove soil particles, centrifuged to remove excess water and weighed. Residues from the five replicate field plots of R, V, and O cover crop monocultures were pooled by species and stored fresh in sealed plastic bags in the fridge at 4°C until the start of the greenhouse experiment on 20th March 2018. Before storage, a subsample was dried at 70°C for 48 h to determine the moisture content of the residues. Ratios of each single species in the four mixtures was determined based on dry weights (Table 5.1), yet were applied in the form of fresh residues similarly as would happen in the field.

Table 5.1 Cover crop ratios measured in the field and used in the greenhouse study. Cover crop ratios, as percentage of total biomass, measured in the field (March 2018) as a result of sowing according to a replacement design, using, for each of the species, 50% or 33% of the seed rate in pure stand, and in line with this the ratios of species, as percentage dry weight, created for the greenhouse experiment.

Code	Cover crop species	Ratios of single species In the field			In the greenhouse		
		R	V	O	R	V	O
R	R. sativus	100%	-	-	100%	-	-
V	V. sativa	-	100%	-	-	100%	-
O	A. strigosa	-	-	100%	-	-	100%
RV	R. sativus + V. sativa	92±2%	8±2%	-	90%	10%	-
RO	R. sativus + A. strigosa	67±8%	-	33±8%	67%	-	33%
VO	V. sativa + A. strigosa	-	5±1%	95±1%	-	10%	90%
RVO	R. sativus + V. sativa + A. strigosa	61±5%	7±1%	32±5%	60%	10%	30%
Fal	Fallow	-	-	-	-	-	-

Soil was collected on the 15th of March from the same field experiment (from plots with no history of cover crops). The soil was air dried overnight and sieved to 2 mm to remove rocks and larger pieces of organic material, stored at 4°C until the start of the experiment. The soil was a loamy sand, characterised by 2% clay, 12% silt, 83% sand; pH 5.3, 3.1% organic matter, 4.47 ± 0.15 mg mineral N/kg soil; 4.63 ± 0.17 mg organic N/kg soil.

5.2.2 Experimental design

The aim of this experiment was to examine how cover crop residues of different species and their mixtures (with different residue qualities: C/N ratio) decompose (using CO_2 as a proxy) and release mineral N over time, and how this affects N_2O emissions and subsequent cash crop growth. We designed the experiment in such a way that we could disentangle the residue quality effect from quantity. Therefore we standardised the quantities used in the treatments by adding the same dry weight equivalent of cover crop residues in each pot, added in the form of fresh residues. Here we only added aboveground biomass because aboveground and belowground residues have different qualities and roots have the extra complication that it is very hard to get them totally free of (particulate) soil organic matter. For the same reasoning of standardizing the litter qualities of the different treatments per species we used the single species to create the mixtures present in this experiment. In order to relate the greenhouse study to the field conditions we used the same ratios of the different species in the mixtures as they established in the field (Table 5.1). Mixtures of cover crop species had very uneven ratios in the field because *Vicia sativa* is a poor competitor during autumn growth (Elhakeem et al., 2019). The standardised total amount of cover crop biomass added per pot was estimated from the field experiment. Wheat seedlings (age: 6 days) were planted at two different dates: 7 days and 14 days after cover crop incorporation. We determined the two planting times according to the accumulated growth degree days in the field (one week in the greenhouse represented 2 weeks in the field). These planting dates (respectively 14 days and 28 days in the field) represent a time at which the bulk of the cover crop residues decompose (Nevins et al., 2020).

We established 8 cover crop treatments: single species R, V and O, 2- species mixtures R+V, R+O, and V+O, a 3-species mixture R+V+O and a fallow (no cover crop added). For each experimental unit 1 kg air dried soil, with 4.7% moisture content, thus 952 g dry weight was brought up to 60% water holding capacity (WHC). Cover crop residues were added as fresh material, cut to two cm and fully mixed through the soil before adding to the pots (volume of 1 L, 12 cm Ø, 11 cm depth, black plastic). The amount of fresh residue added was standardized by the amount of dry matter added to each pot (3.24 g), which is equivalent to adding 2868 kg/ha in the field. The soil moisture content in each pot was gravimetrically adjusted every two days throughout the experimental period.

Summer wheat seeds (*Triticum eastivum* variety Minarat) were germinated in sterilised soil six days before one seedling was transplanted into each pot. We had 8 cover crop residues x 2 wheat planting dates x 6 replicates = 96 pots, plus an additional 8 cover crop residues x 6 replicates = 48 pots that allowed destructive sampling of the soil (Fig 5.1).

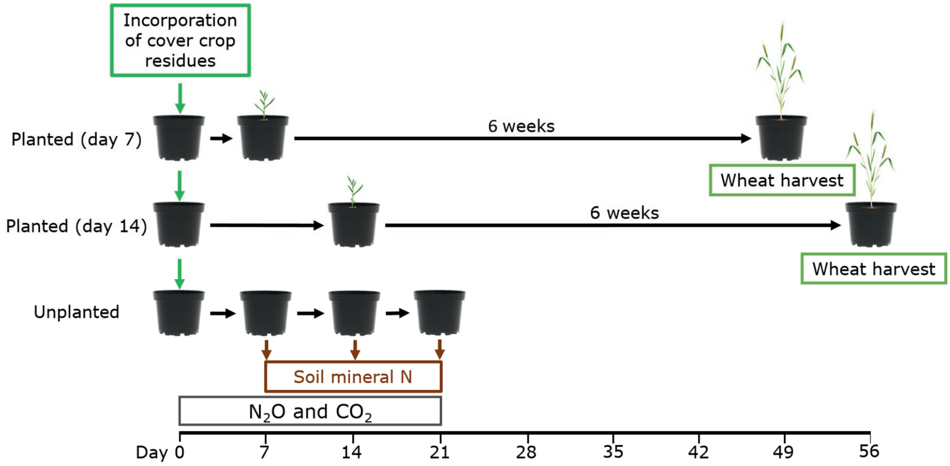

Figure 5.1 Experimental design. Seven cover crop residues were added at day 0 to all pots. Wheat was planted seven or fourteen days after cover crop residue incorporation. Wheat above- and belowground biomass was measured six weeks after planting to measure the early growth. An additional set of unplanted pots (eight cover crop residues x three replicates) were used for soil mineral N sampling on day 7, 14 and 21. Carbon dioxide and N_2O were measured on these unplanted pots.

5.2.3 Plant and soil analysis

The initial cover crop C/N ratios of the different cover crop species were determined by quantifying the C and N concentrations (mg C/g residue and mg N/g residue) on dried (70°C) and ball-milled samples using a CN elemental analyser (LECO, Germany).

To quantify the soil parameters three replicate soil samples were taken before the start of the experiment from the bulk soil. Subsequent soil samples were taken with a soil corer (diameter 1 cm and depth 20 cm, total soil profile) on unplanted pots, one, two, and three weeks after cover crop incorporation. The soil mineral N was determined by extracting dry soil in a 1:10 (w/v) suspension with 0.01 M $CaCl_2$ (Houba et al., 2000b). Soil and $CaCl_2$ were shaken for 2 hours, the suspension was filtered to 45 µm, and the extracts were analysed for total dissolved N (N–Nts), nitrate–N (N–NO_3^- + NO_2^-) and ammonium–N (N–NH_4^+) with segmented flow analysis (SFA; Skalar 6 channel SFA analyser, Skalar, Netherlands, 2003).

Wheat was harvested six weeks after seedling transplantation. Aboveground biomass was cut at soil level, dried at 70°C for 48 hours, and then weighed. Root biomass was washed over a 0.5 mm sieve to remove soil and dried at 70°C for 48 hours before weighing. Aboveground biomass was ground to 1 mm and analysed for C and N content (mg C or N / g dry biomass) using a CN elemental analyser (LECO, Germany).

5.2.4 Greenhouse gas measurements

Carbon dioxide and N_2O fluxes were measured for the first three weeks after cover crop incorporation on unplanted pots. Fluxes were measured 11 times over 21 days: two days in a row after the start of the experiment (cover crop incorporation), then every day for five days the week after, and then two times per week for the rest of the experiment (Fig 5.4 and 5.5A). During the initial two weeks, fluxes were measured on a subset of 96 pots (n = 12, 8 treatments * 12 replicates) and during the third week on a subset of 48 pots (n = 6, 8 * 6 replicates) because the other pots were planted with wheat at this time. Fluxes were measured with a static closed chamber technique (details of the setup can be seen in the appendix, Fig S5.1) (Lubbers et al., 2011). Flux chambers (PFTE) were closed for 30 minutes with an Innova 1312 photo-acoustic infrared gas analyser (LumaSense Technologies A/S, Ballerup, Denmark) fitted with Teflon tubes connected to the flux chambers according to Velthof et al. (2002). A soda-lime filter was attached to the input valve of the gas analyser for N_2O measurements (only) to reduce interference between CO_2 and N_2O. As T0 we measured background levels of N_2O, between each 5 measurement. Values were corrected for background N_2O concentration and for mixing of the gas sample with the previous measurement in the internal volume of the gas analyser (Lubbers et al., 2011). Cumulative emissions were calculated assuming linear changes in gas concentration between measurements (Abalos et al., 2014).

5.2.5 Data analysis

We tested the effect of cover crop residue (treatment factor, 8 levels) and planting date (treatment factor, 2 levels) and block as random factor on soil mineral N, wheat biomass, cumulative CO_2 and N_2O emissions using linear mixed effects models with the function lme from the package nlme (Pinheiro et al., 2019). Significance was determined with analysis of variance (ANOVA) using a type III sum of squares ANOVA, anova (base) (R Core Team, 2018). Normality and homogeneity of model residual variances were assessed by plotting model residuals. Heterogeneous residual variances were addressed using a variance structure in the model with the function varIdent (nlme) (Pinheiro et al., 2019). The model with the variance structure was retained when it was a significantly better fit than the model without, determined by comparing the model's Akaike information criterion (AIC) using the function anova (R Core Team, 2018). To determine significant differences between levels of a factor (i.e. between cover crop residues), a Tukey's honestly significant difference posthoc test (HSD) was used, with the function emmeans (Lenth et al., 2019) and CLD from package multcomp (Hothorn et al., 2008). Correlations between continuous variables (i.e. soil mineral N and time, soil mineral N and cumulative N_2O emissions), linear mixed effects models (lme) and ANOVA (anova) were used, as above. Marginal R^2 was determined with r.squaredGLMM from the package MuMIn (Barton, 2019). To be able to compare the effect of mixed residues on wheat biomass, cumulative CO_2 and N_2O emissions, and soil mineral N with the effect of single residues, we calculated expected values. Expected values of mixtures were calculated by the weighted average of the two respective single species in the mixture:

$$V_{Exp} = \frac{\sum_i f_i V_i}{\sum_i f_i}$$

Where V_i is the value (soil mineral N, wheat biomass, CO_2 and N_2O) after single species incorporation, and f is the fraction of each cover crop species in the mixture. We tested if litter-mixing effects were present, using students t-tests. All statistics were carried out in R version 3.5.1 (R Core Team, 2018). Figures were made using the packages ggplot2 (Wickham, 2016) and cowplot (Wilke, 2019).

5.3 Results

5.3.1 Cover crop quality

Cover crop residue C/N ratio significantly differed among species at the moment of cover crop incorporation ($F_{2,8} = 19.18$, $P < 0.001$, Table 5.2). Vetch had the lowest C/N ratio (10.1), oat had the highest (16.2). This resulted in different total N addition per pot (Table 5.2), with oat residue resulting in the lowest N addition (86.6 mg N/kg soil) and vetch residue resulting in the highest (141.5 mg N/kg soil).

Table 5.2 The C/N ratio of the cover crop residues and the total amount of N added in each cover crop treatment. Mixtures were created from the single species cover crops residues thus C/N ratios of mixtures are not shown. Mixtures were made according to the ratios shown in Table 5.1, thus N additions are calculated from single species C/N ratios. Different letters indicate significant differences between the C/N ratio of the three cover crop species. Means ± SE; n=5.

Code	Cover crop species	C/N ratio	N addition (mg N/kg Soil)
R	R. sativus	12.87 ± 1.05 a	105.1 ± 11.1
V	V. sativa	10.12 ± 0.19 ab	141.5 ± 1.6
O	A. strigosa	16.17 ± 0.57 b	86.6 ± 2.8
RV	R. sativus + V. sativa	-	108.7 ± 10.2
RO	R. sativus + A. strigosa	-	98.9 ± 8.3
VO	V. sativa + A. strigosa	-	92.1 ± 2.7
RVO	R. sativus + V. sativa + A. strigosa	-	103.2 ± 7.7
Fal	Fallow	-	0.0

5.3.2 Soil mineral N

Soil mineral N differed significantly among the cover crop residue treatments, and depended on the sampling time (cover crop: sample time interaction, $F_{14,79} = 5.48$, $P < 0.001$; Table 5.3). With the exception of the fallow treatment, soil mineral N was lowest (at all sample dates) when oat residue was incorporated. Soil mineral N after vetch was significantly higher than after oat residue incorporation. Soil mineral N after radish residue incorporation did not differ from the soil mineral N of either vetch or oat at all sampling dates. The soil mineral N increased over time during the 3 weeks after cover crop incorporation (Table 5.3).

Table 5.3 Soil mineral N (NO_3, NO_2 and NH_4) measured in unplanted pots 7, 14 and 21 days after cover crop incorporation. Different lower case letters indicate significant differences between cover crop treatments for the same sampling date (comparison within columns), different roman numerals indicate significant differences between sampling dates (comparison within rows for the respective N-pool) for each cover crop ($P < 0.05$). Radish (R), vetch (V), oat (O), their 2- and 3-species mixtures (ratio according to Table 5.1) and a fallow (Fal); DAI= days after incorporation of the cover crop residues in the soil.

	$NO_3 + NO_2$ (mg $N\text{-}NO_3 + N\text{-}NO_2$ kg soil^{-1})			NH_4 (mg $N\text{-}NH_4$ kg soil^{-1})			Total mineral N (mg N kg soil^{-1})		
DAI	7	14	21	7	14	21	7	14	21
R	21.5 ab (I)	56.0 bcd (I-II)	67.5 cd (II)	24.0 d (II)	6.17 (I)	5.15 (I)	45.5 abc	62.2 bcd	72.7 cd
V	29.0 b (I)	61.5 cd (II)	71.6 d (II)	16.6 d (II)	5.17 (I)	5.85 (I)	45.6 c (I)	66.6 cd (II)	77.5 d (II)
O	14.0 ab (I)	26.5 b (II)	24.7 b (II)	5.12 b	4.12	4.87	19.1 ab (I)	30.7 b (II)	29.6 b (II)
RV	21.0 ab (I)	59.5 cd (II)	66.2 d (II)	16.6 d (II)	5.25 (I)	4.62 (I)	37.6 bc (I)	64.8 cd (II)	70.8 d (II)
RO	17.9 ab (I)	69.6 d (II)	56.4 cd (II)	17.2 cd (II)	5.25 (I)	4.47 (I)	35.1 abc (I)	74.9 cd (I-II)	60.9 cd (II)
VO	16.7 ab (I)	42.1 c (II)	33.2 bc (II)	8.25 bc	4.70	4.63	25.0 abc (I)	46.8 c (II)	37.9 bc (I-II)
RVO	17.1 ab (I)	66.2 d (II)	57.7 d (II)	10.9 c (II)	4.95 (I)	4.82 (I)	28.0 bc (I)	71.2 d (II)	62.5 d (II)
Fal	9.8 a (I)	13.6 a (II)	10.7 a (I)	1.87 a (I)	3.05 (II)	3.78 (III)	11.7 a (I)	16.7 a (III)	14.5 a (II)
	Cover crop **			Cover crop ***			Cover crop ***		
	Sample date *			Sample date **			Sample date ns		
	Cover crop : Planting date ***			Cover crop : Planting date ***			Cover crop : Planting date ***		

5.3.3 Wheat biomass

Wheat aboveground biomass after 6 weeks of growth depended on the cover crop residue incorporated into the soil (cover crop: $F_{7,30} = 145.77$, $P < 0.001$, Fig 5.2), and the effect of the cover crop treatments differed per planting date (cover crop: planting date, $F_{7,30} = 28.40$, $P < 0.001$). Wheat aboveground biomass was significantly lower when grown on soil with oat residue as compared to the other residue treatments when planted 7 days after cover crop residue incorporation (Fig 5.2). However, this difference was not significant in wheat planted 14 days after cover crop residue incorporation. All cover crop residues promoted plant growth compared to fallow soil and the differences in wheat aboveground biomass response to the different incorporated cover crop residues were larger when wheat was planted 7 days after residue incorporation, compared to 14 days after incorporation. Wheat belowground biomass depended on the incorporated cover crop residue (cover crop: $F_{7,30} = 2.65$, $P < 0.05$, Fig 5.2), and was higher when wheat was planted 7 days after residue incorporation than when planted 14 days after incorporation (planting date: $F_{1,30} = 42.48$, $P < 0.001$).

5.3.4 Wheat aboveground N concentration

The aboveground biomass of wheat varied in total N (cover crop: $F_{7,30} = 36.74$, $P < 0.001$, Fig 5.3A) and C/N ratio (cover crop: $F_{7,30} = 12.37$, $P < 0.001$, Fig 5.3B) depending on the cover crop residue incorporated into the soil. Total N in aboveground wheat biomass was higher when wheat was grown after radish compared to when it was grown after oat. There was no significant effect of planting date on the total N or C/N ratio in the wheat after six weeks of growth. The response of wheat total aboveground N and its C/N ratio to the cover crop residues differed per planting date (cover crop: planting date; $F_{7,30} = 3.05$, $P< 0.05$; $F_{7,30} = 2.43$, $P < 0.05$; and $F_{7,30} = 5.67$, $P < 0.001$ respectively). Particularly wheat grown after a mixture of radish and vetch had a much higher (1.6 x) total N content and lower C/N ratio when it was planted a week later.

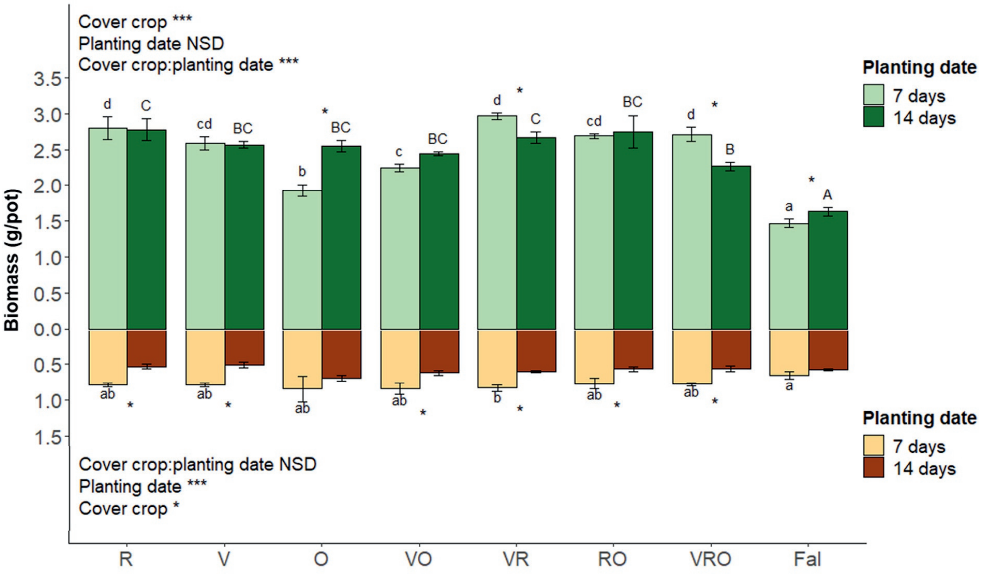

Figure 5.2 Aboveground (above 0 on y-axis) and belowground (below 0 on y-axis) biomass of wheat plants grown for 6 weeks on soil with cover crop residues incorporated on time 0 and wheat plants planted 7 (light color) or 14 (darker color) days later. The different cover crop residue treatments are: R (radish), V (vetch), O (oat) and their combinations, Fal (fallow, i.e. no residue addition). Bars indicate mean ± standard error (n=3), different lower and upper case letters indicate significant differences between above- or belowground biomass when planted 7 days and 14 days (respectively) after cover crop incorporation. Asterisks indicate a significant difference of planting time since cover crop incorporation on above- or belowground biomass.

5.3.5 CO_2 and N_2O emissions

In unplanted soil the cumulative CO_2 differed between cover crop treatment (cover crop: $F_{7,38} = 206.15$, $P < 0.001$, Fig 5.4B). Vetch residue incorporation resulted in the lowest cumulative CO_2 emissions (after the fallow) and radish residue incorporation led to the highest cumulative CO_2 emissions. Similarly cumulative N_2O emissions depended on the cover crop residue incorporated (cover crop: $F_{7,38} = 34.29$, $P < 0.001$, Fig 5.5B). The lowest N_2O emissions (after the fallow) were measured from soil with vetch residue incorporated, while radish residue incorporation resulted in the highest N_2O emissions over the 3 week period. Cover crop residue containing radish caused significantly higher N_2O emissions than cover crop residues without radish (Fig 5.5B).

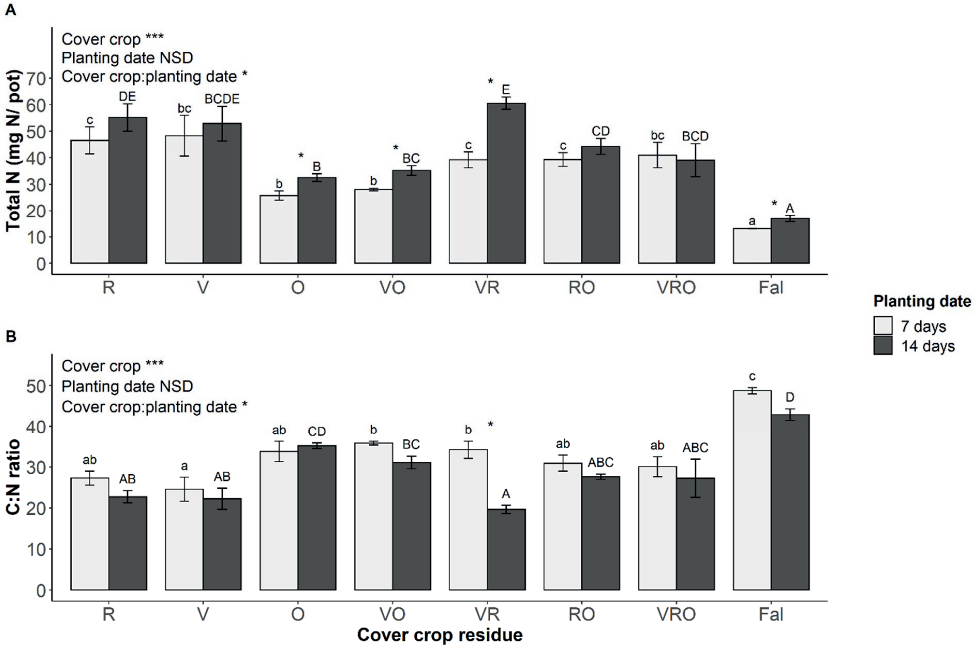

Figure 5.3 Aboveground wheat total N concentration (mg N/pot) (**A**) and C/N ratio (**B**). Wheat was grown on soil with cover crop residue incorporated: R (radish), V (vetch), O (oat) and their combinations or without residue Fal (fallow). Wheat was planted 7 days (light) or 14 days (dark) after cover crop incorporation. Bars indicate mean ± standard error (n=3), different lower case letters indicate significant difference between total N or the C/N ratio of wheat when planted 7 days after cover crop incorporation; different capital letters indicate significant difference between total N or the C/N ratio of wheat when planted 14 days after cover crop incorporation. Stars indicate a significant difference of planting date on total N or C/N ratio.

5.3.6 Mixture effects

There were no significant differences between observed response of mixtures and their respective expected response based on the responses of the respective single species residues, for any of the measured response variables (soil mineral N, wheat above and belowground biomass, wheat N, N_2O and CO_2 emissions; Fig S5.2). This indicates that mixtures of cover crop residues did not behave differently than expected based on the behaviour of the single-species cover crop residue (i.e. there was no synergistic or antagonistic response of combining multiple cover crop residues).

5.3.7 Relationship between soil mineral N , residue C/N and N_2O emissions

In unplanted soil N_2O emissions were positively correlated with soil mineral N overall ($R^2=0.19$, $F_{1,22} = 223.53$, $P < 0.001$, Fig 5.6), and this relationship differed depending on the cover crop residue that was incorporated (Nmin: cover crop; $F_{7,22} = 7.78$, $P < 0.001$). However, this was a weak correlation; soil mineral N only explained 19% of the variation in N_2O emissions. Vetch residue incorporation caused an increase in soil mineral N between 35 - 100 mg N/kg soil, yet this did not result in an increase in N_2O emissions (Fig 5.6). Cover crop C/N ratio was not significantly related to N_2O emissions (Fig S5.3).

5.4 Discussion

In line with our first hypothesis, we found that incorporation of cover crop residues with a low C/N ratio (i.e. vetch and radish) resulted in the highest soil mineral N content, while incorporation of residue with a high C/N ratio (oat) resulted in the lowest soil mineral N. Incorporation of any of the 7 cover crop residues resulted in significantly higher wheat aboveground biomass, compared to the fallow treatment (no cover crop residue incorporated). This is not surprising since the amount of N added in cover crop residues is an overestimation of what is normally added in the field. However, in cover crops the N is applied in organic form and only becomes available for plant uptake upon mineralisation in the soil by the activity of the soil biota and this mineralisation occurs at different rates depending on the cover crop. When standardising for the quantity of cover crop returned to the soil, cover crop quality exerts a considerable impact on the amount of N in the next crop as well as on N_2O emissions when soils are left unplanted.

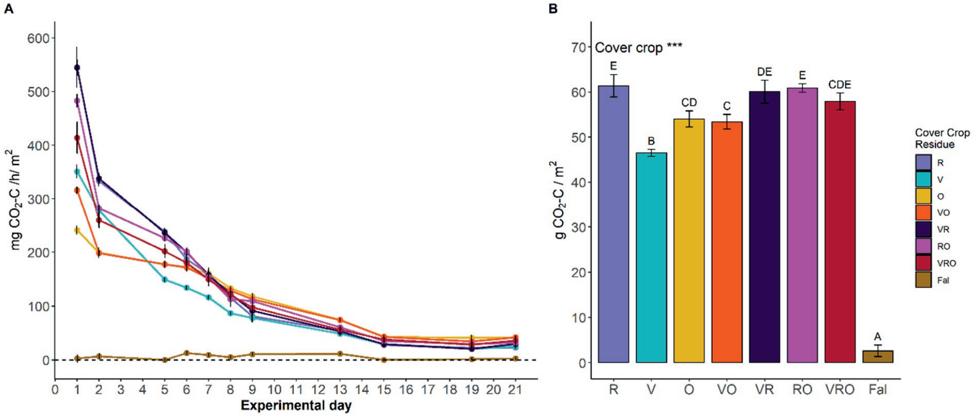

Figure 5.4 Daily CO_2 emissions from unplanted pots in response to the addition of different cover crop residues (**A**) and cumulative emissions of the first 21 days after cover crop incorporation (**B**). Cover crops incorporated in the soil: R (radish), V (vetch), O (oat), and their 2- and 3- species combinations and Fal (fallow). Bars indicate mean ± standard error, different letters indicate significant difference between treatments.

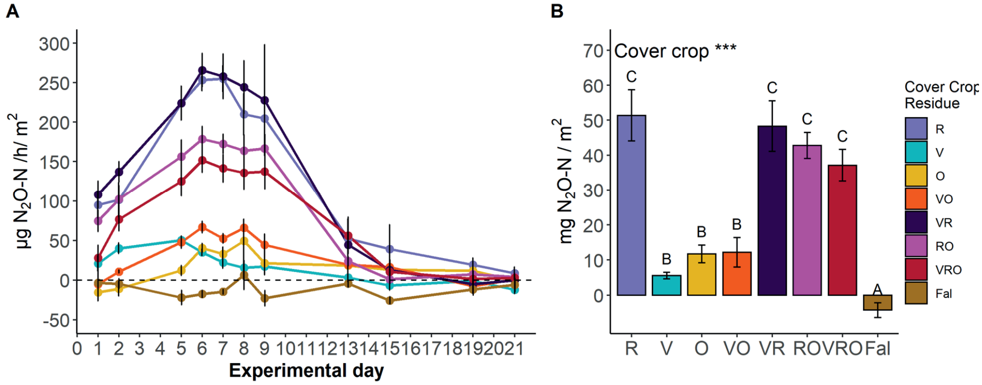

Figure 5.5 Daily N_2O emissions from unplanted pots over time (**A**) and cumulative emissions of the first 21 days after cover crop incorporation (**B**). R (radish), V (vetch), O (oat), and their 2- and 3- species mixtures and Fal (fallow). Bars indicate mean ± standard error, different letters indicate significant difference between treatments.

5.4.1 Timing of N release

Partially in line with our first hypothesis, incorporation of relatively N-rich residue (radish and vetch) resulted in significantly higher wheat aboveground biomass compared to the relatively N-poor oat residue when wheat was planted 1 week after cover crop incorporation. However, this effect was not apparent when wheat was planted 2 weeks after cover crop incorporation. In agreement with our second hypothesis, incorporation of high C/N ratio residue (oat) led to significantly larger wheat aboveground biomass when planted 14, rather than 7 days after incorporation. High C/N residues generally decompose slower (Jahanzad et al., 2016), which means that wheat planted 14 days after incorporation had access to more available N than wheat planted 7 days after incorporation (Table 5.3). Thus, cover crop choice influences N availability over time, and to maximize early main crop performance, cover crop incorporation dates should be adjusted in order to have mineral N available in the soil when the main crop needs it. Especially considering the warmer temperatures in the greenhouse this would translate to approximately 30 days between oat residue incorporation and main crop sowing in the field. Indeed, in a field experiment by Gabriel et al. (2016) where maize was sown directly after cereal cover crop incorporation, maize N uptake was even lower than after a fallow soil.

5.4.2 N_2O and CO_2 emissions

There was only a weak positive relationship between soil mineral N content and N_2O emissions (Fig 5.6). The very low R^2 value (0.19) of this relation signals that factors other than soil mineral N are likely more important in explaining cumulative N_2O emissions in our experiment. Further, N_2O emissions after vetch residue incorporation were significantly lower compared to radish, even though vetch had a lower C/N ratio and resulted in higher soil mineral N than radish. Radish residue incorporation, as a sole crop or in mixtures, increased N_2O emissions proportionally to the amount of radish in the mixture whereas vetch did not. Thus, the cover crop residue effect on soil mineral N availability alone was not the most important factor influencing N_2O emissions in our experiment. This result is surprising since most field studies show higher N_2O emissions after incorporation of residues with a low C/N ratio, and in particular of legumes (Baggs et al., 2000; Basche et al., 2014; Duan et al., 2018; Gomes et al., 2009; Huang et al., 2004). In contrast with field studies, our soils did not have the legacy effect of the cover crops on the soil, because we used the same fallow soil for all our treatments. Field soils with a legacy of legumes generally have a larger N pool than soils after non-leguminous species since legumes fix atmospheric N and generally do not take up as much N from the soil (Basche et al., 2014; Kaspar and Singer, 2011). This could have explained the contrasting results of our greenhouse study with previous field studies.

Nitrous oxide production through denitrification is not only controlled by the soil mineral N content, but by other important factors including the amount of easily decomposable C, oxygen availability, soil pH, and the microbial community structure (Hansen et al., 2019). When N is not limited, the available C in the soil can be a limiting factor in denitrification (Abalos et al., 2019; Liang et al., 2015). In our study, incorporation of vetch residue resulted in the lowest cumulative N_2O emissions (Fig 5.5B), despite having relatively high soil mineral N at all three timepoints (Table 5.3). We found that specifically for vetch residue, soil mineral N was not related with cumulative N_2O emissions (Fig 5.6). Therefore, it could be that because of vetch's low C/N ratio, the N mineralised resulted in less dissolved organic matter compared to the other cover crop residues especially compared to radish which contains relatively high levels of N and C. The C in radish residue is also expected to be easier to decompose by soil microorganisms compared to that of grasses and legumes, as indicated by several decomposition experiments with these species (Barel et al., 2018; Barel et al., 2019). This difference in C-related properties could explain a lack of labile C for the bacterial denitrifiër community with vetch residue and could thus have limited N_2O emissions as compared to radish residue (Pimentel et al., 2015). Easily degradable C is a substrate for microbes, with more microbial activity respiration is increased which results in more CO_2 emissions, which we indeed observed in treatments with radish and much less so with vetch. Moreover, higher CO_2 levels in the soil reduce the soil oxygen content which can create the conditions for net N_2O production and emission (Morley and Baggs, 2010; Weiler et al., 2018).

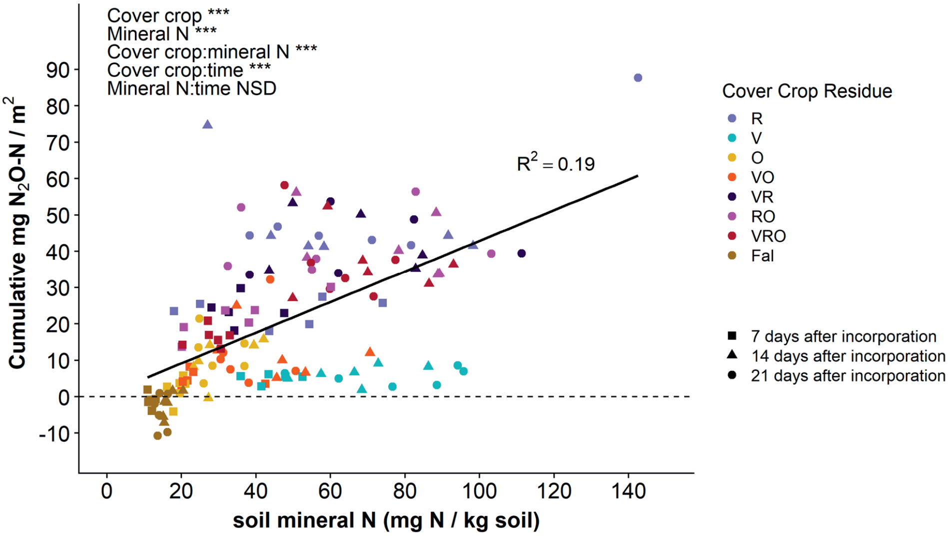

Figure 5.6 In unplanted soil, cumulative N_2O emissions measured on day 7, 15, and 21 were significantly positively related to soil mineral nitrogen (N) measured on day 7, 14, 21. Shapes indicate the time after cover crop incorporation at which the soil mineral N levels were measured. Colours indicate which cover crop residue was incorporated into the soil: R (radish), V (vetch), O (oat), their mixtures and no residue (Fal). The regression line indicates the significant relationship between soil mineral N and cumulative N_2O emissions overall.

5.4.3 Early planting to prevent high N_2O emissions

Our results show that early planting only significantly reduces biomass production and the C/N ratio of wheat when planted in soil with cover crop residue of high C/N ratio. When planted in soil with residue of low C/N ratio early planting can stimulate plant growth and N uptake as good as later planting, however while the soil is unplanted there is a risk of N_2O emissions when radish is incorporated. Yet when wheat is planted earlier after cover crop incorporation, the N uptake from the soil could reduce the total amount of N_2O emitted, in particular to reduce emissions after incorporation of radish. This aspect requires further testing, ideally under field conditions. Overall, there is a potential trade-off between fast release N which gives a large risk of N loss via N_2O emissions in absence of main crop growth, and slow release of N limiting the growth of the main crop. Thus by leaving less time between cover crop incorporation and main crop planting date, high N_2O emissions after incorporation of residue with low C/N and easy decomposable C as in radish can be prevented. Field studies have also shown that peak emissions after cover crop incorporation usually last only up to 40 days, with the highest emissions after 20 days (Baggs et al., 2000; Gomes et al., 2009; Weiler et al., 2018). After this initial flux, soil mineral N levels and N_2O emissions then return to background values because of crop N uptake. These timings are similar to those we found in the greenhouse where we had peak emissions between day 5 and 9 (Fig 5.5A) which represents between day 10 and 18 in the field in terms of degree-days.

5.4.4 Mixture effects

Contrary to what we hypothesised, the responses to cover crop mixtures (soil mineral N, wheat above- and belowground biomass and N_2O and CO_2 emissions) did not differ from the expected responses based on their respective monocultures, i.e. there were no ‘mixture effects’. We had expected that incorporation of cover crop mixtures would provide a more consistent supply of N over time. Mixtures containing oat seemed to mineralise less in the third week compared to the mixture without oat. With more data points through time, the pattern of soil mineral N over time from these mixtures could be better estimated. It is likely that mineralization was fast until day 14 when N was mineralised from most of the easily decomposable material (from the vetch and/or radish). By day 21, only the more difficult to decompose material remained, resulting in lower N mineralization. The lack of mixture effects in our experiment may have been due to the ratios of species in the cover crop mixtures. To mimic the field situation, we created the cover crop mixtures using the ratios of species found in the field, and therefore, the ratio between vetch and the other species was uneven (i.e. mixtures contained 10% vetch residue, Table 5.1). It is important to mimic the field situation in this sense since vetch is a poor competitor against the more robust species like oat and radish (Elhakeem et al., 2019; Wendling et al., 2017). Thus, the cover crop residue being incorporated into the field in reality likely contains a low proportion of vetch. Although this could be different if the cover crop seeding density would be altered to aim at a more even mixture of 50%-50% biomass. Even then, mixture effects reported in the literature are inconsistent, with studies reporting synergistic effects (Anderson and Hetherington, 1999; Schuster et al., 2017) as well as antagonistic effects (Li et al., 2013). Further it is important to note that field studies will always contain a mixture of above- and belowground cover crop residues as well as differences in biomass among the cover crops. Our study solely focussed on the cover crop quality, thus field studies may find contradicting results when there is for example a large biomass difference between the vetch and radish grown in the field, and the below ground influences are taken into account.

5.5 Conclusion

From this greenhouse study we conclude that when cover crops with a standardised quantity and different quality are incorporated into the same soil, low C/N ratio crops provide the plants with higher amounts of mineral N and result in higher wheat biomass as compared to a high C/N crop. We also show that vetch, with a low C/N ratio, produces low cumulative N_2O emissions compared to radish. When considering quality only, vetch gives a similarly high soil N and wheat biomass production compared to radish thus vetch would be the superior cover crop in terms of benefit to the main crop, while limiting N_2O emissions if enough vetch biomass is produced. We can conclude that after incorporation of a N-rich residue, N is released faster, necessitating planting soon after incorporation so the cash crop can take up the N and the N is not lost through N_2O emission. Whereas more time between cover crop incorporation and main crop planting is needed for a good crop yield after incorporation of a cover crop with a high C/N ratio. Using mixtures of cover crop residues resulted in soil mineral N, wheat performance and emissions of CO_2 and N_2O as expected from the respective monocultures. No positive mixing effects were found that would indicate that using mixtures of different qualities can increase the N availability over time and also reduce N_2O emissions after incorporation.

In a field situation cover crops grown in mixtures can change in terms of their quality. Overyielding in mixtures can occur and thus also quantity effects should be considered in the field. Further studies testing the effect of the interaction between quantity and quality of cover crop residue incorporation on mineralisation and N_2O emissions would be valuable in order to grasp what is happening in field situations. Additionally, further studies should take legacy effects of cover crop growth into account since these may influence the soil quality as well as the microbial community and thus also affect N_2O emissions.

5.6 Acknowledgments

This study is a part of the Clever Cover Cropping project which is a collaboration between the Soil Biology Group and the Centre for Crop Systems Analysis (Wageningen University & Research), the Centre for Soil Ecology (CSE) and the Netherlands Institute of Ecology (NIOO-KNAW). This study was supported by a grant from the Netherlands Organization for Scientific Research (NWO green, grant number 870.15.071) with co-financing from seed producers (Agrifirm, Vandinter Semo, P.H. Petersen Saatzucht, and Joordens Zaden). We are grateful to Mirza Haque, Veronica Spinelli, and Anna-Reetta Salonen for help with the experimental work.

5.7 Supporting information

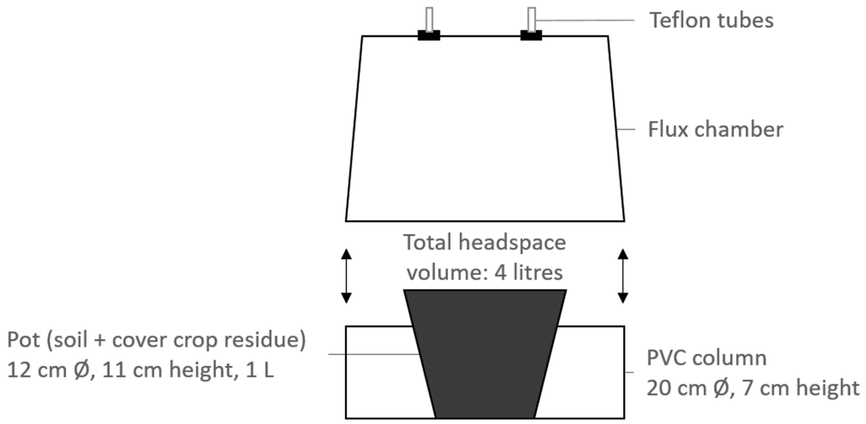

Figure S5.1 Mesocosm in PVC column. Adapted from Lubbers et al. (2011).

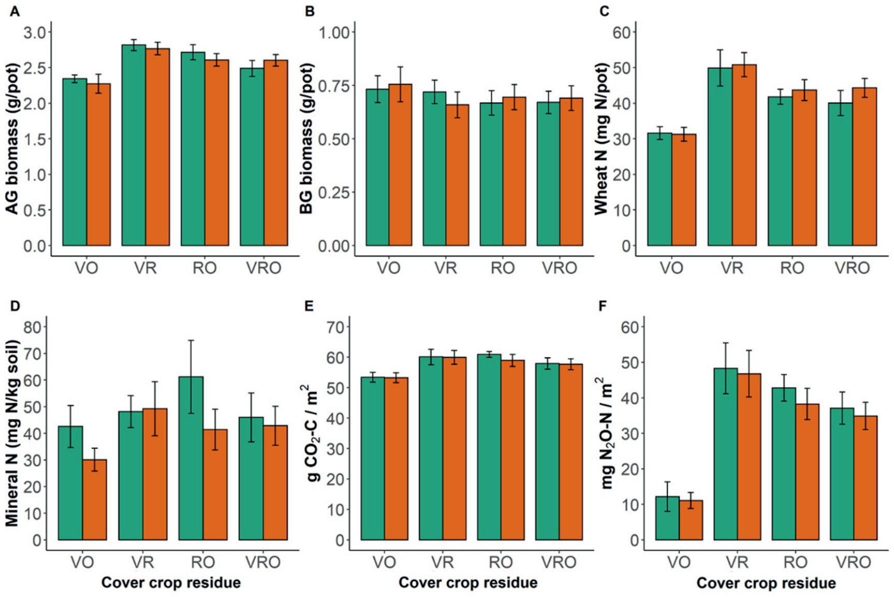

Figure S5.2 Observed and expected above ground biomass (**A**), belowground biomass (**B**), Wheat N (**C**), soil mineral N (**D**), cumulative CO_2 (**E**) and cumulative N_2O (**F**) of the three 2-species mixture and the 3-species mixture. The expected value is the weighted average of the two respective single species in the mix, proportions in mixtures as shown in Table 5.1. R (radish), V (vetch), O (oat). Bars indicate mean ± standard error, none of the differences between observed (green) and expected (orange) were significant (*P*<0.05).

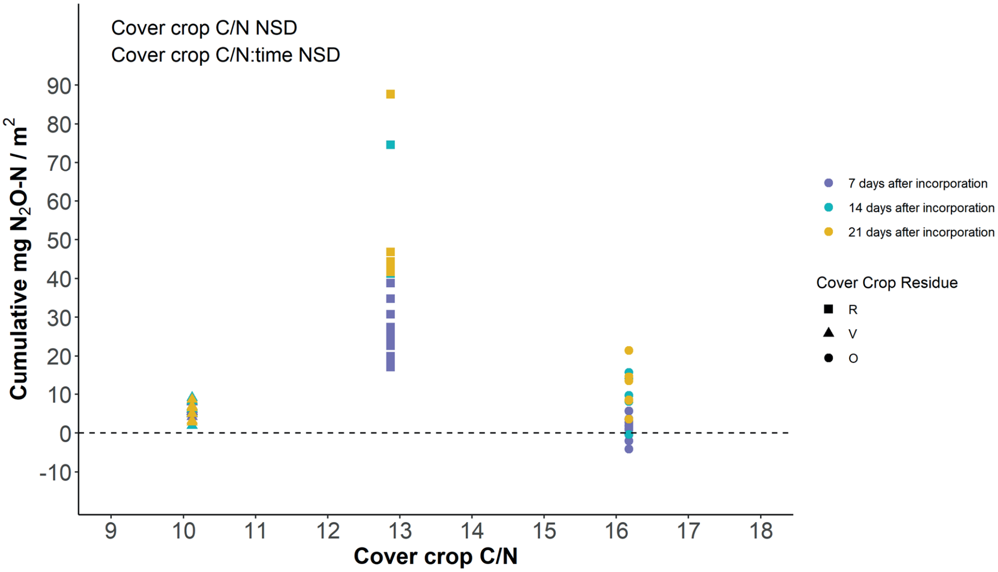

Figure S5.3 In unplanted soil, cumulative N_2O emissions measured on day 7, 15, and 21 were not significantly related to cover crop C/N ratio (single species only). Colours indicate the time after cover crop incorporation at which the N_2O emissions were measured. Shapes indicate which cover crop residue was incorporated into the soil: R (radish), V (vetch) and O (oat).

Chapter 6

Do cover crops change levels of soil organic carbon in arable soil?

Rima J. Porre, Mathilde Hagens, Gerlinde B. De Deyn, Ali Elhakeem, Sytske M. Drost, Leon Brooijmans, Ellis Hoffland

Abstract

Cover crops, grown to retain soil and nutrients till planting the next cash crop, are incorporated into the soil at the end of their growing season and provide the soil with organic matter. It has been advocated that cover crops promote soil C sequestration, however the extent to which this is achieved varies, likely due to differences in biomass quantity and quality of different cover crops. Recently the use of cover crop mixtures is promoted since these promote biodiversity and can enhance the quality and quantity of biomass input into the soil. Ideally, diverse residue inputs improve soil C sequestration while also improving soil fertility. Cover crop (mixtures) could increase both the labile and stable pools of organic matter depending on their biomass quality that is incorporated in the soil. In this study we examined if cover crop (mixtures) impacted both the labile and the stable fractions of C. We hypothesised that cumulative cover crop biomass is larger in mixtures than monocultures and relates positively to soil C pools, and that higher quality cover crop biomass (lower C:N ratio) results in relatively more stable SOC. We established a field experiment on sandy soil comprising an agricultural rotation of cash crops and cover crops for the duration of four years. Single cover crops (*Avena strigosa*, *Vicia sativa* and *Raphanus sativus*) were compared to all possible two- and three-species mixtures, and fallow soil was included as reference. Soil organic matter, total soil C and C in the several fractions (ranging from labile to stable) were quantified in soil samples collected in the spring of the 3rd and 4th year of the experiment. The cover crops did not have any significant effect on the total soil C content or the different C pools compared to the fallow, even after 4 years. However, SOM was slightly increased in two of the cover crop mixture treatments after 4 years. Our results suggest that 4 years of cover cropping on tilled sandy soil is short in order to observe increases in the stable soil C pool. Or, the quantity and quality of the three cover crops interfere in such a way that the net effects on labile and stable soil C are similar.

6.1 Introduction

SOM is important for soil functioning thus levels need to be maintained (Reeves, 1997), and cover crops can be instrumental in this. Sequestration of carbon (C) in arable soils can offset CO_2 emissions (Minasny et al., 2017) while increasing the SOC content. Moreover, soil organic matter (SOM) is important for soil structure, water holding capacity, it feeds soil life and is a source of nutrients (N and P) and is thus essential for crop biomass production (Liu et al., 2006). One way to increase SOC stocks in soils is by adding more plant biomass to the soil. Cover crops, usually grown in between two growing seasons of main crops, are incorporated into the soil before the next cash crop which makes them a potential tool to restore agricultural SOC pools (Kaye and Quemada, 2017; Poeplau and Don, 2015).

Cover crops, depending on the crop type, have been estimated to increase the soil C content with up to 0.32±0.08 Mg C ha^{-1} y^{-1} in a recent publication using data from 37 studies worldwide (Poeplau and Don, 2015). A meta-analysis by Jian et al. (2020) showed that by adding cover crops in a rotation in a temperate climate, the soil organic C content increased with 2.2 Mg ha^{-1} on average, with the majority of studies lasting less than 5 years. In their meta-analysis the focus was solely on the total SOC pool. Yet the effect of cover crops on the soil quality and soil C storage could vary depending on the pools in which the C is stored. This is relevant, because different pools may support different soil functions.

Generally labile C fractions are essential for crop production by providing nutrients whereas stable C fractions are essential for C sequestration. Labile C fractions often mentioned are (free) particulate organic matter (POM), dissolved organic carbon (DOC), permanganate oxidisable carbon POX-C (Bongiorno et al., 2019) and are distinguished based on their fractionation methodology (Haynes, 2005). Stable C fractions are usually defined as C bound to minerals (MAOM) and C occluded in aggregates (Gulde et al., 2008). Important to note is that aggregate stability, and thus the stability of C in aggregates depends on management practices (such as tillage) (Gulde et al., 2008). Cover crops provide the soil with plant biomass as well as low molecular weight compounds such as root exudates and leaf leachates (Liang et al., 2017). When plant biomass enters the soil it forms particulate organic matter (POM) after being partially decomposed or fragmented to a size smaller than 2 mm (Benbi et al., 2014; Castellano et al., 2015). POM can persist in soil through inherent recalcitrance or physical protection in aggregates (Cotrufo et al., 2019) or undergo decomposition, provided enough N is available. The decomposition products can become part of the stable C fraction by bonding to mineral surfaces as mineral-associated organic matter (MAOM) (Haddix et al., 2020).

Recent studies indicated that easily decomposed biomass has a greater effect on C stabilisation than biomass which is difficult to break down (Cotrufo et al., 2013). Fast decomposable compounds are easily and efficiently utilised by soil biota, which results in increased microbial necromass of low molecular weight (Cotrufo et al., 2013; Haddix et al., 2020; Kallenbach et al., 2015; Liang et al., 2017; Schmidt et al., 2011; Wieder et al., 2013). This microbial necromass binds to minerals and thus end up in the stable MAOM fraction (Cotrufo et al., 2013; Haddix et al., 2020). Liang et al. (2019) showed that more than 50% of total SOC in agricultural soils consisted of microbial necromass. This would suggest that fast decomposable residues can be considered as precursor of the more stable C fractions (Lehmann and Kleber, 2015) whereas materials that decompose slowly build up in the more labile POM fractions. In a recent publication by Lavallee et al. (2020) they plea for a distinction between POM, with a mean residence time of <10 years to decades, and MAOM with a mean residence time of decades to centuries.

In agroecosystems the proportion of C that is added to the soil that eventually gets stored in the soil depends on the quantity and quality of the biomass input, as well as on soil type and management practices (Jian et al., 2020). Generally SOM storage is increased when higher quantities of C are supplied to the soil (Lal, 2004). C storage in the MAOM pool has a saturation limit, depending on the silt and clay content of the soil, whereas POM accrual does not (Lavallee et al., 2020). Plant biomass quality, and thus decomposability, is determined by several factors such as the N, lignin and polyphenol contents and the C:N ratio of the substrate, the latter which is most frequently reported (Porre et al., 2020). Generally, biomass with a low C:N ratio and low lignin and polyphenol concentrations shows fast initial decomposition, while high C:N ratio biomass with high lignin and polyphenol concentrations is known to decompose more slowly in the early stage of decomposition (Schmatz et al., 2017). High C:N litters could contribute more to POM accrual (Cotrufo et al., 2013). Whereas, plant residues with a low C:N ratio are expected to end up in the soil stable C pools, by interaction with the soil mineral matrix (Dungait et al., 2012; Liang et al., 2017).

Besides residue quality, residue diversity can increase microbial biomass and functional diversity and hence could affect the stable SOM pools (Drost et al., 2020; Lange et al., 2015). This means stabilisation of C can be stimulated by diverse and high quality residue input. Plant mixtures can benefit from complementarity in their rooting systems due to distinct spatial orientation, which leads to an enhanced ability for spacing and thus nutrient uptake (Cong et al., 2015). Moreover, some plant species, e.g. legumes, have the ability to fix nitrogen (N) from the atmosphere which could result in enhanced growth of its neighbours when grown mixed. This can eventually result in a higher biomass production in the mixture than expected from the species in monoculture, called overyielding (Vandermeer, 1989). Cover crop mixtures containing legumes can thus have a high biomass production as well as a high N content and might thus increase stable SOM to a greater extent than crops grown in mono-culture. Besides increasing residue quantity, cover crop mixtures are able to increase residue quality and diversity (Duval et al., 2016) and hence might increase C stabilisation. A meta-analysis by Jian et al. (2020) showed that cover crop mixtures increased the SOC content relative to the control (no cover crop), whereas grasses grown alone did not. Moreover, studies on cover crop mixtures in relation to SOC are limited and no indication is given to which C pools, labile or stable or both pools, were increased by cover crop residue addition.

The aim of this study is to examine the effect of cover crops and their mixtures on soil organic matter maintenance in a sandy soil. This effect is evaluated by quantifying different SOM fractions, ranging from labile to stable. We hypothesised that, I) mixtures produce more biomass and a higher cover crop biomass input results in a higher total SOM content and II) incorporation of cover crops with a lower C:N ratio results in more stable SOM.

6.2 Materials and methods

6.2.1 Experimental design & sampling

The field experiment was located on a sandy soil (2% clay, 12 % silt, 86% sand) with a pH of 5.3 and an initial SOM content of 3.1%, located at the Wageningen University & Research experimental farm (51°59'42.6"N 5°39'36.8"E), the Netherlands. The long term annual average temperature is 9.4°C and annual average rainfall is 780 mm. An agricultural rotation was established in 2016 with a main crop in spring and summer and cover crops in autumn and winter. The cover crop treatments consisted of *Vicia sativa*, *Avena strigosa* and *Raphanus sativus* in monocultures as well as all possible two- and three-species mixtures. The seven cover crop treatments and a fallow were replicated 5 times in a complete randomized block design with 8 plots per block totalling 40 plots, each plot being 10 m x 6 m. The cover crops were sown in August of each year (Table 6.1) with seeding rates of 30 kg ha^{-1} (oilseed radish), 110 kg ha^{-1} (vetch) and 90 kg ha^{-1} (black oat) according to farming practices.

Table 6.1 Specifications of the timing of cover crop sowing, harvesting, incorporation as well as the amounts and timing of N fertilisation. The field experiment started in August of 2016.

Cover crop				
Sowing date	N fertilisation date	Amount N fertilised	Date subsample harvest	Incorporation
15-08-2016	23-08-2016	30 kg N/ha	21-11-2016	23-03-2017
28-08-2017	20-09-2017	30 kg N/ha	20-11-2017	08-03-2018
16-08-2018	23-08-2018	30 kg N/ha	12-11-2018	18-02-2019
16-08-2019	28-08-2019	30 kg N/ha	11-11-2019	02-03-2020

Species were mixed according to a replacement design, using, for each of the species, 50% for the two-species mixtures and 33% for the three-species mixture of the seed rate in the pure stand. Each year, after sowing, the cover crops were fertilised with calcium ammonium nitrate (CAN) at a rate of 30 kg N/ha in order to ensure establishment. Cover crops were incorporated into the soil in March of each year (Table 6.1) before planting the main crop (Table 6.2).

Table 6.2 Specifications of the cash crop: timing of planting or sowing, harvesting and fertilisation

Cash crop				
Crop type	Planting date	Date fertilisation	Amount N fertilised	Harvest date
Maize	20-04-2017	02-05-2017	95 kg N/ha	21-08-2017
Potato	26-03-2018	12-04-2018	110 kg N/ha	02-07-2018
Barley	03-04-2019	04-04-2019	60 kg N/ha	23-07-2019

6.2.2 Cover crop biomass

Above- and belowground cover crop biomass were harvested in November of each year (Table 6.1). Aboveground biomass was cut just above the soil surface from two randomly allocated 1m^2 squares in the centre of each plot. Weeds were separated from the harvested samples. Following species separation in the mixtures, shoots were rinsed with water to remove any adhering soil particles and dried at 70°C for 48 h. Belowground biomass was harvested by taking four soil cores (8 cm Ø, 30 cm depth) from each plot (two within rows and two between rows). Roots were washed to remove soil and organic matter particles and oven-dried at 70 °C for 48 h. Dried biomass (both above- and belowground) was ground to a fine powder. Concentration of C and N in shoot and root were determined separately using combustion in an elemental analyser (Flash EA 1112, Thermo Scientific).

6.2.3 Soil sampling

Soil samples were taken from all plots at the start of the experiment (August 2016) and three and four years later two weeks after cover crop incorporation in March 2019 and 2020. Eight samples (2 cm Ø, 20 cm depth) were taken in a random design from each plot. After collection the soil was air dried for 24 h at room temperature and sieved (2mm).

6.2.4 Soil organic matter

Total soil organic matter (SOM) was determined by loss on ignition. Soil samples were dried at 105°C for 24 h and 20 g of dried soil was heated up to 550°C in a Nabertherm oven for a minimum of 3 hours and weighed before and after.

6.2.5 Labile C

Labile C was quantified by analysing dissolved organic carbon (DOC) and permanganate oxidisable carbon (POX-C) (Haynes, 2005). Additionally soil microbial biomass C (MBC, as an indication of the soil microbial C pool) and macroaggregates were measured.

6.2.5.1 Dissolved organic C (DOC)

Dry soil, sieved over 2 mm, was extracted in a 1:10 (w/v) suspension with 0.01 M $CaCl_2$ (Houba et al., 2000a). Soil and $CaCl_2$ were shaken for 2 hours, the suspension was filtered to 0.45 µm, and the filtrates were analysed for total dissolved C on a TOC-5050A analyser (Shimadzu Corporation, Kyoto, Japan).

6.2.5.2 Microbial biomass C (MBC)

Fresh soil samples (sieved on a 2 mm sieve) were fumigated with chloroform in order to determine the amount of microbial carbon (MBC) in the soil (Brookes et al., 1985; Klug, 1999). Fresh soil samples and fumigated soil samples were subsequently extracted with a 0.5 M K_2SO_4 solution (with a soil to solution ratio of 1:4, w/v). C in both samples was analysed on a San^{++} 6 channel segmented flow analyser (SFA-TOC) (Skalar, The Netherlands).

6.2.5.3. Permanganate oxidisable C (POX-C)

The permanganate oxidisable carbon (POX-C) was analysed following the procedure of Weil et al. (2003) and modified according to Bongiorno et al. (2019). In short, 18 mL of deionised water and 2 mL of 0.2 M potassium permanganate ($KMnO_4$) were added to 2.5 g of air-dried soil and shaken for 2 min. at 120 rpm. The solutions were left undisturbed for 10 min. Subsequently 49.5 mL of deionised water was added to 0.5 mL of the supernatant. The absorbance of the samples was measured with a spectrophotometer at 550 nm. To calculate the amount of POX-C in the treatments a calibration curve was established with 0.005, 0.01, 0.015

and 0.02 M $KMnO_4$;. The intercept and slope represent parameters *a* and *b*, respectively, in equation 1.

$$POX - C\ (mg\ kg^{-1}) = [0.02\ mol\ L^{-1} - (a + b * Abs)] * (9000\ mg\ C\ mol^{-1}) * (0.02\ L\ solution\ Wt^{-1})$$

Where $KMnO_4$ has a molar concentration of 0.02 mol L^{-1}, Abs represents the absorbance of the individual treatment, the amount of C which is oxidised by 1 mole of MnO_4 is 9000 mg C mol^{-1}; the volume of the stock solution is 0.02 L ; and Wt^{-1} is the sample dry weight used, in this case 2.5 g.

6.2.5.4 Macroaggregates

Air-dried soil (80 grams, sieved <2 mm) was slaked for 5 min. in a 250 µm sieve which was placed in a basin with a water level of 1 cm above the sieve according to (Six et al., 2000). After slaking, the sieve was moved up and down 50 times in 2 min. with an amplitude of approximately 3 cm. Then, the material on the sieve was backwashed into pre-weighed drying pans and oven-dried at 105 ºC (overnight) and weighed.

6.2.6 Stable C (fractionation)

We chose to use the fractionation by Poeplau et al. (2018); (Six et al., 2002), described below (Fig 6.1). This extensive fractionation was only done on a subset of the field treatments. First, we excluded block five since it had a larger SOM content compared to the other blocks. Second, we did not expect large differences between the single cover crop treatments and the mixtures. Thus we only examine the stable C pools on the single cover crop treatments (3 cover crops and a fallow * 4 replicates = 16 samples). In order to get both the POM and MAOM pool, described by Lavallee et al. (2020) we summed the obtained POM and i(POM) to get the total POM and we summed the silt + clay and the i(silt + clay) to get the MAOM pool.

6.2.6.1 Microaggregate isolation

In short, 30 g of air-dried soil (sieved to <2 mm) was slaked for 5 min in 50 mL deionised water. The soil was placed on the 250 µm mesh of the microaggregate isolator and gently shaken (150 rpm) together with 50 glass beads (4 mm; thus macroaggregates were broken up) and a constant flow of deionised water in order that the microaggregates were immediately flushed on a 53 µm sieve. The 53 µm sieve was moved up and down in a water bath in order to fully sieve this fraction. Ultimately, POM (>250 µm), micro-aggregates (53-250 µm) and the silt + clay fraction (<53 µm) were isolated. All fractions were oven-dried at 105 ºC (overnight) and weighed.

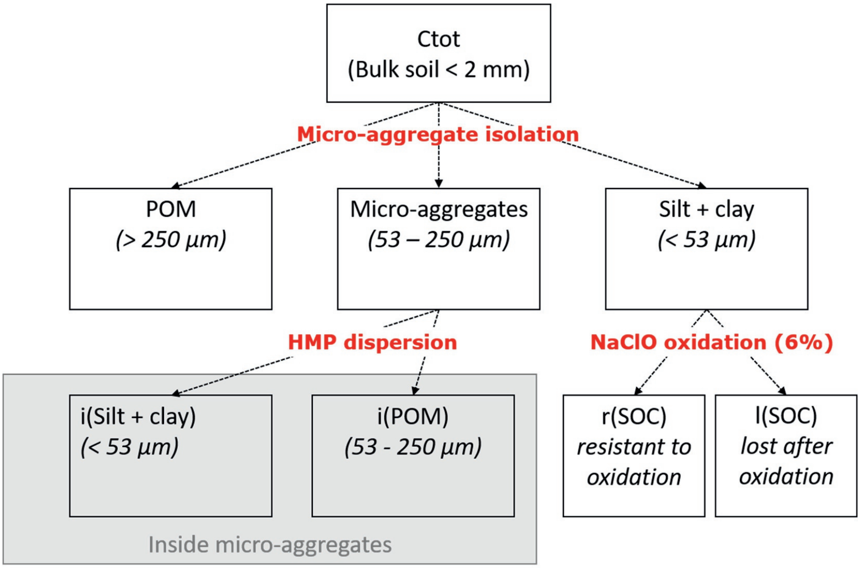

Figure 6.1 Soil C fractionation scheme adapted from Poeplau et al. (2018); Six et al. (2002). Ctot is the C measured in the bulk soil. POM is the coarse POM that is free in soil or occluded in macroaggregates (the macroaggregates are broken up in the first step). Microaggregates and the silt + clay fraction are separated based on size. Microaggregates are dispersed by sodium-hexametaphosphate (NaHMP) the intra-aggregate fractions (i(silt + clay) and i(POM)) are separated based on size. The silt + clay fraction is oxidised with NaClO and results in fractions resistant to oxidation (r(SOC)) and lost on oxidation (l(SOC)). The method originally presented included an extra oxidation step of i(Silt + clay), we could not include this step since our soil did not contain enough i(Silt + clay) for oxidation.

6.2.6.2 Dispersion and oxidation

Microaggregates were dispersed by transferring 5g of the microaggregate fraction to 15 ml of 5 g L^{-1} sodium hexametaphosphate (NaHMP) and 10 glass beads. The solution was shaken on a reciprocal shaker for 18 hours at a frequency of 90 rpm. Subsequently, the suspensions were sieved (53 µm) in order to collect the intra-aggregate POM (iPOM) and the silt + clay fraction originating from the microaggregates (i(Silt + clay). The silt + clay fraction was oven-dried at 105 ºC (24h). Due to the low silt + clay content of the soil, this procedure was repeated thrice in order to collect a sufficient amount for oxidation.

The silt + clay fractions originating from the bulk soil was oxidised by adding 50 mL of 6% sodium hypochlorite (NaClO) to 1g of silt + clay. The pH was adjusted to 8 with concentrated hydrochloric acid (HCl) and the solution was kept undisturbed for 18 hours at 25 ºC and subsequently centrifuged at 1000 g for 15 min. The silt + clay fraction was oxidised twice and washed with deionised water to collect the resistant SOC (rSOC) fraction. The received fraction was oven-dried at 105 °C (24h) and weighed.

6.2.6.3 C/N-analysis of the C fractions

The different pools: Ctot, POM, micro-aggregates, silt + clay, i(silt+clay), and r(SOC), were colloid grinded and (subsamples of 200 – 300 mg) were analysed on a LECO Truspec CHN analyser (LECO Corporation, St. Joseph, MI, USA).

6.2.7 Statistical analysis

We tested the effect of cover crop residue (factor, 8 levels, average biomass of 4 years) on the several different organic matter fractions measured using linear mixed effects models with the function lme from the package nlme (Pinheiro et al. 2016) with 'block' as a random factor. Significance was determined with analysis of variance (ANOVA), using the function anova (R Core Team, 2018). Normality and homogeneity of model residual variances were assessed by plotting model residuals. Heterogeneous residual variances were addressed using a variance structure in the model with the function varIdent (nlme) (Pinheiro et al., 2019). The model with the variance structure was retained when it was significantly better fit than the model without, determined by comparing the model's akaike information criterion (AIC) using the function anova (R Core Team, 2018). To determine significant differences between levels of a factor (i.e. between cover crop residues), a Tukey's honestly significant difference posthoc test (HSD) was used, with the function emmeans (Lenth et al., 2019) and CLD from package multcomp (Hothorn et al., 2008). A two-sided t-test (base R) was used to test if SOM was significantly different from zero. For correlations between continuous variables (i.e. cover crop C and SOM), linear mixed effects models (lme) and ANOVA (anova) were used, as above. Marginal R^2 was determined with r.squaredGLMM from the package MuMIn (Barton, 2019). All statistics were carried out in R version 3.5.1 (R core team 2016). Figures were made using the packages ggplot2 (Wickham, 2016) and cowplot (Wilke, 2019).

6.3 Results

6.3.1 Cover crop

Cover crops yielded, on average 2 - 6 Mg/ha, per growing season, depending on the crop species (Fig 6.2A). Vetch produced significantly lower biomass (~2 Mg/ha) compared to all other cover crop (mixtures) that yielded >5 Mg/ha ($F_{6,24} = 40.41$, $p < 0.001$). Cover crop mixture biomass was similar to the biomass of oat and radish. Weed growth in fallow fields amounted to 1.3 Mg/ha, on average over the years. Via their biomass the incorporated cover crops represented between 0.87 and 2.55 Mg C/ha average input per year over the four years of the experiment (Fig 6.2B). Vetch contributed the lowest amount of C and oat and the vetch-radish mixture the highest amount of C ($F_{6,24} = 31.52$, $p < 0.001$). The C:N ratio of the biomass was significantly different between the cover crops ($F_{6,24} = 40.18$, $p < 0.001$, Fig 6.2C). Vetch had the lowest C:N ratio (12.1) and oat the highest (23.2). The C:N ratios of mixtures were close to those of the highest yielding species, which was either oat or radish.

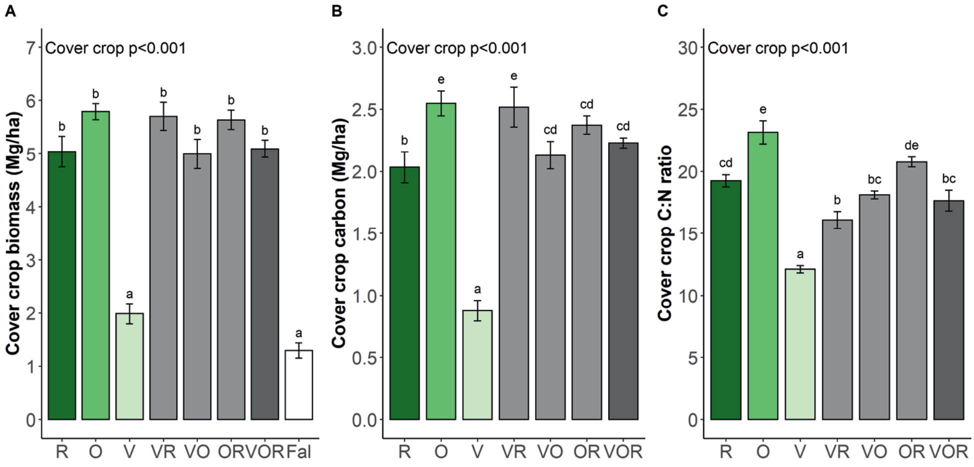

Figure 6.2 The 4- year average (2016-2019) of cover crop (above- and belowground biomass combined) A) biomass in Mg/ha, here we also indicate weed biomass for the fallow, B) C in Mg/ha and C) the C:N ratio. The different cover crop treatments are: R (radish), O (oat), V (vetch) and their combinations, Fal (fallow, i.e. no cover crop). Bars indicate mean ± standard error (n=5), different lower case letters indicate significant difference between cover crop treatments ($p < 0.05$).

6.3.2 Soil organic matter

The SOM concentration in the soil at the start of the experiment varied significantly among the blocks between 2.5 and 3.6% (Fig 6.3A; $F_{4,35} = 34.61$, $p < 0.001$). In 2019 there was no change in SOM or any of the treatments compared to the start of the experiment (Fig 6.3B). In 2020 there was a significant, yet small, increase of SOM for the mixture vetch + radish (Fig 6.3B; $T_4 = 2.92$, $p = 0.04$, $\Delta SOM = 0.07\%$) and the three species mixture vetch + radish + oat (Fig 6.3B; $T_4 = 3.24$, $p = 0.03$, $\Delta SOM = 0.06\%$).

6.3.3 Soil C fractions

The amounts of dissolved organic carbon (DOC, Fig 6.4A-B), microbial biomass carbon (MBC, Fig 6.4C-D), total C (Fig 6.4E) and the permanganate oxidisable carbon (POX-C, Fig 6.4F) fractions did not differ significantly between soils with cover crops and soil left fallow between the main crops after three and four years of imposing the treatments.

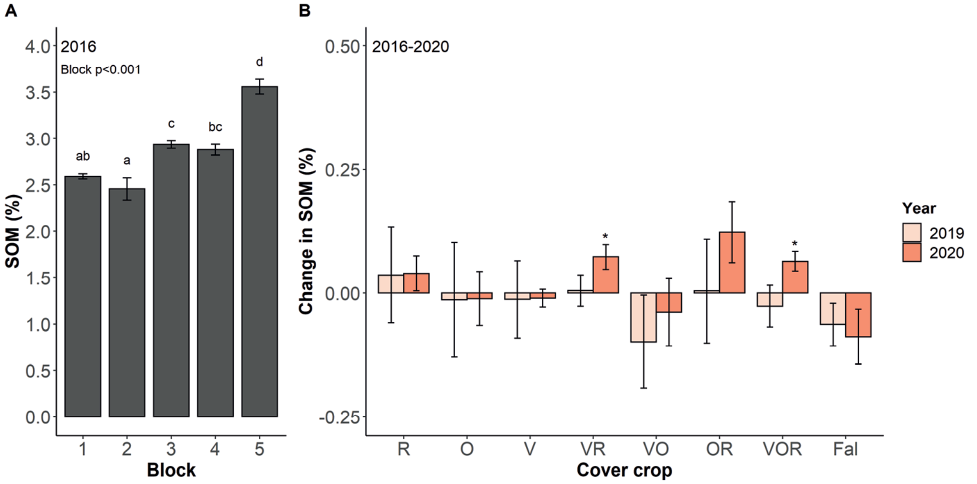

Figure 6.3 Soil organic matter (SOM, in %) measured at the start of the field experiment in 2016 (A) and the change in SOM (B) measured in 2019 and 2020 compared to the initial SOM % in 2016 (Change in SOM (%) = % SOM_t - $\%SOM_{2016}$; t = 2019 or 2020). The different cover crop treatments are: R (radish), O (oat), V (vetch) and their combinations, Fal (fallow, i.e. no cover crop). Bars indicate mean ± standard error (n=5). Asterisks above the bars indicate a significant difference from zero ($P < 0.05$).

On average the soil consisted of 63% POM, 28% microaggregates and 8% silt and clay in terms of dry weight. The total recovery of C after fractionation was 110±14%. Forty-five percent of the C in the soil was in the silt and clay fraction (Fig 6.6), while the C present in microaggregates (34%) was similar to that in POM (33%). Over 80% of the C bound to the silt and clay fraction (not occluded in micro-aggregates) was lost after oxidation with NaClO. In microaggregates over two-third of the C was present in POM, the remaining C was associated with the silt and clay fraction. The C contents in particulate organic matter (POM, Fig 6.5A), micro-aggregates (Fig 6.5B), silt + clay associated C (Fig 6.5C), resistant SOC (Fig 6.5D), silt + clay associated C in micro-aggregates (Fig 6.5E), total POM and MAOM (Fig 6.5F) were not significantly different between the cover crops and the fallow, as quantified in the third year. Additionally, no effect of cover crop incorporation on the amount of macroaggregates in the soil was seen (Fig S6.1, appendix). The average cover crop biomass input, C:N ratio or total C added in cover crop biomass were not related to any of the C fractions in the soil ($p > 0.10$; Fig S6.2-S6.5, supporting information).

6.3.5 C saturation

The C saturation of the soil was determined by the silt and clay protected C (<53 µm organo-mineral complexes). Our field plots reached a silt and clay C saturation that is close to the lower limit of the 95% confidence interval predicted by Six et al. (2002) (Fig 6.7). This indicates that our silt and clay fraction was close to being saturated with C.

6.4 Discussion

6.4.1 Biomass input & soil C

The objective of this study was to test whether the soil C content increased as compared to fallow soil as result of cover crop biomass input of monocultures and enhanced biomass input of cover crop mixtures. Contrary to what we expected, cover crops with a larger biomass did not result in an increased soil C content compared to cover crops with a smaller biomass. This is in contrast with results from a meta-analysis by (Poeplau and Don, 2015) in which biomass input and soil C accumulation were found to be positively correlated. However, the majority of studies in their analysis lasted for more than 5 years. In our study the cover crops were grown for up to 4 years, which could explain why we hardly detected changes in the soil C content in response to cover crop incorporation, other than a slight increase in SOM for two of the mixture treatments in 2020 compared to 2016. In addition, C accumulation is, among other factors, dependent on soil texture (i.e. clay and silt percentage), and higher responses are generally expected in soils which are richer in clay and silt (Stewart et al., 2007). The field used for this experiment had a relatively low clay and silt content, 2% and 12%, respectively. Previous studies indicated that 79% of all C added in crop residues is lost after 1 year due to decomposition (de Sa Pereira et al., 2017). Using this assumption and based on the maximum amount of C added in our study, 2.55 Mg C ha^{-1} y^{-1}, this would translate into an increase of soil C of 0.53 Mg ha^{-1} y^{-1}. Our soil contained ~25 Mg C ha^{-1} in the topsoil at the start of the experiment. Thus an addition of 0.53 C Mg ha^{-1} y^{-1} would be a negligible amount especially considering the variability in C per block. In a study by Goidts et al. (2009) a field scale variability was reported in croplands of up to 22%, indicating that changes in SOC content would only be visible after years of high C addition to the soil. Additionally it is possible that the addition of cover crop biomass, all with a C:N ratio below 30, lead to microbial community growth which primed old SOC breakdown (Fontaine et al., 2004). This was also reported by Poeplau and Don (2015) where they found a decrease in SOC in 13 out of 139 plots (all with an age <6 years). In our study none of the treatments, including the fallow, caused a decrease in SOM.

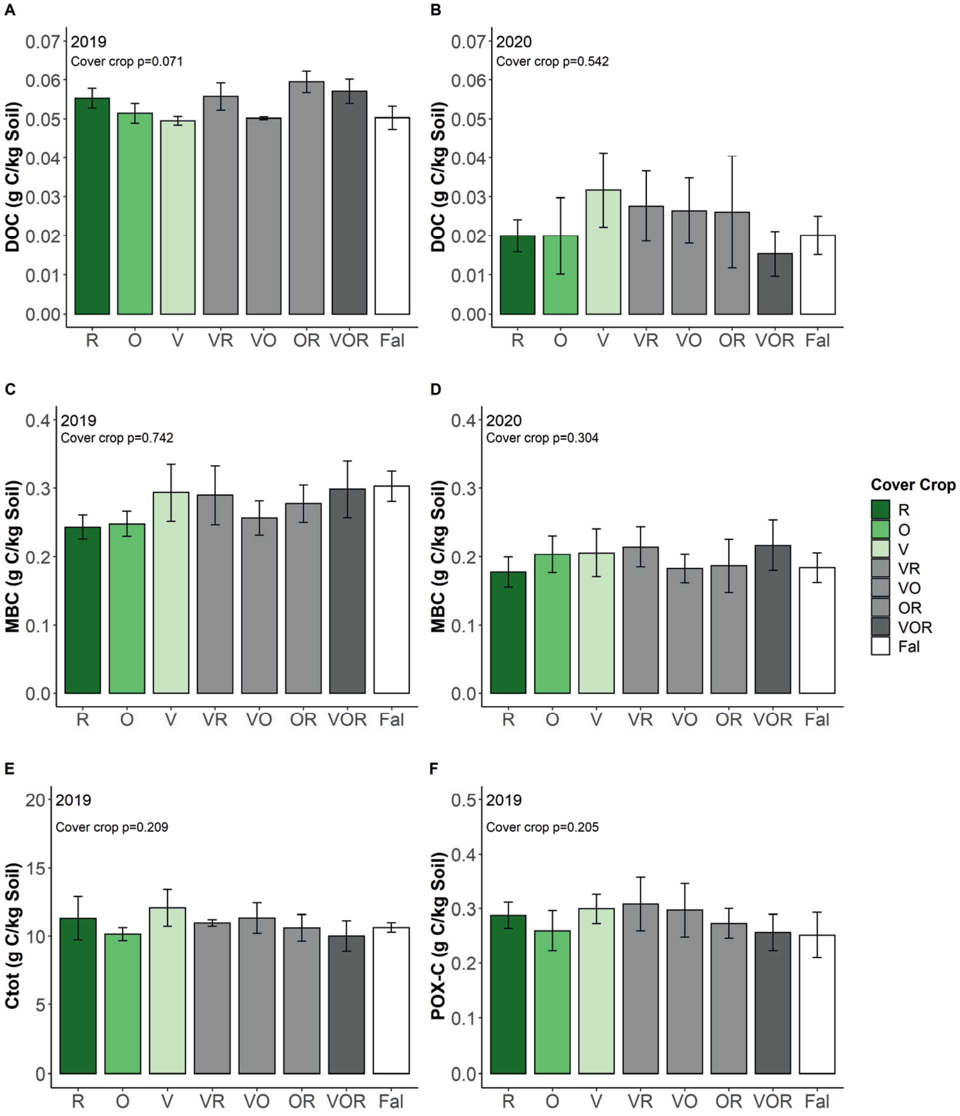

Figure 6.4 Dissolved organic C in A) 2019 and B) 2020, microbial biomass C in C) 2019 and D) 2020. Total C in 2019 (E) and permanganate oxidisable C in 2019 (F). The different cover crop treatments are: R (radish), O (oat), V (vetch) and their combinations, Fal (fallow, i.e. no cover crop). Bars indicate mean ± standard error (n=5 for MBC and DOC measurements and n=4 for total C and POX-C). None of the soil C pools were significantly affected by cover crop.

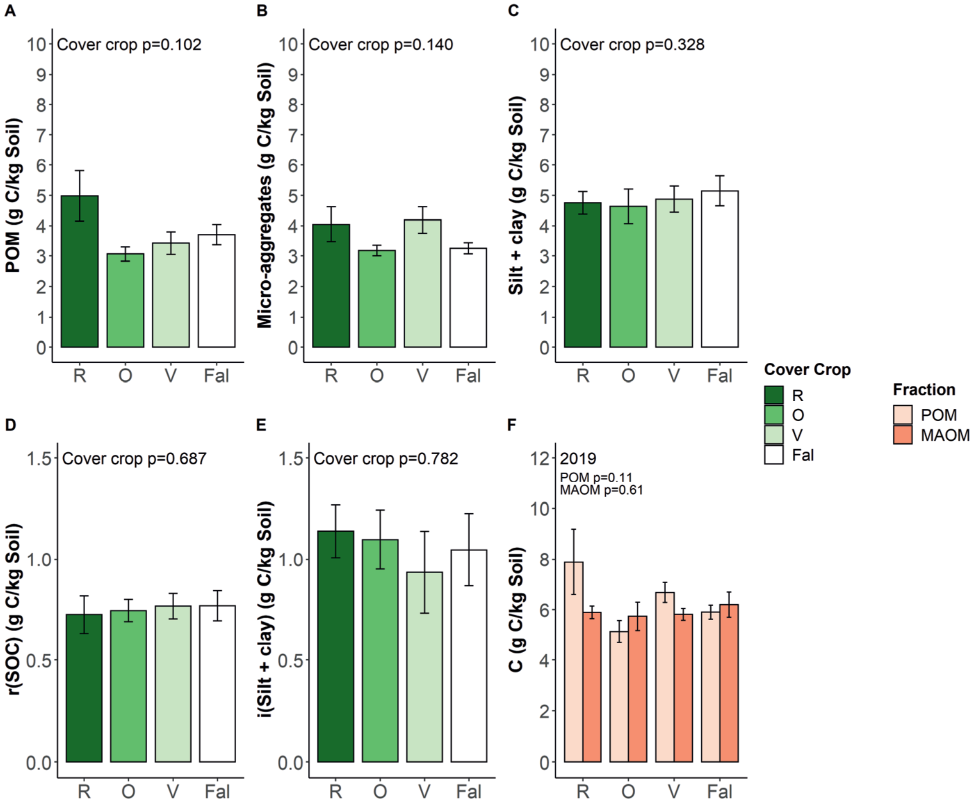

Figure 6.5 C present in the different fractions measured in 2019. C present in A) in particulate organic matter, B) in micro-aggregates, C) silt and clay associated C, D) resistant soil organic C and E) silt and clay associated C present in microaggregate and F) total POM and MAOM. The different cover crop treatments are: R (radish), O (oat), V (vetch) and Fal (fallow, i.e. no cover crop). Bars indicate mean ± standard error (n=4). None of the soil C pools were significantly affected by cover crop ($p < 0.05$).

In this study a tilled cropland was used as field experiment. In tilled (disturbed) soils microaggregate formation within macroaggregates can be reduced with a factor close to 2 compared to undisturbed soils since macroaggregates have a shorter turnover rate in disturbed soils (Six et al. 2000). In addition, undisturbed soils have a higher microbial activity (Balota et al., 2003) which could explain the divergent results we found compared to studies in which long-term undisturbed soils were used (Mitchell et al., 2018). Besides, Mitchell et al. (2018) used biomass input levels ranging from 5 to 15 Mg/ha while cover crop input in our field ranged between 2 to 6 Mg/ha.

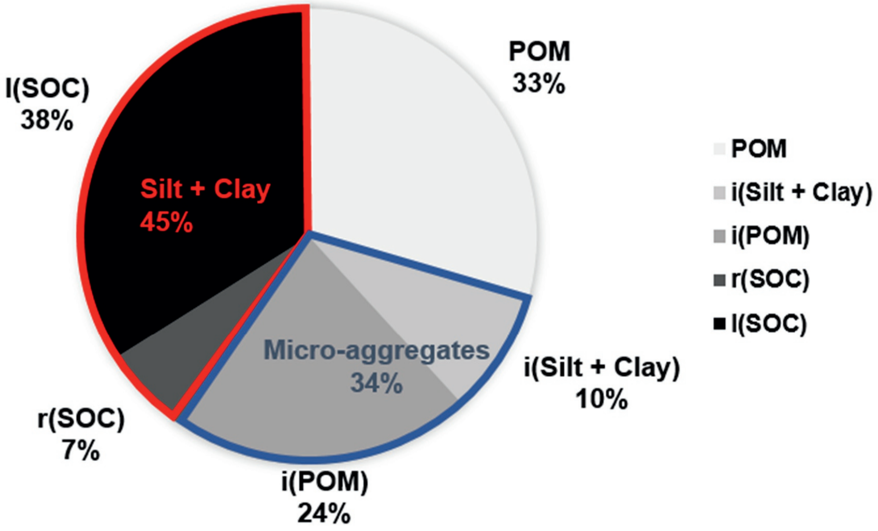

Figure 6.6 Soil C fractions (average of the single cover crop species and fallow) measured in 2019. Particulate organic matter (POM), Silt and Clay (i(Silt + Clay) and POM (iPOM) within micro-aggregates and SOC bound to the silt and clay fraction not occluded in microaggregates. Here we have SOC resistant to oxidation (r(SOC)) and lost upon oxidation (l(SOC). On average the recovery was 110%.

6.4.2 Residue quality effect on stable SOM

We expected a higher C content in the MAOM pool after vetch residue incorporation compared to oat and radish because vetch has a lower C:N ratio. However, vetch had a much lower biomass input compared to radish and oat. It is thus surprising that C contents in the stable fractions (microaggregate, S+C and rSOC) were similar for all three cover crops (Fig 6.5). Stabilisation of C could have thus been much more efficient in vetch and even the fallow soil which had some weed growth. This is in line with previous research in which C stabilisation in the MAOM and microaggregate fraction is negatively correlated with residue quality (Córdova et al., 2018; Kallenbach et al., 2015; Lavallee et al., 2018). The C in the soil after vetch is either a result from new input or perhaps less C was lost from the old C (priming) in the soil, or perhaps both (Kuzyakov, 2010). In field experiments often no significant effect of residue quality on micro-aggregate C content is reported (Gentile et al., 2011; Wang et al., 2017). This is most likely a result of a difference in biomass that could mask the residue quality effect on stable C accumulation. Another possibility is that inherent limitation in C stabilisation was caused by soil saturation levels which are determined by the soil's clay and silt percentage (Gulde et al., 2008; Stewart et al., 2007). The soil at our study site was a loamy sand (with a silt and clay content of 14 and 2 %, respectively). The silt and clay fraction in our soil was close to saturation (i.e. scattering around the lower 95% confidence interval for saturation) (Gulde et al., 2008; Six et al., 2002). Indeed, most of the stable C in our field was located in the S+C fraction (Fig 6.4). Often, microaggregates contain most of the stable C in sandy soils, since sandy soils have a low silt and clay content and thus a low capacity for organo-mineral associations (Puttaso et al., 2013). Tillage in our field could have inhibited this microaggregate formation. Thus cover crops, including mostly low C:N residues, might have only a limited capacity in increasing stable C storage on sandy soils since the MAOM is already close to saturated.

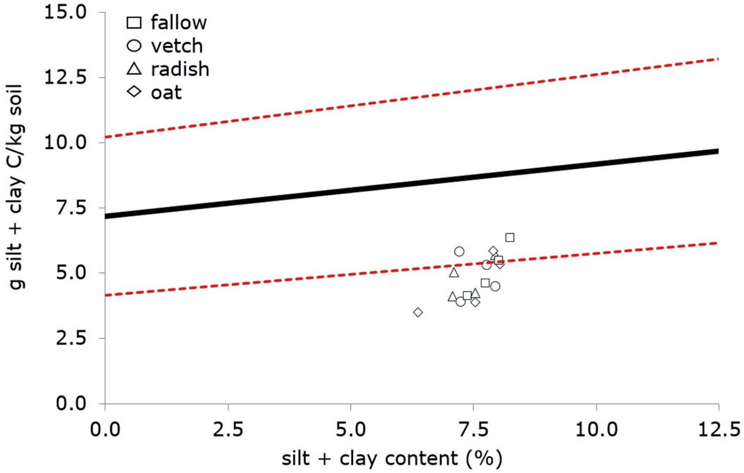

Figure 6.7 The relationship between silt + clay content (%) and silt + clay associated C (g silt + clay C/kg soil) obtained from Six et al. (2002). The black line ($y = 7.18 + 0.2x$) with the 95% confidence (red dashed line) indicates the relationship reported by Six et al. (2002) on silt and clay fractions (<50 µm) in cultivated soils. Data points indicate our sampled data with squares, circles, triangles and diamonds representing fallow, vetch, radish and oat plots respectively.

6.4.3 Labile SOM

Unexpectedly, after 4 years of cover crops, the dissolved organic carbon (DOC), microbial biomass carbon (MBC) and POX-C were not increased in the soil. POX-C has previously been shown to be highly sensitive to land management practices as a representation of the labile SOM pool that quickly respond to changes in management (Bongiorno et al., 2019; Culman et al., 2012; Weil et al., 2003). Additionally higher quality residue could have led to higher DOC concentrations due to the release of products as a result of microbial consumption. Yet we found no such relationship between the C:N ratio of the cover crops and the labile C pool. Possibly the C:N ratio in November differs from the C:N ratio of the biomass incorporated in March. During the winter period, depending on the climate, cover crops either still accumulate biomass or they senesce. Additionally, incorporation of residues with a low C:N ratio generally show a high initial decomposition rate. However this is time sensitive and affected by temperature and humidity. Thus a peak in labile C can be missed when measuring at just one timepoint. We initially expected higher labile C after vetch, however again the low biomass of vetch could have masked the effect of residue quality.

6.4.4 Cover crop mixtures

Contrary to what we expected, mixtures did not produce a higher biomass than expected from the single cover crops. Unsurprisingly also no differences in total soil C and microbial C were found as a result of incorporating cover crop mixtures. However, we did see that in 2020 the mixture of vetch + radish and the three-species mixture caused a slight increase in SOM compared to 2016. This was not seen in any of the other cover crop treatments. Plant species enrichment and residue diversity enrichment are repeatedly reported to be positively correlated to microbial C and soil C accumulation (Lange et al., 2015; Santonja et al., 2017). In a biodiversity experiment of Fornara and Tilman (2008) they found an average soil C increase of 69.5 g C m^{-3} y^{-1} in a 16 species mixture compared to an increase of 13.9 g C m^{-3} y^{-1} under monocultures. It was suggested that this was largely the effect of an increase in belowground biomass, as result of complementarity in rooting systems. However mixing results on soil C are often reported in undisturbed systems such as grasslands. Thus, in a conventional arable system it is expected to take much longer to see an effect of mixing on soil C.

6.5 Conclusion

None of the single cover crops and only two of the cover crop mixtures increased the total SOM content after 4 years of including cover crops on a sandy soil. After 3 years of including single cover crops no differences were found in any of the C fractions (labile to stable) compared to the fallow soil. Residue quality, as indicated by the C:N ratio, did not correlate with any of the soil C fractions. Based on these results it would be highly relevant to see if some high yielding cover crop mixtures can increase SOM on a longer term. Additionally mixtures could potentially be of importance for C sequestration on clay soil, which has a lower saturation limit.

6.6 Acknowledgements

This study is a part of the Clever Cover Cropping project which is a collaboration between the Soil Biology Group, the Centre for Crop Systems Analysis (Wageningen University &

Research), the Centre for Soil Ecology (CSE) and the Netherlands Institute of Ecology (NIOO-KNAW). This study was supported by a grant from the Netherlands Organization for Scientific Research (NWO green, grant number 870.15.071) with co-financing from seed producers (Agrifirm, Vandinter Semo, P.H. Petersen Saatzucht, and Joordens Zaden). We are grateful to Gerlinde Vink, Willeke van Tintelen, Tamás Salanki and Péter Garamszegi for help with the experimental work.

6.7 Supporting information

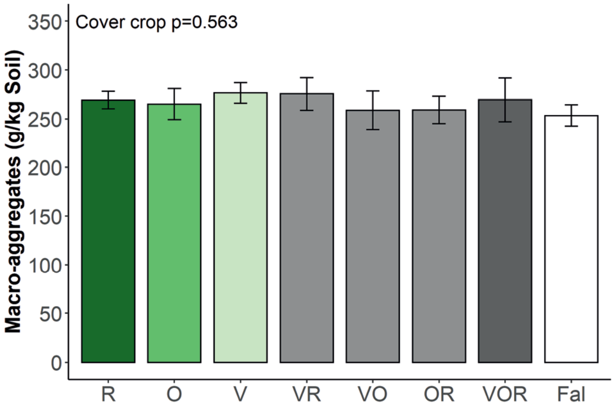

Figure S6.1 The amount of macroaggregates in the soil (in g macroaggregate/kg soil) measured in 2019. The different cover crop treatments are: R (radish), O (oat), V (vetch) and their combinations, Fal (fallow, i.e. no cover crop). Bars indicate mean ± standard error (n=4).

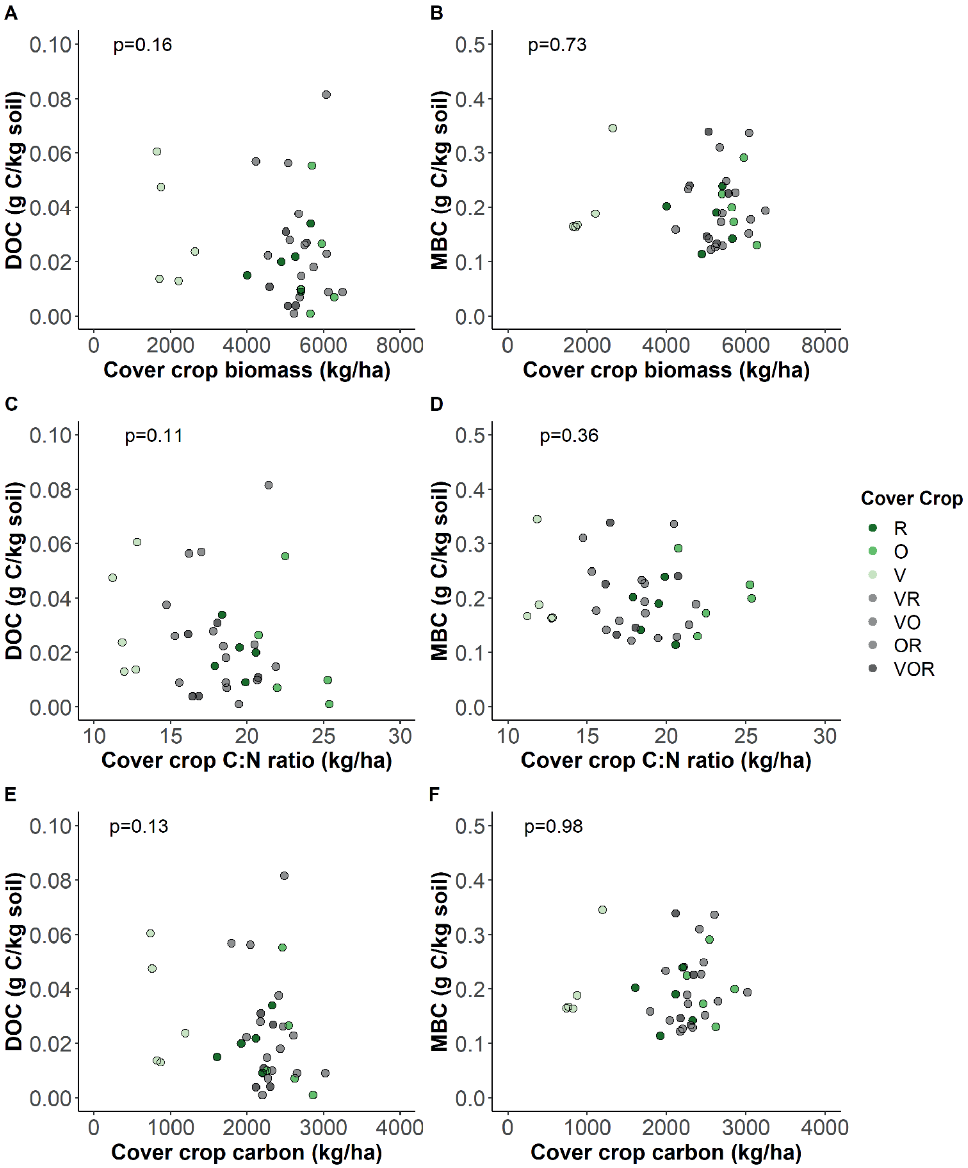

Figure S6.2 The relationship between the cover crop biomass and A) dissolved organic carbon and B) microbial biomass carbon. The relationship between the cover crop C:N ratio and C) DOC and D) MBC. The relationship of C added by the cover crops and E) DOC and F) MBC. DOC and MBC were measured in 2020. Cover crop parameters are related to the total biomass (above- and belowground) and are averages over the 4 years (2016-2019). The colours indicate the different cover crops: R (radish), O (oat), V (vetch) and their combinations. None of the relationships were significant ($p < 0.05$).

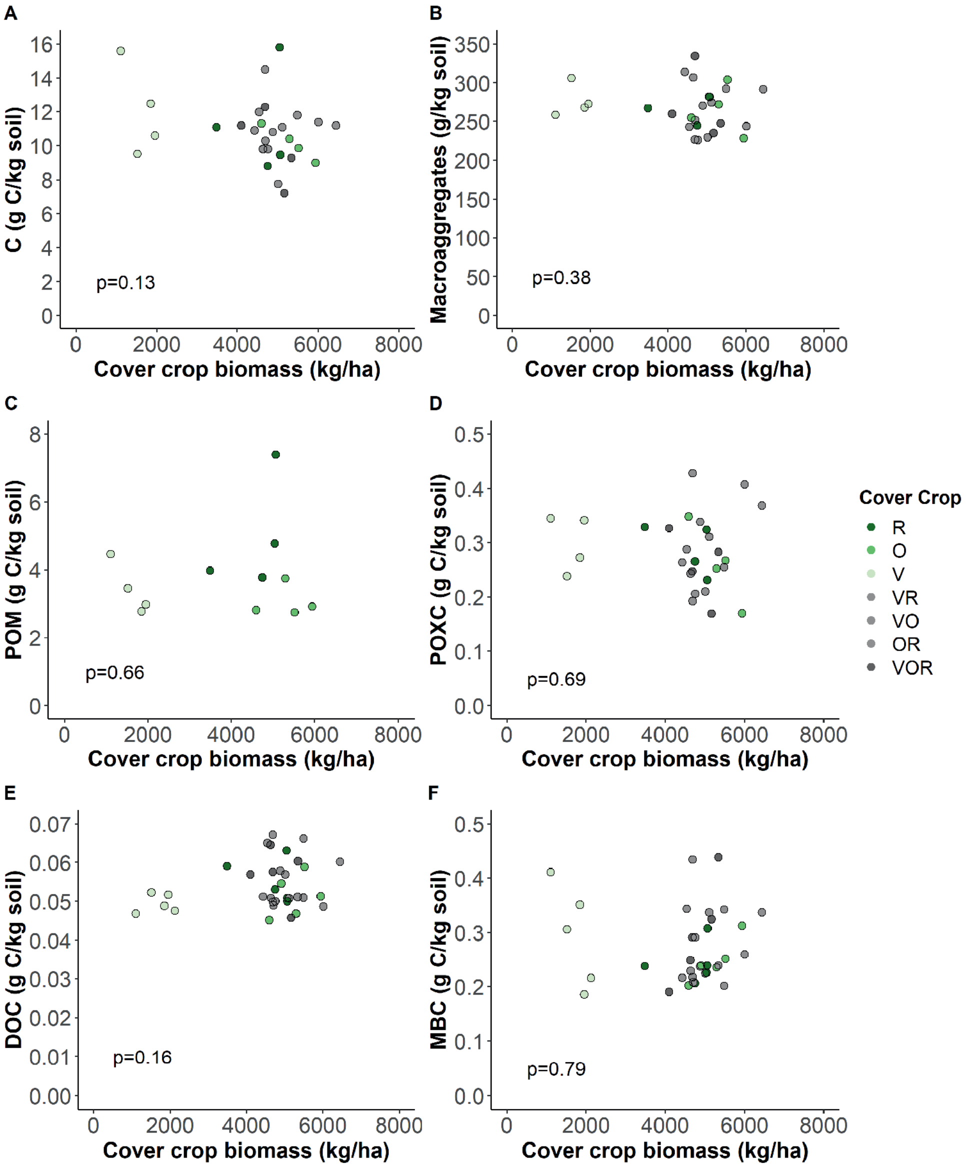

Figure S6.3 The relationship between the cover crop biomass (above and belowground combined) and the A) total carbon, B) amount of macroaggregates, C) particulate organic matter, D) permanganate oxidisable organic matter, E) dissolved organic carbon and F) microbial biomass carbon measured in 2019. Cover crop parameters are related to the total biomass (above- and belowground) and are averages over the 3 years (2016-2018). The colours indicate the different cover crops: R (radish), O (oat), V (vetch) and their combinations. None of the relationships were significant ($p < 0.05$).

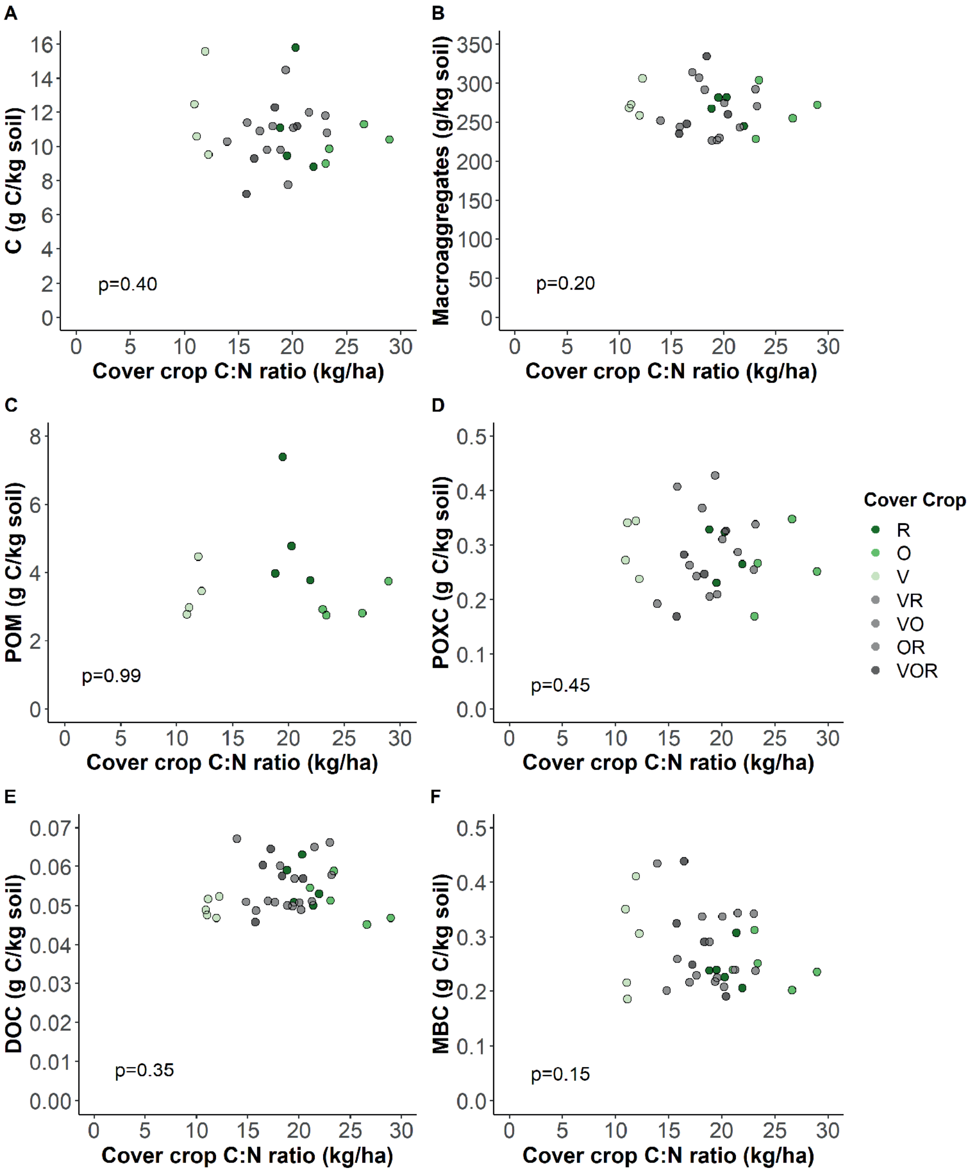

Figure S6.4 The relationship between the cover crop C:N ratio and the A) total organic carbon, B) amount of macroaggregates, C) particulate organic matter, D) permanganate oxidisable organic matter, E) dissolved organic carbon and F) microbial biomass carbon measured in 2019. Cover crop parameters are related to the total biomass (above- and belowground) and are averages over the 3 years (2016-2018). The colours indicate the different cover crops: R (radish), O (oat), V (vetch) and their combinations. None of the relationships were significant ($p < 0.05$).

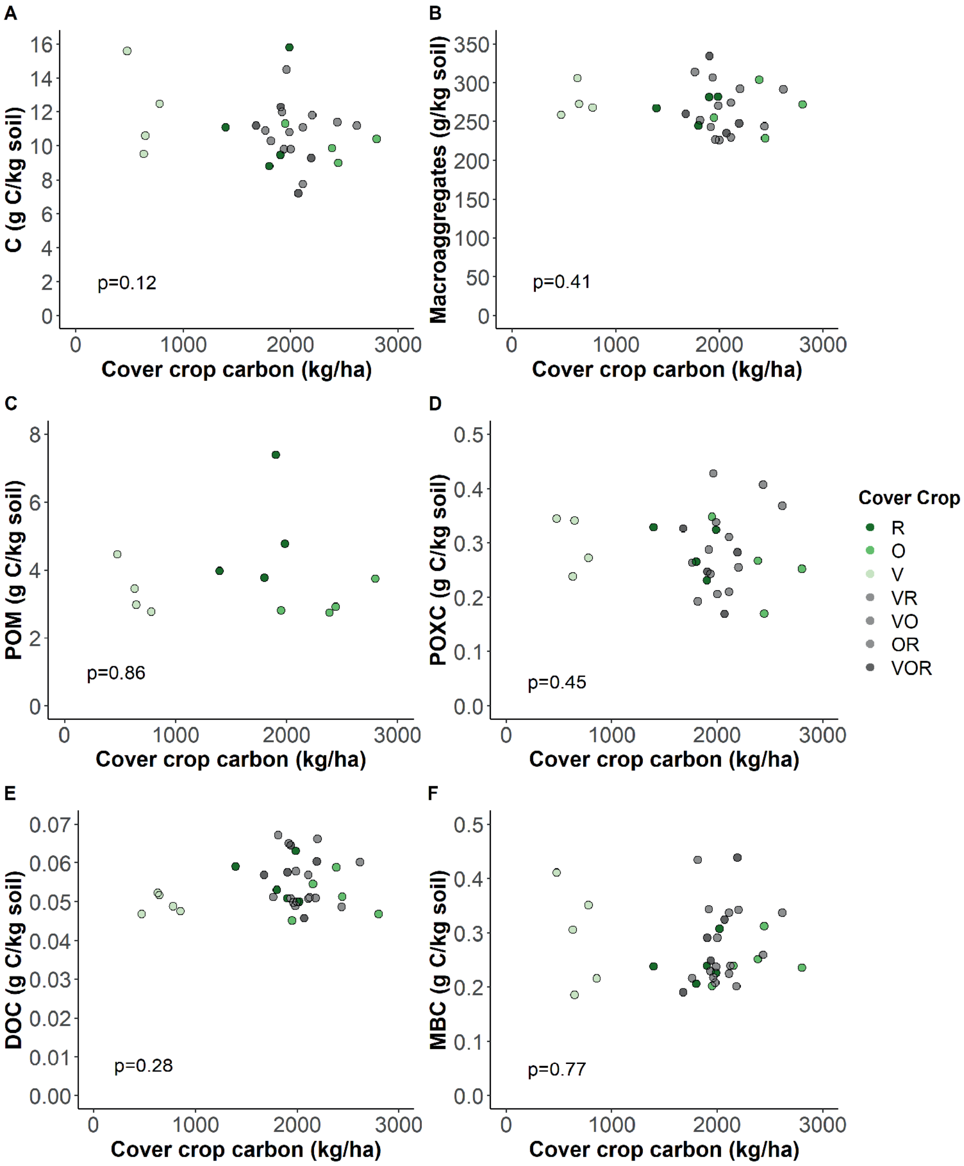

Figure S6.5 The relationship between the cover crop carbon (above- and belowground C combined, averaged over 2016-2018) and the A) total organic carbon, B) amount of macroaggregates, C) particulate organic matter, D) permanganate oxidisable organic matter, E) dissolved organic carbon and F) microbial biomass carbon measured in 2019. Cover crop parameters are related to the total biomass (above- and belowground) and are averages over the 3 years (2016-2018). The colours indicate the different cover crops: R (radish), O (oat), V (vetch) and their combinations. None of the relationships were significant ($p < 0.05$).

Chapter 7

General discussion

Rima J Porre

7.1 Introduction

Cover crops have been shown to be beneficial for many ecosystem functions such as nutrient leaching prevention (Francis et al., 1998), erosion control (De Baets et al., 2011), improving soil structure (Blanco-Canqui et al., 2015), soil organic carbon (SOC) accumulation (Jian et al., 2020), weed and disease suppression (Brust et al., 2011a), and promoting biodiversity. It is thus not surprising that growing cover crops has been promoted, however, it remains unclear which cover crops are best at promoting different functions and whether species mixtures of cover crops could combine the beneficial properties of individual cover crop species. Cover crops provide two main functions related to the C and N cycle. They function as 1) catch crops; crops planted after the cash crop take up any remaining nutrients from the soil and prevent N leaching and as 2) green manures; after incorporation of the crop biomass into the soil mineral N is released upon mineralisation of the cover crop residues and can be taken up by the next cash crop, and SOC levels are increased. Cover crops thus affect carbon and nutrient cycling during their growth as well as through legacy effects after their growth. From natural systems it is known that species mixtures could be beneficial for promoting yield and yield stability (Cardinale et al., 2007). Therefore, cover crop mixtures are potentially an important tool to further increase the efficiency in agroecosystems of nutrient cycling and carbon accumulation during their growth. However, at least as important are the legacy effects cover crop mixtures provide to the growth and nutrient uptake of the cash crop. Similarly, on the longer-term, soil carbon accumulation is important to evaluate if mixing plant species results in carbon accumulation as compared to impacts of single species.

In my thesis I aimed to answer the question: “Do cover crop mixtures improve element cycling in an agricultural rotation?”. Here I divide the research in this thesis in three sections; during cover crop growth, after cover crop incorporation and long term effects (Fig 7.1).

During cover crop growth: based on the hypothesis that cover crop mixtures exhibit transgressive overyielding due to resource complementarity (**1**) I expected that cover crop mixtures produce more biomass and take up more N and thus are more effective at reducing N leaching during growth compared to single cover crops (**2**) and, I expected that fertilising non leguminous cover crops with N will kick start cover crop growth and thereby reduce N leaching, whereas fertilising leguminous cover crops will increase N leaching (**3**).

After cover crop incorporation: I expected that mixtures that accumulated most biomass with a low C:N ratio during their growth, result in higher amounts of N leached after cover crop incorporation (**2**). However, mixed residues/litters are often hypothesised to interact during decomposition. This interaction often leads to faster decomposition than expected from the single litters (Gartner and Cardon, 2004). Therefore, I examined if chemical litter dissimilarity in leaf litter mixtures will cause larger non-additive mass loss in litter mixtures compared to litter mixtures of species with high trait similarity (**4**). When only the quality of residues is considered I expected that single cover crop species that have a low C:N ratio might decompose very quickly and thus risk losses of N. Whereas cover crops with a high C:N ratio might cause immobilisation and thus might hamper cash crop growth. Therefore I expected that mixtures of cover crop residues of different qualities will simultaneously provide N for plant growth and will limit N_2O emissions, relative to soil with residues of the individual species (**5**).

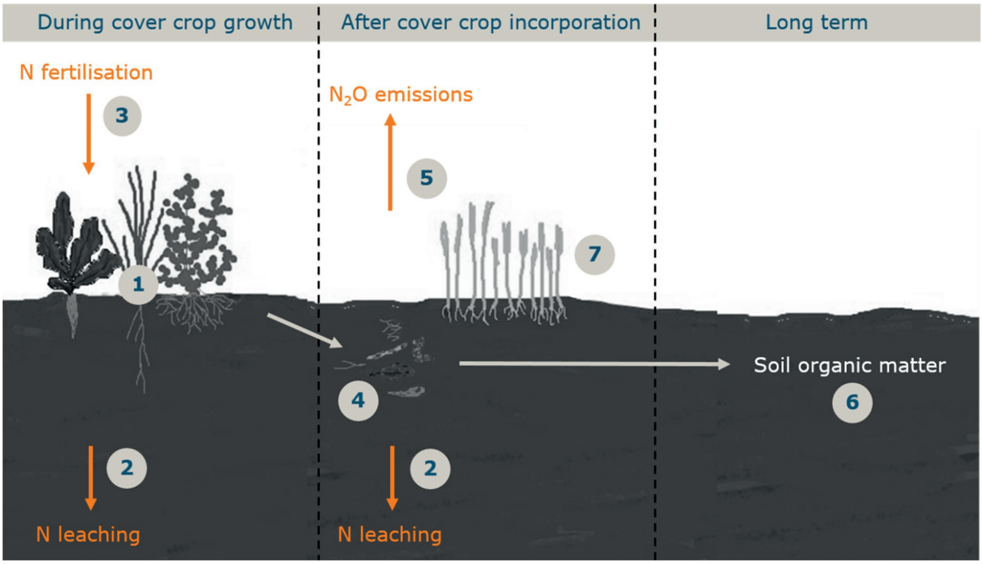

Figure 7.1 Diagram of the processes examined during cover crop growth, after cover crop incorporation and how this affected long term soil organic matter storage. Numbers indicate the different processes I reflect on in this thesis.

Long term: SOM can be increased in soil when biomass is added to the soil. The accrual of SOM in soil is dependent on the biomass quantity, quality as well as the soil type and management practices. I hypothesised that, in a sandy tilled soil, cover crop (mixtures) increase labile and stable soil organic carbon (**6**). And finally I expected that cover crop mixtures increased cash crop yield (quality) as well as the agronomic N efficiency (**7**).

In this general discussion I reflect on my main hypotheses and the results obtained and the implications of my results for the role of cover crop mixtures in promoting nitrogen use efficiency/retention and carbon accumulation in agroecosystems. First, in section 7.2, I will discuss to which extent the theoretical expectation of increased yield of species mixtures hold in my field experiment making use of concepts from (agro)ecology and biodiversity-ecosystem functioning research. Subsequently in section 7.3 to 7.6 I will discuss how the performance of mixtures affected the three processes mentioned above to pinpoint potential trade-offs and synergies between the different impacts of the cover crops over time (i.e. during their growth and via their legacy effects). Since for economic, societal and environmental reasons cover crop management should not negatively impact cash crop performance, I will discuss how cover crop mixtures affected cash crop growth and cash crop (N) yield (section 7.7). In Section 7.8 I will evaluate how my research findings can contribute to play a role in solving some of the mayor issues in sustainable agriculture and finally, I will give directions for future research.

7.2 Cover crop mixture growth

7.2.1 Overyielding and transgressive overyielding

In natural systems species mixtures often overyield or even exhibit transgressive overyielding (Cardinale et al., 2007). Overyielding occurs when cover crop mixtures produce a higher biomass than the average biomass of the respective monocrops whereas transgressive overyielding signifies that the mixtures produced a higher biomass than the most productive monocrop (Hector, 2006). Recent studies have indeed shown that overyielding (Finney et al., 2016; Florence et al., 2019; Smith et al., 2014) and sometimes transgressive overyielding (Wendling et al., 2017) occurs in cover crop mixtures. This is especially suggested for plant mixtures that have complementary growing strategies. In this thesis I used cover crops from three families; Fabaceae (vetch), Poaceae (oat) and Brassicaceae (radish) to create the mixtures. These cover crops are complementary in their growing strategies in multiple ways. The vetch can fix atmospheric N_2 and thus can facilitate growth of oat or radish when grown together (Thorup-Kristensen et al., 2003). These three species have different rooting patters, a thick taproot of radish with many fine root hairs, fibrous roots of oat and shallow thicker roots if vetch (Burr-Hersey et al., 2017). Therefore complementarity in terms of special niche differentiation at depth was expected.

In chapter 2 I showed that mixtures in my field, on average produced a similar biomass as radish, often the most productive monoculture. None of the mixtures exhibited transgressive overyielding which is in accordance with most studies on cover crop mixtures. Possibly cover crop species are not complementary enough, they are all fast growing species with a relatively low C:N ratio. Or the time of growth is too short in order for complementary growing strategies to take effect (Finn et al., 2013). Indeed, in intercropping systems overyielding is mostly caused by the complementarity effect (Li et al., 2020) due to temporal niche differentiation (Yu et al., 2015). This temporal niche differentiation is absent in cover crop mixtures. Additionally the system rich in N (due to fertilisation), previous studies have indicated that systems with nutrient deficiencies more often result in transgressive overyielding (Wendling et al., 2017). As expected we found overyielding in the legume and non-legume mixtures, the mixture of vetch + radish overyielded in three of the years and vetch + oat in two of the years (Fig 7.2). This is possibly related to the facilitation caused by vetch in terms of N availability (Weidlich et al., 2018; White et al., 2017). Moreover, radish in monoculture is subjected to a high amount of intraspecific competition. Radish mixed with vetch, which is less competitive, thus reduces the intraspecific competition allowing radish to produce a similar amount or even more in mixtures compared to when it is grown alone (Wendling et al., 2017; Wortman et al., 2012). Indeed, this is what I found in the mixtures of radish and vetch, in all years radish in the mixture produced a higher amount than expected from the monoculture (Fig 7.2). It is not surprising that the oat + radish mixture did not overyield since these have similarly high N demand, thus competing for the same resources. And, not only N plays a role, also light interception could have been important in terms of complementarity (Evers et al., 2019). Although Elhakeem et al. (2019) showed that a mixture of radish and oat could perform better when sown in alternate rows (reduced competition for light), this effect was not consistent over the years. Competition for light is thus not the most important driving factor that affects the mixtures performance of radish and oat. In our study the three species mixture did not exhibit overyielding in any of the years. Perhaps the inter- and intra- specific competition of oat and radish is not reduced enough in the three species mixture since vetch only represents a small amount of the biomass (Fig 7.2). This is further corroborated with studies using species mixtures with a diversity higher than two, here overyielding is less often found, especially on the short term (Cardinale et al., 2007; Isbell et al., 2011). I would thus argue that more species in the mixture might only be relevant if the additional species add more in terms of complementarity, or the seeding rate of the different species should be altered in order to reduce competition. This needs to be tested further.

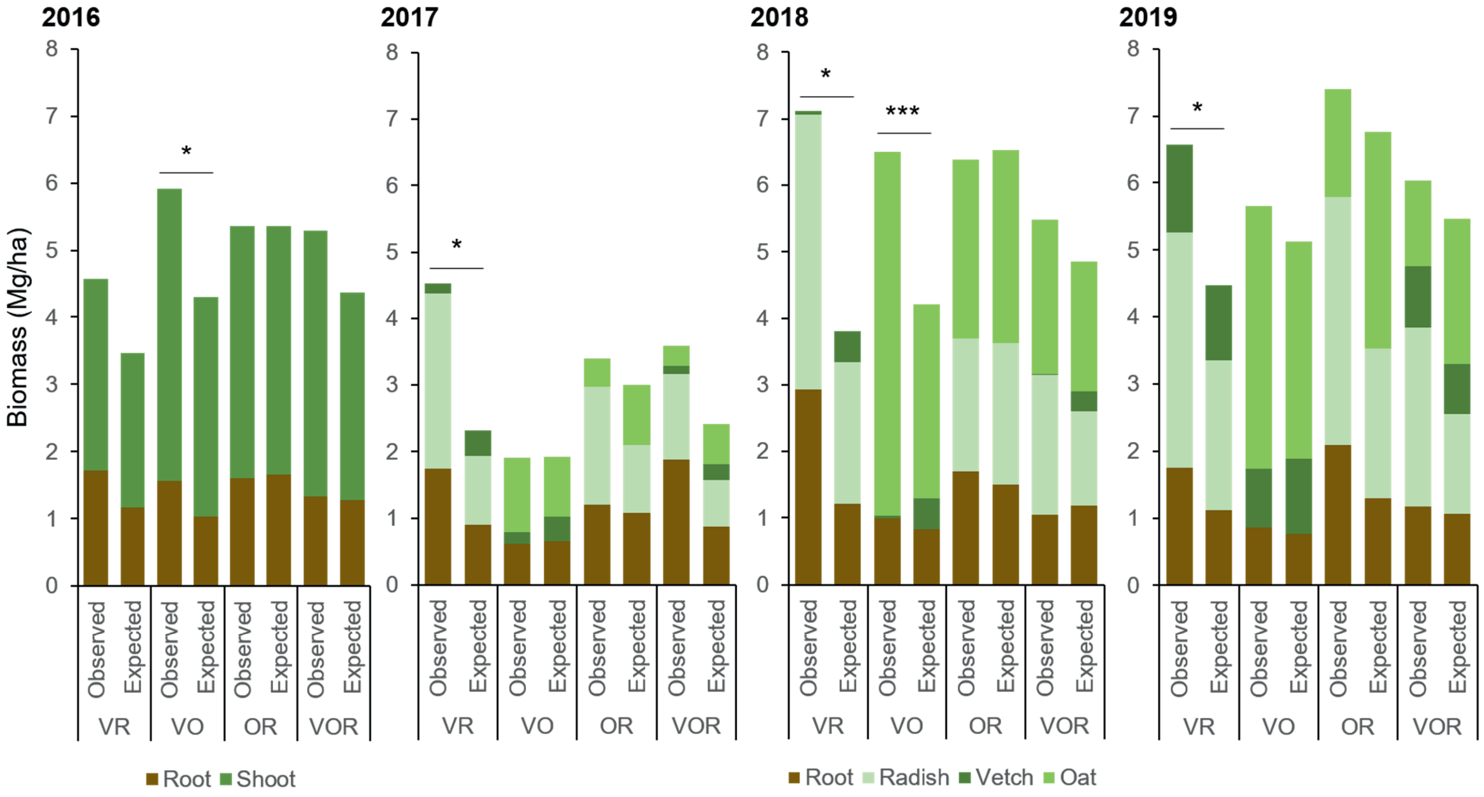

Figure 7.2 Observed and expected total cover crop biomass of the mixtures in 2016-2019. Expected biomass of the mixtures is calculated as the average biomass of the corresponding monocultures, based on seeding rates of 50-50% in the 2- species mixtures and 33-33-33% in the 3-species mixture (n = 5). Colours in 2016 represent the shoot and root biomass (aboveground biomass was not separated according to species that year). Colours in 2017-2019 represent root biomass (brown) and the aboveground biomass separated by species (green). Root biomass was not separated by species in any of the years. Significant differences are indicated by * ($p < 0.05$) and *** ($p < 0.001$).

7.2.2 *Seeding density in the mixtures*

In this study I sowed the species mixtures according to a replacement design (De Wit, 1960; Schmid et al., 2008), i.e. the species in mixtures were sown at a reduced seeding rate compared to the monocultures of 50% in the two-species mixture and 33% in the three-species mixture. As mentioned in the previous paragraph, this design will reduce intraspecific competition in species, thus likely giving a yield advantage to radish. Based on this, uneven ratios between vetch and the radish and oat biomass were expected. Yet, as can be seen in figure 7.2, observed biomass of vetch was even lower than expected (especially in 2018). This is likely caused by the N fertilisation (30 kg/ha), previous studies have shown that fertilisation mainly favours the non-legume species (Möller et al., 2008). Indeed in Chapter 3 (data not shown), although the presence of vetch was low the three species mixture, I did see that the amount of vetch in the mixture declined even further at higher fertilisation levels. Perhaps vetch is such a poor competitor and other legumes would result in better performance of a legume - non legume mixture. This leaves two questions 1) If seeding rates would be altered, will this improve the competitive strength of vetch in the mixture? I expect not, radish and oat have quick initial growth and vetch does not, thus vetch will probably be outcompeted. And 2) Is it desirable to have a more even ratio between vetch and oat or radish? I would propose that even ratios between the legume and non-legume might decrease the benefits that our mixtures now had in terms of N leaching reduction (see section 7.3) as well as for supplying N to the following cash crop (section 7.7). I would argue that the overall yield might be lower if more vetch is more dominant in the mixture, thus a lower N supply to the soil and cash crop can be expected. Additionally N leaching would most likely increase since less N would be taken up from the system.

7.2.3 *Year to year variation*

Year to year variation in cover crop (mixture) biomass may be explained by several main factors, namely cover crop sowing date, temperature, rainfall, light as well as N availability. This year to year variation is often found in studies on plant performance. In the biodiversity experiments on grasslands it has been shown that monocultures show the largest year to year variation (Finn et al., 2013). Similar results were found in other studies of cover crops (Thomsen and Hansen, 2014). However a recent study by Elhakeem et al. (2020) (currently under review) showed that year to year variability was not reduced in mixtures compared to monocultures. Similarly I found that the biomass of mixtures was not consistent over the years. In 2017, when the sowing was at the end of August, biomass of all cover crops was lower than in the other 2 years (when sowing was 2 weeks earlier). Therefore studies should always be performed on multiple years in order to quantify the effect of mixtures (Finn et al., 2013). Biomass of the vetch + oat mixture was especially low in 2017 (~1.8 Mg/ha). It is likely that the late sowing and thus reduced growth degree days and daylight affected the competition between vetch and oat. This is in close relation with the temperature and sunlight hours which are still relatively high in August, but quickly decline in September. Thus cover crop sowing date is important when considering the efficiency of cover crops in terms of N leaching reduction, green manuring potential as well as weed and disease suppression.

7.2.4. N yield and C:N ratio

In the previous sections I solely focused on cover crop biomass. Not only biomass but also N yield is important when considering cover crop effects on the C and N cycling in soil. A high N yield can mean that less N is lost from the system during cover crop growth, and that more N is available for mineralisation in spring after cover crop incorporation. Additionally the quality (C:N ratio) of the biomass can play a role in the rate of nutrient release in spring (section 7.5) as well as in C storage (section 7.6). The N yield in mixtures is generally expected to follow the same trend as the biomass of mixtures (Blesh et al., 2019; Couëdel et al., 2018) which is corroborated with my findings; the N yield (the total amount of N in the cover crop biomass) in my study followed a similar pattern as the biomass (Chapter 2). It has been shown in natural grasslands that species in the mixture without legumes can have a plastic response in terms of C:N ratio (Van Ruijven and Berendse, 2005), i.e. when N is lacking the C:N ratio of the high N demanding species increases and it decreases when N is plentiful. Only for oat did the C:N ratio vary over the years, the C:N ratio was lower in 2017 compared to 2018 and 2019. Thus, in years where oat had a high biomass it also had a higher C:N ratio, indicating that it may have invested more in shoot growth compared to leaf growth (Van Ruijven and Berendse, 2005). The C:N ratio of the species did not change consistently when it was grown alone or grown in mixture (Fig 7.3). Thus there was not a strong change in quality of the biomass in mixtures, and therefore this will not likely have a strong effect of mineralisation rates after cover crop incorporation.

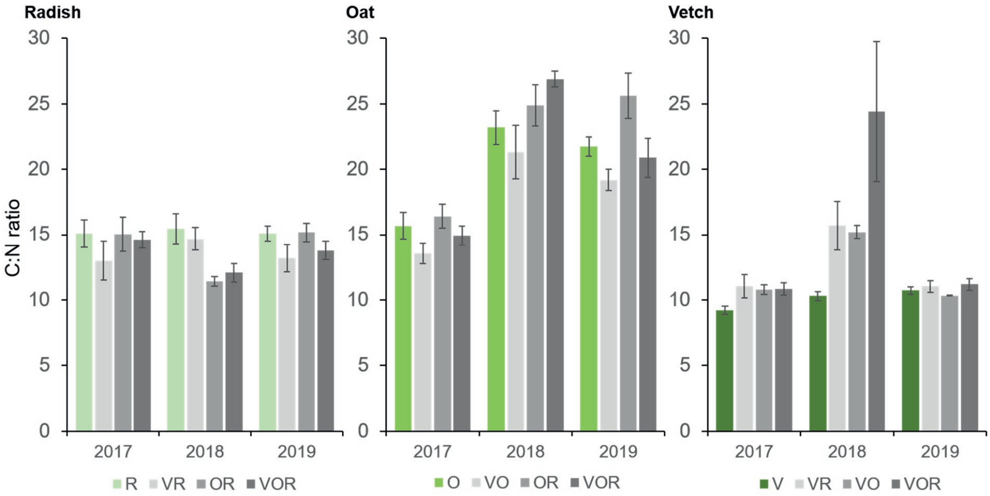

Figure 7.3 The C:N ratio measured in the (aboveground biomass) of the different species in monocrops and in mixtures. Error bars show the standard error (n = 5)

7.3 Leaching reduction by cover crop mixtures

7.3.1 during cover crop growth

Single cover crops have commonly been reported to reduce N leaching (Abdalla et al., 2019; Thapa et al., 2018). As mixtures are hypothesised to overyield compared to single species they are often expected to reduce leaching equally well as single cover crops or even more (Kaye et al., 2019; White et al., 2017). In the second chapter of my thesis I showed that cover crop mixtures generally have a similar biomass as oat or radish (the best performing mono cultures in terms of biomass) and they similarly reduced N leaching during their growth. Generally N leaching during cover crop growth was lowest under the radish monocrop and the oat + radish mixture, although all cover crop mixtures containing radish were as effective as radish grown alone at reducing N leaching. My results corroborate findings by Couëdel et al. (2018) who showed that crucifer-legume mixtures were as effective as crucifers grown alone in reducing N leaching. (Couëdel et al., 2018). In my experiment N leaching was negatively correlated to total cover crop biomass as well as to root biomass. Above a total biomass threshold of 3 or 4 Mg/ha (depending on the year) leaching was reduced to almost zero. Similarly, Finney et al. (2016) reported a threshold biomass of 7 Mg/ha above which no more N was leached. I propose that cover crop productivity is the main driver of N leaching reduction up to a threshold productivity level and I expect that this threshold depends on soil mineral N levels at the start of the cover crop season, the amount of cash crop residues present, N fertilisation and the amount of rainfall. Not only the biomass reached, also the rate at which the soil is covered is important. Ground cover is related to leaching because it reduces the total amount of water leached (Gabriel et al., 2012), radish has an especially quick initial growth and ground cover (Chapter 2) which results in lower N leaching. I propose that quick initial growth it crucial for leachate reduction, since N left in the soil after the cash crop and (possible) additional fertiliser are likely to leach most at the end of summer when temperatures and thus mineralisation rates in soil are still high.

7.3.2 After cover crop incorporation

Bi-culture cover crop mixtures of legumes and non-legumes are often suggested to be effective at reducing N leaching (which I indeed found, as discussed in the previous paragraphs) as well as supplying N to the soil after cover crop incorporation (White et al., 2017). Mixtures with legumes generally obtain a similar yield to non-legumes grown as monocrop since the presence of legumes can facilitate the growth of the non-legume (Weidlich et al., 2018). The total amount of N in the mixture biomass is expected to be larger due to the extra N obtained through biological N fixation of the legume (Andersen et al., 2014; Couëdel et al., 2018; Weidlich et al., 2018; White et al., 2017). I expected that this increased supply of N to the soil and following cash crop would increase the risk of N leaching, since biomass with a low C:N ratio is expected to mineralise fast (Bradford et al., 2016) and thus release N before the cash crop can take it up. Contrary to what I expected, cover crops that took up most N in their biomass during their growth did not cause more N leaching during cash crop growth. However, the earliest measurements of leaching I took were in May, and cover crops were incorporated into the soil in March. The soil analysis in March indicated that cover crops with a high N yield resulted in higher soil mineral N (Chapter 2). Since soil mineral N is positively correlated to N leaching it is likely that the risk of N leaching was higher in these treatments just after cover crop incorporation. Due to practical constraints in field preparations for planting the cash crops and the in situ tools required to collect leachates the measurements of leaching in my field experiment were less frequent than desired. This was especially the case during the spring when cover crops were incorporated into the soil and the field was prepared for cash crop sowing/planting. Therefore, in order to test how cover crop (mixtures) affect N leaching in spring I suggest to measure N leaching continuously throughout the year.

7.3.3 Caveats and potential solutions

Considering temporal variation of cover crop growth and decomposition I refrained from extrapolation of N leaching beyond the days on which soil pore water was collected. Thus I may have missed differences between crops that now remain undetected. Although the methods used in this thesis give a relatively straightforward way to collect soil pore water, accurate estimations of N leaching losses are based on the concentration of N in the pore water and modelled water flows (Vogeler et al., 2020). Additionally the ceramic suction cup method is most challenging in sandy soil since significant rainfall is needed in order to obtain a large enough sample for analysis (Zotarelli et al., 2007). Moreover, ceramic suction cups had to be removed when the soil is ploughed and prepared for the following crop. Thus, the biggest gap in our dataset were leaching losses that were not measured in the month after cover crop incorporation. Although there are downsides to the method used in this thesis, I am confident that we have been able to accurately portray differences between cover crop treatments on the days of measurements. A useful addition to our measurements could have been the use of resin bags (Finney et al., 2016). Resin bags are buried below the soil surface at a depth of ~30 cm, subsequently they are left in the field for months and after collection cumulative NO_3^- leaching can be quantified. The downside of this method, and why we choose to use porous suction cups, is that multiple holes need to be dug in the soil before sowing the cover crops and twice more throughout the year. I considered this to give too much soil disturbance, in terms of soil structure and water flow, in our field because it was a multi-year experiment.

I conclude from my study that high biomass producing cover crop (mixtures) are most effective at reducing N leaching during their growth. Since mixtures of radish with vetch generally produced the highest biomass it is as effective as monocultures oat or radish in N leaching reduction. This mixture generally has a higher N content and therefore also has a higher potential for mineral N supply to the cash crop upon mineralisation in the soil of the incorporated cover crops. This mixture does not only have a high N content, yet also residues of different quality, in section 7.5 I will discuss how this can affect decomposition and mineralisation rates and potentially affect losses of N to the environment. Based on my results and previous studies I would recommend using mixtures of a legume and a crucifer for improving both N retention in autumn and N supply in spring. On the longer term differences in cover crop biomass quality can also be relevant from the perspective of soil carbon sequestration potential (see 7.6).

7.4 N fertilisation of cover crops

Farmers across Europe and in the Netherlands are advised to fertilise cover crops with N to kick-start the growth (Smit et al., 2019; Van Leeuwen-Haagsma et al., 2019). However, this advice is not based on any scientific studies. In order to improve nutrient efficiency and reduce risks of N loss to the environment, the impact of N fertilisation of the cover crops warranted investigation. Plant growth responses of monocultures and mixtures to different levels of N application can provide some insight into the role of N availability in overyielding of mixtures. Previous studies indicated overyielding occurs more often when there is a lower availability of N (Hooper et al., 2005). Thus I expected that the three-species mixture showed higher overyielding when 0 kg N/ha was applied compared to the other fertilisation levels. Yet this is not what I found, the mixture performed similarly at all fertilisation levels compared to the monocrops (Chapter 3). Considering the time at which cover crops grow, when daylight hours and temperature declines in autumn, these resources are possibly more limiting that the N availability in soil (Evers et al., 2019). Additionally, in I hypothesised that N fertilisation increases biomass production of radish and oat, which have a high N demand. However I found that the effect of fertilising cover crops with N on biomass was minor and only observed for radish in one of the 2 years (2018). In this year, N uptake of radish and oat increased with N fertilisation. Here we saw oat had a lower C:N ratio at higher fertilisation levels, confirming that the cover crops quality can change according to nutrient availability (Van Ruijven and Berendse, 2005). However, the recovered amounts of N were less than the applied amounts, indicating that at higher fertilisation levels, higher losses of N are expected (N leaching or possibly N_2O emissions). I conclude that application of N fertiliser to cover crops growing on sandy soils does not improve N capture service. Most likely there was enough N present in the soil and light or temperature, not N, was the factor limiting cover crop growth.

In all four years we fertilised the cover crops with 30 kg N/ha in the long term field experiment. Yet, here I have shown that N fertilisation is not necessary in order to kick-start the cover crop growth, at least not in the soils we used. This suggests that cover crop use can become more sustainable.

7.5 Mixed litter decomposition and mineralisation

7.5.1 litter mixture decomposition

It has often been shown that litter (plant) mixtures can decompose faster than expected from their respective monoculture (synergistic mass loss) (Gartner and Cardon, 2004). Most commonly it is hypothesised litter trait differentiation causes synergistic mass loss (Handa et al., 2014; Pérez Harguindeguy et al., 2008). The N transfer hypothesis is most commonly mentioned as potential explaining mechanism (Hättenschwiler et al., 2005). However, in chapter 4 I showed that mass loss of mixed litters is equal to the weighted average of the mass loss of the constituent single litters in the majority of litter mixing studies and on average. I also showed that a larger litter trait diversity did not relate to synergistic litter decomposition. The effects of litter mixing are in many cases predictable from the decomposition rates of the individual species. However, I also found cases of synergistic and antagonistic mixture effects and concluded that any of these interactive effects (positive or negative) between litter species are contextual, and cannot be generalised and predicted beyond the context in which the results were obtained. Thus, to truly predict litter mixing effects, all parameters involved would need to be taken into account (Fig 7.4). This does not only involve chemical traits of litter, but also physical traits. Moreover interactions between plant, soil (biotic and abiotic properties) and (micro) climate exist. i.e. when a several plant species grow together (instead of a monoculture) the plants can affect each other in terms of quality, they can affect the microclimate (more shading) as well as the soil quality, such as an increased microbial diversity. These properties in turn then affect the decomposition of the litter mixture. Thus I propose it is nearly impossible to predict litter mixture effects, other than the weighted average mass loss of the constituent species, based on the principle that a small change in the initial scenario, could cascade through the system, eventually changing the outcome of the direction of the litter mixing effect.

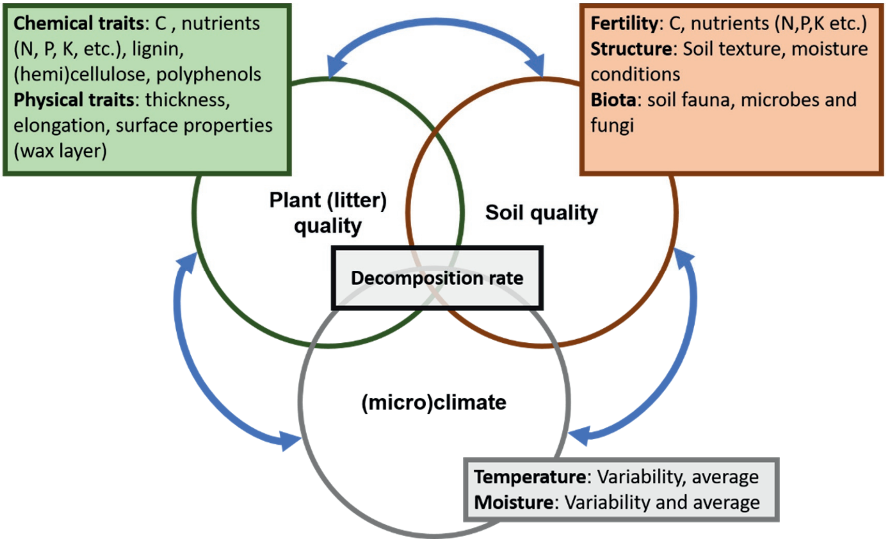

Figure 7.4 Conceptual diagram of controls of litter mixture decomposition. Blue arrows indicate interactions between plant and soil and climate.

These results as shown in chapter 4 are obtained from a meta-analysis on litterbag studies. Thus, it does revolve around a slightly artificial decomposition environment. I can imagine that litters decomposing directly in contact with soil will have a different microclimate and the litters are in direct contact with each other, which might not be the case when the litters are loosely present in the soil or litter layer. I do not think that this would favour either antagonistic or synergistic mass loss. More likely it would dampen mixture effects.

7.5.2 Cover crop mixture decomposition

The results from this meta-analysis can be translated into cover crop decomposition. Based on chapter 4 I would not expect any synergistic mixing effects when residues are decomposed in mixtures as compared to single species residues. In the field the cover crop monocultures and mixtures differ both in their quantity and quality of biomass that is returned to the soil upon their incorporation. Moreover, also the soil microbial community under the different cover crops can diverge (Barel et al. 2019). Therefore, to specifically test the impact of cover crop quality differences between the cover crop treatments on decomposition and mineralisation I established a lab incubation in which I used field soil from the fallow plots and standardised the quantity of fresh litter added to the soil. I incubated cover crop species (collected from my field experiment) separately and in mixtures, additionally I incubated root and shoot residues of the cover crops separately and in mixtures on a sandy soil. I measured CO_2 as a proxy for decomposition rate. Here, also no synergistic litter mixing effects were found in cover crop species mixtures (Fig 7.5A) and neither in shoot + root mixtures (Fig 7.5B).

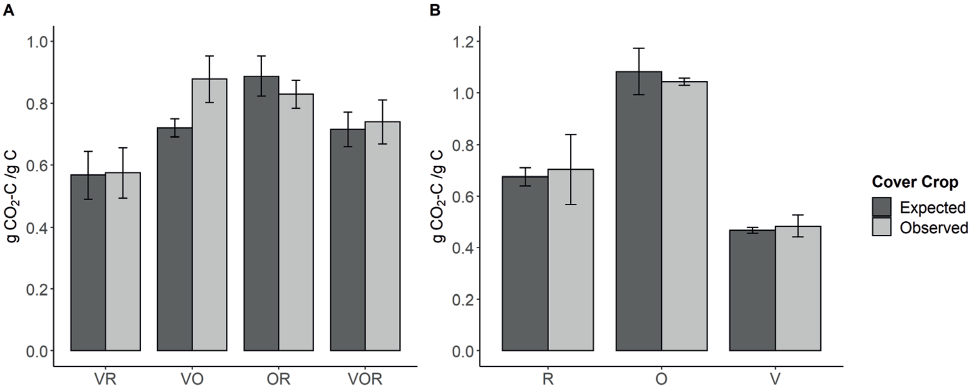

Figure 7.5 Cumulative CO_2 flux during a 16 week decomposition experiment with A) cover crop species mixtures and (1:1 and 1:1:1 ratios) B) shoot and root cover crop mixtures (1:1 ratio) added to sandy soil. The cover crops are (combinations of) radish (R), oat (O) and vetch (V). Light coloured bars are observed cumulative CO_2 of the mixture, dark bars are expected cumulative CO_2 calculated as the average of CO_2 measured when the A) species or B) shoot and root material decomposed alone (courtesy of Tsering Zam).

Again, decomposition rates can be predicted from individual component decomposing alone. Similarly, I established a greenhouse study (Chapter 5) in which I used field soil from the fallow plots and standardised the quantity of fresh litter (standardised according to dry weight equivalent) added to the soil. I incubated cover crop species separately and in mixtures, this time according to the species ratios in the mixtures as found in the field. Also here I did not see any interaction effects of mixtures on mineral N availability, CO_2 production and N_2O production. In part, it could have been that the cover crops were too similar in terms of residue quality (C:N ratio). Cover crops are fast growing species and thus have a similar chemical make-up. Yet, the meta-analysis indicated that litter trait dissimilarity (thus also cover crop quality) is not related to synergistic litter decomposition. Thus when matching decomposition rates and N-release to cash crop uptake we can expect average release rates of N compared to monocultures.

Since I focussed only on quality and not on the combination of quality and quantity (like in Chapter 2), here I expected cover crops with a low C:N ratio (radish and vetch) to increase soil mineral N soon after incorporation in the soil compared to the cover crop with a high C:N ratio (oat). I hypothesised that the timing of cover crop incorporation and cash crop planting is important in order to promote cash crop growth and to decrease losses of N to the environment (N_2O emissions in this chapter). Indeed, wheat planted shortly after oat had poor growth due to the low levels of soil mineral N whereas early planting after vetch and radish resulted in good wheat growth. Surprisingly N_2O emissions after incorporation of oat and vetch were similarly low, whereas they were much higher after radish. Thus we did see that, unexpectedly, mineralisation of vetch results in low N_2O emission and radish in very high emissions even though their impact on mineral N in soil was similar. The reason for this difference is likely due to the inherent quality difference between radish and vetch, or through the effect that these residues had on the soil environment. Given that N_2O emissions are tightly linked to the balance between the microbial communities that produce N_2O and those that consume N_2O (Bateman and Baggs, 2005; Hallin et al., 2018) in future studies it would be very interesting to explore the potential shifts in microbial community composition between the different cover crops.

Chapter 5 results are based on a greenhouse pot experiment. In this experiment I used the same (fallow) field soil in each pot and incubated the residues in the greenhouse with a relatively constant temperature and constant soil moisture content. In this greenhouse experiment I solely wanted to understand how quality of cover crop residues affected the mineralisation rates as well as interactions between mixtures. I suggest caution when extrapolating these results to the field. The interactions between plant-soil, plant-climate and climate-soil (Fig 7.4) are largely ignored. I expect that plant-climate interactions are similar since this study no longer focussed on growing plants. There may have been a small discrepancy in interactions between climate and soil, in the field rainfall fluctuates and temperatures will be lower, I expect especially rainfall could have affected N_2O emissions in a different way than what we saw in the lab. N_2O is emissions are largely controlled by soil moisture (Pimentel et al., 2015), thus when soil was temporally dry or wet could have affected the N_2O emissions. However, the interaction between plant and soil is in my opinion the largest oversight. Foremost is the importance of residue quantity in the field, which has been shown to be most important, next to the quality (Barel et al., 2018). Further, cover crops have been documented to leave a legacy in the soil (Barel et al., 2018; Barel et al., 2019). Cover crops changed not only the soil N content (Barel et al., 2018) but also the microbial community (Barel et al., 2019). Thus the plant legacy can affect decomposition rates of newly added litters, as was shown by Barel et al. (2019). This also relates to literature on home-field advantage where the soil is trained to decompose own plants grown on own soil better than residues grown elsewhere (Veen et al., 2015).

7.6 Labile and stable soil C

Cover crops have been shown previously to increase SOC (Jian et al., 2020; Poeplau and Don, 2015). Assuming absence of any synergistic mass loss (Chapters 4 and 5) I expected that cover crop (mixtures) with a larger biomass would cause higher increase in SOC. Moreover, since recent studies indicate that biomass that is easily decomposed increases the stable SOC pool (Cotrufo et al., 2013), I expected that cover crops with a low C:N ratio would cause a larger increase in stable SOC. I expected to see the largest differences in SOC change among the single species since they had the most diverse biomass and quality. Thus, I decided to do the extensive soil fractionation on the single cover crop treatments only. In chapter 6 I showed that the SOM content of the soil was only increased after 4 years of cover cropping with the vetch + radish and the vetch + oat + radish mixture. The other cover crop (combinations) did not change the SOM content. The SOM increased with 0.07 and 0.06% over a period of 4 years, which relates to an increase of 0.50 ± 0.17 Mg ha^{-1} y^{-1} and 0.44 ± 0.13 Mg ha^{-1} y^{-1} SOM for vetch + radish and vetch + oat + radish respectively. And, using the conventional conversion factor of 58% C within SOM (Pribyl, 2010) we get to 0.29 ± 0.10 Mg ha^{-1} y^{-1} and 0.26 ± 0.08 Mg ha^{-1} y^{-1} SOM. This is on the lower side of the 0.32 ± 0.08 Mg ha^{-1} y^{-1} SOC increase reported by Poeplau and Don (2015). However, considering the sandy soil in our tilled arable system I would have expected lower increases in SOC compared to the average reported in a study that includes other soil types as well.

Cover crop biomass and cover crop quality were not related to any of the soil C fractions (POM, MAOM, micro aggregate-C) measured in 2019. Either four years of cover cropping on a sandy soil is too short in order to observe increases in the stable soil C fractions. Or the quantity and quality of cover crops interfere in such a way that it does not matter if you add a high yielding, high C:N crop or a low yielding, low C:N crop.

Here I chose to study the effect of cover crops on a sandy soil, because these soils are prone to leaching losses and are generally low in OM. However, stable SOM build-up is more likely in soils with a finer texture. The high clay content in these soils results in a high potential of organic matter binding to minerals due to the large reactive surface area (Gulde et al., 2008; Haddix et al., 2020). Indeed I showed that the soil in my study was close to saturation in terms of binding to minerals. Thus, I expect that cover crop (mixture) effects are larger in clayey soils. Indeed the meta-analysis by Jian et al. (2020) showed that cover crops were more effective in SOM accrual in soils with a higher clay content. Since I found an increase in SOM for two of the mixtures (that generally produced a high biomass), it would be interesting to see if POM is increased for these mixtures. Considering the sandy soil, build-up of POM can be of higher importance than MAOM (Lavallee et al., 2020).

In our study we only considered the plow layer (0-20 cm depth) in terms of changes in soil C. Previous studies have indicated that C accumulation often occurs lower down (Rumpel and Kögel-Knabner, 2011). Therefore, to get a better insight in cover crop effects on C sequestration, I suggest the entire soil profile should be taken into account. Although the amount of SOM is usually higher in the top soil layer, the turnover of this layer is higher compared to soil deeper down (Fontaine et al., 2007). Carbon that is stored in deeper layers is more stable and thus might be of higher importance when considering C sequestration (Rumpel and Kögel-Knabner, 2011).

7.7 Cash crop performance

7.7.1 Cash crop (N) yield

Cover crops have been shown to accumulate nutrients in the biomass in the fall (chapter 2 and 3), and after incorporation in spring, these nutrients mineralise and become available in the soil and can be taken up by the following cash crop (chapter 5). Studies on single cover crops often state that cereals in an agricultural rotation can hamper cash crop growth (chapter 5) (Thapa et al., 2018), whereas vetch increases cash crop growth due to its quick N supply (Wagger et al., 1998). These studies often did not include any additional fertilisation of the cover crop and cash crop. However, in our study the cover crops received 30 kg N/ha and the cash crops received a 95 (maize), 110 (potato) and 60 (barley) kg N/ha each. These amounts applied to the cash crop were slightly lower than what was recommended by seed suppliers for growth on sandy soil. The cover crops, measured in November, at maximum standing biomass, had an average of 70 (2016), 140 (2017) and 160 kg N/ha (2018). Here we show that cover crops in the first year (2017) had no effect (compared to the fallow) on cash crop growth (biomass and N uptake; Fig 7.6).

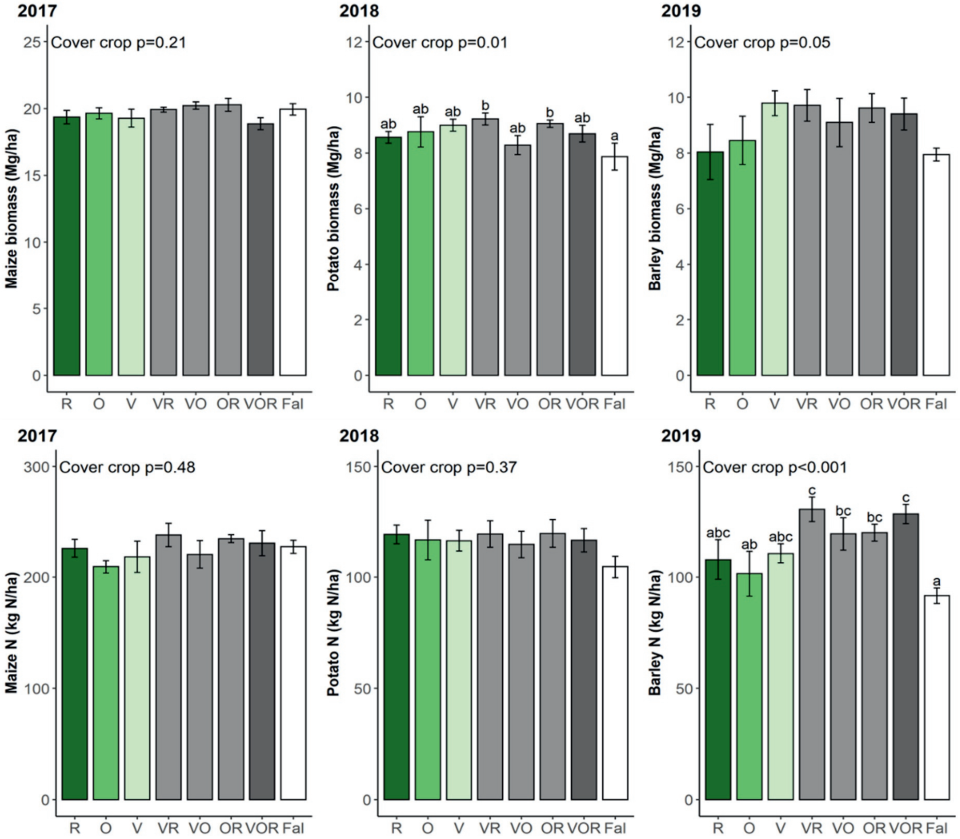

Figure 7.6 Biomass (above) and N uptake (below) of maize (2017, potato (2018) and barley (2019). The cover crops treatments are: R (radish), O (oat), V (vetch) and all their combinations. Bars are means, error bars denote the standard error (n = 5). P values refer to the level of significance. Bars with the same letter are not significantly different. *These data are obtained from the field experiment of the Clever Cover Cropping project, in cooperation with Sytske M. Drost and Ali Elhakeem.*

However, in 2018 two of the four mixtures caused a significant increase in potato biomass, and after three years of cover crops, all cover crop mixtures promoted N uptake by barley compared to the fallow, whereas the monocrops did not. The reason for these differences between monoculture and mixture cover crop legacies on barley may be the amount of soil mineral N after cover crop incorporation in March. This amount was was similar or higher in the mixtures compared to all three sole crops. This trend seemed to get stronger over the years (Chapter 2). Potentially mixtures, especially vetch + radish and the 3-species mixture, that have a high biomass production and increased the SOM content (Chapter 5) improved the soil fertility (nutrient content) over time. Of course, it could be argued that I planted a different cash crop each year. And specific interactions between the cover crop and following cash crop are possible. However it is striking that the time between cover crop incorporation and cash crop planting was largest in 2019 (45 days as opposed to 20-30 days in the other years) when effects of mixtures on the cash crop where strongest. Thus I do not expect that it is solely an effect of the biomass quality of that year, since cover crop biomass is generally quickly mineralised in the first few weeks after incorporation (Baggs et al., 2000). And solely based on the N content I would have expected oat to affect the cash crop similarly well as the 3-species mixture (Chapter 2).

7.8 Societal relevance & Outlook

To answer the main research question I posed in this thesis: “Do cleverly chosen cover crop mixtures, with diverse litter traits improve element cycling in an agricultural rotation?” I made an overview of the cover crop performance and the effect that each cover crop (mixture) had on the C and N properties that I measured in this thesis (Table 7.1). From this table I conclude that the mixture of vetch and radish performed best overall. The reason that vetch + radish performed well is most likely a combination of the lower intraspecific combination of radish in the mixture, combined with the facilitation caused by N_2 fixation of vetch.

I have also shown that, although the three species mixture performs well in terms of mineral N and SOM supply, it does not perform better than the two-species mixture. Surprisingly, as I mentioned in chapter 4 and 5, litter trait diversities did not play a role in determining the mixture effect on element cycling. Rather, the absolute amount of cover crop biomass and N yield were important, as can be seen in table 7.1. Additionally I showed that the root length density did not increase in the cover crop mixture and thus did not explain mixture effects on N leaching and biomass N uptake.

Table 7.1 Overview of the cover crop performance on N leaching, soil quality and the cash crop parameters. Cover crop biomass and N yield as well as N leaching and soil mineral N are averaged over the years. Root length density (RLD, only 2019), soil organic matter (SOM, only 2020) and cash crop biomass and N yield (only 2020) are data from the final year. Cover crop performances are indicated with a +/- when they are different compared to the averages across treatments or with a ++/-- when they are a minimum of 20% different from the average. Effect sizes on the soil are quantified in a similar fashion, although here they are compared to the fallow soil. Zeros indicate that there was no significant difference among the cover crops or from the fallow ($p < 0.05$).

	Cover crop production			**N leaching reduction**		**Soil**		**Cash crop**	
	Biomass	N yield	RLD	Cover crop season	Cash crop season	Mineral N	SOM	Biomass	N yield
Radish	+	-	0	++	0	++	0	0	+
Vetch	--	--	0	0	0	+	0	0	++
Oat	+	+	0	++	0	0	0	0	+
Radish + vetch	++	++	0	++	0	++	+	0	++
Vetch + oat	-	-	0	0	0	++	0	0	++
Radish + oat	+	-	0	++	0	++	0	0	++
Vetch + oat + radish	+	+	0	0	0	++	+	0	++

In the last decade a policy was set in place in the Netherlands (and other European countries) to increase biodiversity in agriculture by means of cover crop mixture (van Leeuwen-Haagsma et al., 2019, Smit et al., 2019). This thesis has shown that farmers that comply with these greening measures not only receive a subsidy, yet also can benefit in terms of soil fertility and cash crop performance.

7.8.1 Solving the N challenge

A part in solving the global reactive nitrogen challenge is increasing the N-use efficiency for food, fiber and fuel production (Houlton et al., 2019). Cover crop mixtures (including radish) are equally efficient as commonly used cover crops in reduction of N leaching. Therefore only based on the N cycle, I would recommend a mixture that involves both leguminous and non-leguminous plants for increasing N use efficiency in the agricultural rotation. This would serve to reduce N leaching losses and as a supply of N to the following cash crop (i.e. green manure) and would remove the negative effects that high C:N cover crops could have on hampering cash crop growth. In chapter 3 I showed that there are no consistent benefits in fertilising cover crops with N. Thus further reduction of N fertilisation is possible in agricultural rotations. By using cover crop mixtures, after a couple of years, N fertilisation demand of the cash crop might be further decreased compared to when monoculture cover crops are used.

7.8.2 C sequestration

In chapter 6 I showed that some of the cover crop mixtures increased SOM after 4 years of cover cropping. This indicates that on the longer time scales mixtures can be beneficial in promoting SOM. Studies on SOM accrual in soil often mention the trade-off between carbon sequestration and soil fertility (Janzen, 2006). In this thesis I could show that cover crop (mixtures) did not have this negative trade-off effect on cash crop growth. The reason for this may be that the species used are generally decomposing fast and the recent view that fast decomposition does not need to imply reduced C sequestration as it is thought that microbial metabolites, so an active microbial community and its turnover, play an important role in C sequestration (Cotrufo et al., 2013). Sandy soils have a lower saturation limit than clay soil due to the lower surface area for C binding (Six et al., 2002). Thus, cover crop mixtures will not be able to increase the carbon content to a great amount in sandy soil unless stable aggregates are formed. I suggest that further studies, with the aim of increasing C sequestration, should focus on soil types with a higher clay content.

7.8.3 Further research

This thesis focused solely on the C and N cycle and the cash crop performance. Based on the results of this thesis mixtures of a legume and a crucifer perform equally well as monoculture cover crops. And I showed that a higher cover crop biomass production is related to positive effects on N leaching and SOM increase. Further research could further focus on the mechanisms involved that caused high cover crop yields. I expect that the N fertilisation of cover crops prevented transgressive overyielding in the legume – non-legume mixture. Second, I suspect that the strong competition of oat and radish prevented overyielding in the three species mixture. This thesis focussed solely on three cover crop species, from three plant families often used as cover crops, and their mixtures on a sandy soil. Ideally more species should be tested, while varying the seeding rates to avoid intraspecific competition as well as dominance in mixtures.

My research, which showed that mixtures of legumes-non legumes are best in terms of N cycling and C sequestration is in close agreement with recent literature on cover crop mixtures. However, more studies are needed in order to assess if similar results are obtained on other soil types, especially to quantify the C sequestration potential. Additionally more diverse species mixtures would be ideal when considering the decline in biodiversity worldwide. Thus, research how multi-species mixtures affect yield stability and C and N cycling are recommended.

Cover crops are not only relevant in terms of C and N cycling. Often cover crops are incorporated in an agricultural rotation for other purposes such as weed suppression, erosion control, disease and nematode suppression. Similar to N leaching reduction, cover crop biomass is positively related to weed suppression (Finney and Kaye, 2017; Florence et al., 2019) and erosion control (Blanco-Canqui et al., 2015). However in terms of disease and nematode suppression other factors play a role such as cover crops that are not a host for specific plant parasitic nematodes (Scheepens et al., 2003). Additionally some cover crops such as brassicas exude glucosinolates upon decomposition which can inhibit nematodes and pathogens (Petersen et al., 2001). Further research should indicate if mixing cover crops does not decline the efficiency in nematode community reduction. I expect it is likely that mixing a cover crop that is a host for certain plant parasitic nematodes with a cover crop aimed at plant parasitic nematode reduction will dilute this suppression effect.

References

Abalos, D., De Deyn, G.B., Kuyper, T.W., van Groenigen, J.W. (2014) Plant species identity surpasses species richness as a key driver of N_2O emissions from grassland. Global Change Biology 20, 265-275.

Abalos, D., van Groenigen, J.W., Philippot, L., Lubbers, I.M., De Deyn, G.B. (2019) Plant trait-based approaches to improve nitrogen cycling in agroecosystems. Journal of Applied Ecology 56, 2454-2466.

Abdalla, M., Hastings, A., Cheng, K., Yue, Q., Chadwick, D., Espenberg, M., Truu, J., Rees, R.M., Smith, P. (2019) A critical review of the impacts of cover crops on nitrogen leaching, net greenhouse gas balance and crop productivity. Global Change Biology 25, 2530-2543.

Abdollahi, L., Munkholm, L.J. (2014) Tillage system and cover crop effects on soil quality: I. chemical, mechanical, and biological properties. Soil Science Society of America Journal 78, 262-270.

Aerts, R. (1997) Climate, leaf litter chemistry and leaf litter decomposition in terrestrial ecosystems: A triangular relationship. Oikos 79, 439-449.

Andersen, S.N., Dresbøll, D.B., Thorup-Kristensen, K. (2014) Root interactions between intercropped legumes and non-legumes-a competition study of red clover and red beet at different nitrogen levels. Plant and Soil 378, 59-72.

Anderson, J.M., Hetherington, S.L. (1999) Temperature, nitrogen availability and mixture effects on the decomposition of heather [*Calluna vulgaris* (L.) Hull] and bracken [*Pteridium aquilinum* (L.) Kuhn] litters. Functional Ecology 13, 116-124.

Baggs, E.M., Rees, R.M., Smith, K.A., Vinten, A.J.A. (2000) Nitrous oxide emission from soils after incorporating crop residues. Soil Use and Management 16, 82-87.

Balota, E.L., Colozzi-Filho, A., Andrade, D.S., Dick, R.P. (2003) Microbial biomass in soils under different tillage and crop rotation systems. Biology and Fertility of Soils 38, 15-20.

Barantal, S., Schimann, H., Fromin, N., Hättenschwiler, S. (2014) C, N and P fertilization in an Amazonian rainforest supports stoichiometric dissimilarity as a driver of litter diversity effects on decomposition. Proceedings of the Royal Society B: Biological Sciences 281.

Barel, J.M., Kuyper, T.W., de Boer, W., Douma, J.C., De Deyn, G.B. (2018) Legacy effects of diversity in space and time driven by winter cover crop biomass and nitrogen concentration. Journal of Applied Ecology 55, 299-310.

Barel, J.M., Kuyper, T.W., Paul, J., de Boer, W., Cornelissen, J.H.C., De Deyn, G.B. (2019) Winter cover crop legacy effects on litter decomposition act through litter quality and microbial community changes. Journal of Applied Ecology 56, 132-143.

Barton, K., (2019) MuMIn: Multi-model inference., R package version 1.43.6. ed.

Basche, A.D., Miguez, F.E., Kaspar, T.C., Castellano, M.J. (2014) Do cover crops increase or decrease nitrous oxide emissions? a meta-analysis. Journal of Soil and Water Conservation 69, 471-482.

Bateman, E.J., Baggs, E.M. (2005) Contributions of nitrification and denitrification to N_2O emissions from soils at different water-filled pore space. Biology and Fertility of Soils 41, 379-388.

Behnke, G.D., Villamil, M.B. (2019) Cover crop rotations affect greenhouse gas emissions and crop production in Illinois, USA. Field Crops Research 241.

Benbi, D.K., Boparai, A.K., Brar, K. (2014) Decomposition of particulate organic matter is more sensitive to temperature than the mineral associated organic matter. Soil Biology and Biochemistry 70, 183-192.

Bichel, A., Oelbermann, M., Echarte, L. (2017) Impact of residue addition on soil nitrogen dynamics in intercrop and sole crop agroecosystems. Geoderma 304, 12-18.

Blanco-Canqui, H. (2018) Cover crops and water quality. Agronomy Journal 110, 1633-1647.

Blanco-Canqui, H., Shaver, T.M., Lindquist, J.L., Shapiro, C.A., Elmore, R.W., Francis, C.A., Hergert, G.W. (2015) Cover crops and ecosystem services: Insights from studies in temperate soils. Agronomy Journal 107, 2449-2474.

Blesh, J. (2018) Functional traits in cover crop mixtures: Biological nitrogen fixation and multifunctionality. Journal of Applied Ecology 55, 38-48.

Blesh, J., VanDusen, B.M., Brainard, D.C. (2019) Managing ecosystem services with cover crop mixtures on organic farms. Agronomy Journal 111, 826-840.

Bommarco, R., Kleijn, D., Potts, S.G. (2013) Ecological intensification: harnessing ecosystem services for food security. Trends in Ecology & Evolution 28, 230-238.

Bonanomi, G., Capodilupo, M., Incerti, G., Mazzoleni, S. (2014) Nitrogen transfer in litter mixture enhances decomposition rate, temperature sensitivity, and C quality changes. Plant and Soil 381, 307-321.

Bonanomi, G., Cesarano, G., Gaglione, S.A., Ippolito, F., Sarker, T., Rao, M.A. (2017) Soil fertility promotes decomposition rate of nutrient poor, but not nutrient rich litter through nitrogen transfer. Plant and Soil 412, 397-411.

Bongiorno, G., Bünemann, E.K., Oguejiofor, C.U., Meier, J., Gort, G., Comans, R., Mäder, P., Brussaard, L., de Goede, R. (2019) Sensitivity of labile carbon fractions to tillage and organic matter management and their potential as comprehensive soil quality indicators across pedoclimatic conditions in Europe. Ecological Indicators 99, 38-50.

Bouma, T.J., Nielsen, K.L., Koutstaal, B. (2000) Sample preparation and scanning protocol for computerised analysis of root length and diameter. Plant and Soil 218, 185-196.

Bradford, M.A., Berg, B., Maynard, D.S., Wieder, W.R., Wood, S.A. (2016) Understanding the dominant controls on litter decomposition. Journal of Ecology 104, 229-238.

Brennan, E.B., Smith, R.F. (2018) Mustard Cover Crop Growth and Weed Suppression in Organic, Strawberry Furrows in California. Hortscience 53, 432-440.

Brooker, R.W., Bennett, A.E., Cong, W.F., Daniell, T.J., George, T.S., Hallett, P.D., Hawes, C., Iannetta, P.P.M., Jones, H.G., Karley, A.J., Li, L., McKenzie, B.M., Pakeman, R.J., Paterson, E., Schöb, C., Shen, J., Squire, G., Watson, C.A., Zhang, C., Zhang, F., Zhang, J., White, P.J. (2015) Improving intercropping: A synthesis of research in agronomy, plant physiology and ecology. New Phytologist 206, 107-117.

Brookes, P.C., Landman, A., Pruden, G., Jenkinson, D.S. (1985) Chloroform fumigation and the release of soil nitrogen: A rapid direct extraction method to measure microbial biomass nitrogen in soil. Soil Biology and Biochemistry 17, 837-842.

Brust, J., Gerhards, R., Karanisa, T., Ruff, L., Kipp, A. (2011a) Why undersown and cover crops become important again for weed suppression in European cropping systems. Gesunde Pflanzen 63, 191-198.

Brust, J., Gerhards, R., Karanisa, T., Ruff, L., Kipp, A. (2011b) Why Undersown and Cover Crops Become Important Again for weed Suppression in European Cropping Systems. Gesunde Pflanzen 63, 191-198.

Bukovsky-Reyes, S., Isaac, M.E., Blesh, J. (2019) Effects of intercropping and soil properties on root functional traits of cover crops. Agriculture, Ecosystems and Environment 285.

Burr-Hersey, J.E., Mooney, S.J., Bengough, A.G., Mairhofer, S., Ritz, K. (2017) Developmental morphology of cover crop species exhibit contrasting behaviour to changes in soil bulk density, revealed by X-ray computed tomography. Plos One 12.

Butterbach-Bahl, K., Baggs, E.M., Dannenmann, M., Kiese, R., Zechmeister-Boltenstern, S. (2013) Nitrous oxide emissions from soils: How well do we understand the processes and their controls? Philosophical Transactions of the Royal Society B: Biological Sciences 368.

Cameron, K.C., Di, H.J., Moir, J.L. (2013) Nitrogen losses from the soil/plant system: A review. Annals of Applied Biology 162, 145-173.

Campbell, E.E., Parton, W.J., Soong, J.L., Paustian, K., Hobbs, N.T., Cotrufo, M.F. (2016) Using litter chemistry controls on microbial processes to partition litter carbon fluxes with the Litter Decomposition and Leaching (LIDEL) model. Soil Biology and Biochemistry 100, 160-174.

Cardinale, B.J., Duffy, J.E., Gonzalez, A., Hooper, D.U., Perrings, C., Venail, P., Narwani, A., MacE, G.M., Tilman, D., Wardle, D.A., Kinzig, A.P., Daily, G.C., Loreau, M., Grace, J.B., Larigauderie, A., Srivastava, D.S., Naeem, S. (2012) Biodiversity loss and its impact on humanity. Nature 486, 59-67.

Cardinale, B.J., Wright, J.P., Cadotte, M.W., Carroll, I.T., Hector, A., Srivastava, D.S., Loreau, M., Weis, J.J. (2007) Impacts of plant diversity on biomass production increase through time because of species complementarity. Proceedings of the National Academy of Sciences of the United States of America 104, 18123-18128.

Castellano, M.J., Mueller, K.E., Olk, D.C., Sawyer, J.E., Six, J. (2015) Integrating plant litter quality, soil organic matter stabilization, and the carbon saturation concept. Global Change Biology 21, 3200-3209.

Chu, M.W., Jagadamma, S., Walker, F.R., Eash, N.S., Buschermohle, M.J., Duncan, L.A. (2017) Effect of Multispecies Cover Crop Mixture on Soil Properties and Crop Yield. Agricultural & Environmental Letters 2.

Cong, W.F., Hoffland, E., Li, L., Six, J., Sun, J.H., Bao, X.G., Zhang, F.S., Van Der Werf, W. (2015) Intercropping enhances soil carbon and nitrogen. Global Change Biology 21, 1715-1726.

Cong, W.F., van Ruijven, J., Mommer, L., De Deyn, G.B., Berendse, F., Hoffland, E. (2014) Plant species richness promotes soil carbon and nitrogen stocks in grasslands without legumes. Journal of Ecology 102, 1163-1170.

Conn, C., Dighton, J. (2000) Litter quality influences on decomposition, ectomycorrhizal community structure and mycorrhizal root surface acid phosphatase activity. Soil Biology and Biochemistry 32, 489-496.

Córdova, S.C., Olk, D.C., Dietzel, R.N., Mueller, K.E., Archontouilis, S.V., Castellano, M.J. (2018) Plant litter quality affects the accumulation rate, composition, and stability of mineral-associated soil organic matter. Soil Biology and Biochemistry 125, 115-124.

Cornwell, W.K., Cornelissen, J.H.C., Amatangelo, K., Dorrepaal, E., Eviner, V.T., Godoy, O., Hobbie, S.E., Hoorens, B., Kurokawa, H., Pérez-Harguindeguy, N., Quested, H.M., Santiago, L.S., Wardle, D.A., Wright, I.J., Aerts, R., Allison, S.D., Van Bodegom, P., Brovkin, V., Chatain, A., Callaghan, T.V., Díaz, S., Garnier, E., Gurvich, D.E., Kazakou, E., Klein, J.A., Read, J., Reich, P.B., Soudzilovskaia, N.A., Vaieretti, M.V., Westoby, M. (2008) Plant species traits are the predominant control on litter decomposition rates within biomes worldwide. Ecology Letters 11, 1065-1071.

Cotrufo, M.F., Ranalli, M.G., Haddix, M.L., Six, J., Lugato, E. (2019) Soil carbon storage informed by particulate and mineral-associated organic matter. Nature Geoscience 12, 989-994.

Cotrufo, M.F., Wallenstein, M.D., Boot, C.M., Denef, K., Paul, E. (2013) The Microbial Efficiency-Matrix Stabilization (MEMS) framework integrates plant litter decomposition with soil organic matter stabilization: do labile plant inputs form stable soil organic matter? Global Change Biology 19, 988-995.

Couëdel, A., Alletto, L., Tribouillois, H., Justes, É. (2018) Cover crop crucifer-legume mixtures provide effective nitrate catch crop and nitrogen green manure ecosystem services. Agriculture, Ecosystems and Environment 254, 50-59.

Creissen, H.E., Jorgensen, T.H., Brown, J.K.M. (2016) Increased yield stability of field-grown winter barley (Hordeum vulgare L.) varietal mixtures through ecological processes. Crop Protection 85, 1-8.

Crews, T.E., Peoples, M.B. (2005) Can the synchrony of nitrogen supply and crop demand be improved in legume and fertilizer-based agroecosystems? A review. Nutrient Cycling in Agroecosystems 72, 101-120.

Crist, E., Mora, C., Engelman, R. (2017) The interaction of human population, food production, and biodiversity protection. Science 356, 260-264.

Culman, S.W., Snapp, S.S., Freeman, M.A., Schipanski, M.E., Beniston, J., Lal, R., Drinkwater, L.E., Franzluebbers, A.J., Glover, J.D., Grandy, A.S., Lee, J., Six, J., Maul, J.E., Mirksy, S.B., Spargo, J.T., Wander, M.M. (2012) Permanganate oxidizable carbon reflects a processed soil fraction that is sensitive to management. Soil Science Society of America Journal 76, 494-504.

Cúpina, B., Vujić, S., Krstić, D., Radanović, Z., Čabilovski, R., Manojlović, M., Latković, D. (2017) Winter cover crops as green manure in a temperate region: The effect on nitrogen budget and yield of silage maize. Crop and Pasture Science 68, 1060-1069.

Dabney, S.M., Delgado, J.A., Reeves, D.W. (2001) Using winter cover crops to improve soil and water quality. Communications in Soil Science and Plant Analysis 32, 1221-1250.

De Baets, S., Poesen, J., Meersmans, J., Serlet, L. (2011) Cover crops and their erosion-reducing effects during concentrated flow erosion. CATENA 85, 237-244.

De Carvalho, A.M., Coelho, M.C., Dantas, R.A., Fonseca, O.P., Júnior, R.G., Figueiredo, C.C. (2012) Chemical composition of cover plants and its effect on maize yield in no-tillage systems in the Brazilian savanna. Crop and Pasture Science 63, 1075-1081.

De Carvalho, A.M., Marchão, R.L., Bustamante, M.M.C., de Alcântara, F.A., Coser, T.R. (2014) Characterization of cover crops by NMR spectroscopy: Impacts on soil carbon, nitrogen and phosphorus under tillage regimes. Revista Ciencia Agronomica 45, 968-975.

De Deyn, G.B., Quirk, H., Yi, Z., Oakley, S., Ostle, N.J., Bardgett, R.D. (2009) Vegetation composition promotes carbon and nitrogen storage in model grassland communities of contrasting soil fertility. Journal of Ecology 97, 864-875.

De Sa Pereira, E., Galantini, J.A., Duval, M.E. (2017) Use of a three-compartment model to evaluate the dynamics of cover crop residues. Archives of Agronomy and Soil Science 63, 1623-1629.

De Wit, C.T. (1960) On competition. Verslagen landbouwkundige onderzoekingen 66, 1-82.

Delgado-Baquerizo, M., García-Palacios, P., Milla, R., Gallardo, A., Maestre, F.T. (2015) Soil characteristics determine soil carbon and nitrogen availability during leaf litter decomposition regardless of litter quality. Soil Biology and Biochemistry 81, 134-142.

Di, H.J., Cameron, K.C. (2002) Nitrate leaching in temperate agroecosystems: Sources, factors and mitigating strategies. Nutrient Cycling in Agroecosystems 64, 237-256.

Dijksma, S., (2014) Wijzigingen in invulling van de vergroening GLB, in: DGA-ELV/14119366, B.a.d.v.v.d.T.K.d.S.-G. (Ed.).

Drost, S.M., Rutgers, M., Wouterse, M., de Boer, W., Bodelier, P.L.E. (2020) Decomposition of mixtures of cover crop residues increases microbial functional diversity. Geoderma 361, 114060.

Duan, Y.F., Hallin, S., Jones, C.M., Priemé, A., Labouriau, R., Petersen, S.O. (2018) Catch crop residues stimulate N_2O emissions during spring, without affecting the genetic potential for nitrite and N_2O reduction. Frontiers in microbiology 9.

Dungait, J.A.J., Hopkins, D.W., Gregory, A.S., Whitmore, A.P. (2012) Soil organic matter turnover is governed by accessibility not recalcitrance. Global Change Biology 18, 1781-1796.

Duval, M.E., Galantini, J.A., Capurro, J.E., Martinez, J.M. (2016) Winter cover crops in soybean monoculture: Effects on soil organic carbon and its fractions. Soil and Tillage Research 161, 95-105.

Elhakeem, A., Bastiaans, L., Makowski, D., Houben, S., Couwenberg, T., van der Werf, W. (2020) High productivity and stability in cover crop mixtures.

Elhakeem, A., van der Werf, W., Ajal, J., Lucà, D., Claus, S., Vico, R.A., Bastiaans, L. (2019b) Cover crop mixtures result in a positive net biodiversity effect irrespective of seeding configuration. Agriculture, Ecosystems & Environment 285, 106627.

Erisman, J.W., Galloway, J.N., Seitzinger, S., Bleeker, A., Dise, N.B., Petrescu, A.M., Leach, A.M., de Vries, W. (2013) Consequences of human modification of the global nitrogen cycle. Philos Trans R Soc Lond B Biol Sci 368, 20130116.

Erisman, J.W., Sutton, M.A., Galloway, J., Klimont, Z., Winiwarter, W. (2008) How a century of ammonia synthesis changed the world. Nature Geoscience 1, 636-639.

Evers, J.B., Van Der Werf, W., Stomph, T.J., Bastiaans, L., Anten, N.P.R. (2019) Understanding and optimizing species mixtures using functional-structural plant modelling. Journal of Experimental Botany 70, 2381-2388.

Fageria, N.K., Baligar, V.C., Bailey, B.A. (2005) Role of cover crops in improving soil and row crop productivity. Communications in Soil Science and Plant Analysis 36, 2733-2757.

Feddes, R.A., Kowalik, P.J., Zaradny, H., (1978) Simulation of field water use and crop yield. Pudoc, Wageningen.

Finn, J.A., Kirwan, L., Connolly, J., Sebastià, M.T., Helgadottir, A., Baadshaug, O.H., Bélanger, G., Black, A., Brophy, C., Collins, R.P., Čop, J., Dalmannsdóttir, S., Delgado, I., Elgersma, A., Fothergill, M., Frankow-Lindberg, B.E., Ghesquiere, A., Golinska, B., Golinski, P., Grieu, P., Gustavsson, A.-M., Höglind, M., Huguenin-Elie, O., Jørgensen, M., Kadziuliene, Z., Kurki, P., Llurba, R., Lunnan, T., Porqueddu, C., Suter, M., Thumm, U., Lüscher, A. (2013) Ecosystem function enhanced by combining four functional types of plant species in intensively managed grassland mixtures: a 3-year continental-scale field experiment. Journal of Applied Ecology 50, 365-375.

Finney, D.M., Kaye, J.P. (2017) Functional diversity in cover crop polycultures increases multifunctionality of an agricultural system. Journal of Applied Ecology 54, 509-517.

Finney, D.M., White, C.M., Kaye, J.P. (2016) Biomass production and carbon/nitrogen ratio influence ecosystem services from cover crop mixtures. Agronomy Journal 108, 39-52.

Florence, A.M., Higley, L.G., Drijber, R.A., Francis, C.A., Lindquist, J.L. (2019) Cover crop mixture diversity, biomass productivity, weed suppression, and stability. PLoS ONE 14.

Foley, J.A., Ramankutty, N., Brauman, K.A., Cassidy, E.S., Gerber, J.S., Johnston, M., Mueller, N.D., O'Connell, C., Ray, D.K., West, P.C., Balzer, C., Bennett, E.M., Carpenter, S.R., Hill, J., Monfreda, C., Polasky, S., Rockström, J., Sheehan, J., Siebert, S., Tilman, D., Zaks, D.P.M. (2011) Solutions for a cultivated planet. Nature 478, 337-342.

Fontaine, S., Bardoux, G., Abbadie, L., Mariotti, A. (2004) Carbon input to soil may decrease soil carbon content. Ecology Letters 7, 314-320.

Fontaine, S., Barot, S., Barré, P., Bdioui, N., Mary, B., Rumpel, C. (2007) Stability of organic carbon in deep soil layers controlled by fresh carbon supply. Nature 450, 277-280.

Fornara, D.A., Tilman, D. (2008) Plant functional composition influences rates of soil carbon and nitrogen accumulation. Journal of Ecology 96, 314-322.

Francis, G.S., Bartley, K.M., Tabley, F.J. (1998) The effect of winter cover crop management on nitrate leaching losses and crop growth. Journal of Agricultural Science 131, 299-308.

Freschet, G.T., Aerts, R., Cornelissen, J.H.C. (2012) A plant economics spectrum of litter decomposability. Functional Ecology 26, 56-65.

Gabriel, J.L., Alonso-Ayuso, M., García-González, I., Hontoria, C., Quemada, M. (2016) Nitrogen use efficiency and fertiliser fate in a long-term experiment with winter cover crops. European Journal of Agronomy 79, 14-22.

Gabriel, J.L., Muñoz-Carpena, R., Quemada, M. (2012) The role of cover crops in irrigated systems: Water balance, nitrate leaching and soil mineral nitrogen accumulation. Agriculture, Ecosystems & Environment 155, 50-61.

Galloway, J.N., Leach, A.M., Erisman, J.W., Bleeker, A. (2017) Nitrogen: the historical progression from ignorance to knowledge, with a view to future solutions. Soil Research 55, 417-424.

García-Palacios, P., Milla, R., Delgado-Baquerizo, M., Martín-Robles, N., Álvaro-Sánchez, M., Wall, D.H. (2013) Side-effects of plant domestication: Ecosystem impacts of changes in litter quality. New Phytologist 198, 504-513.

García-Palacios, P., Shaw, E.A., Wall, D.H., Hättenschwiler, S. (2016) Temporal dynamics of biotic and abiotic drivers of litter decomposition. Ecology Letters, n/a-n/a.

García-Palacios, P., Shaw, E.A., Wall, D.H., Hättenschwiler, S. (2017) Contrasting mass-ratio vs. niche complementarity effects on litter C and N loss during decomposition along a regional climatic gradient. Journal of Ecology 105, 968-978.

Gartner, T.B., Cardon, Z.G. (2004) Decomposition dynamics in mixed-species leaf litter. Oikos 104, 230-246.

Geiger, R. (1954) Klassifikation der Klimate nach W. Köppen. Zahlenwerte und Funktionen aus Physik, Chemie, Astronomie, Geophysik und Technik 3.

Gentile, R., Vanlauwe, B., Kavoo, A., Chivenge, P., Six, J., (2011) Residue quality and N fertilizer do not influence aggregate stabilization of C and N in two tropical soils with contrasting texture. Springer Netherlands, Dordrecht, pp. 795-805.

Goidts, E., Van Wesemael, B., Crucifix, M. (2009) Magnitude and sources of uncertainties in soil organic carbon (SOC) stock assessments at various scales. European Journal of Soil Science 60, 723-739.

Gomes, J., Bayer, C., de Souza Costa, F., de Cássia Piccolo, M., Zanatta, J.A., Vieira, F.C.B., Six, J. (2009) Soil nitrous oxide emissions in long-term cover crops-based rotations under subtropical climate. Soil and Tillage Research 106, 36-44.

Gulde, S., Chung, H., Amelung, W., Chang, C., Six, J. (2008) Soil carbon saturation controls labile and stable carbon pool dynamics. Soil Science Society of America Journal 72, 605-612.

Haddix, M.L., Gregorich, E.G., Helgason, B.L., Janzen, H., Ellert, B.H., Cotrufo, F.M. (2020) Climate, carbon content, and soil texture control the independent formation and persistence of particulate and mineral-associated organic matter in soil. Geoderma 363, 114160.

Hallin, S., Philippot, L., Löffler, F.E., Sanford, R.A., Jones, C.M. (2018) Genomics and ecology of novel N_2O-reducing microorganisms. Trends in Microbiology 26, 43-55.

Handa, I.T., Aerts, R., Berendse, F., Berg, M.P., Bruder, A., Butenschoen, O., Chauvet, E., Gessner, M.O., Jabiol, J., Makkonen, M., McKie, B.G., Malmqvist, B., Peeters, E.T.H.M., Scheu, S., Schmid, B., Van Ruijven, J., Vos, V.C.A., Hättenschwiler, S. (2014) Consequences of biodiversity loss for litter decomposition across biomes. Nature 509, 218-221.

Hansen, S., Frøseth, R.B., Stenberg, M., Stalenga, J., Olesen, J.E., Krauss, M., Radzikowski, P., Doltra, J., Nadeem, S., Torp, T., Pappa, V., Watson, C.A. (2019) Reviews and syntheses: Review of causes and sources of N_2O emissions and NO_3 leaching from organic arable crop rotations. Biogeosciences 16, 2795-2819.

Haramoto, E.R., Gallandt, E.R. (2004) Brassica cover cropping for weed management: A review. Renewable Agriculture and Food Systems 19, 187-198.

Hättenschwiler, S., Jørgensen, H.B. (2010) Carbon quality rather than stoichiometry controls litter decomposition in a tropical rain forest. Journal of Ecology 98, 754-763.

Hättenschwiler, S., Tiunov, A.V., Scheu, S. (2005) Biodiversity and litter decomposition in terrestrial ecosystems. Annual Review of Ecology, Evolution, and Systematics 36, 191-218.

Hättenschwiler, S., Vitousek, P.M. (2000) The role of polyphenols in terrestrial ecosystem nutrient cycling. Trends in Ecology and Evolution 15, 238-242.

Hauggaard-Nielsen, H., Jensen, E.S., (2005) Facilitative root interactions in intercrops, Root physiology: From gene to function. Springer, pp. 237-250.

Haynes, R.J., (2005) Labile organic matter fractions as central components of the quality of agricultural soils: An overview, Advances in Agronomy. Academic Press, pp. 221-268.

Hector, A. (2006) Overyielding and stable species coexistence. New Phytologist 172, 1-3.

Heuermann, D., Gentsch, N., Boy, J., Schweneker, D., Feuerstein, U., Gross, J., Bauer, B., Guggenberger, G., von Wiren, N. (2019) Interspecific competition among catch crops modifies vertical root biomass distribution and nitrate scavenging in soils. Scientific Reports 9.

Hoekstra, N.J., Suter, M., Finn, J.A., Husse, S., Lüscher, A. (2015) Do belowground vertical niche differences between deep- and shallow-rooted species enhance resource uptake and drought resistance in grassland mixtures? Plant and Soil 394, 21-34.

Holmes, A.A., Thompson, A.A., Lovell, S.T., Villamil, M.B., Yannarell, A.C., Dawson, J.O., Wortman, S.E. (2019) Nitrogen provisioned and recycled by cover crops in monoculture and mixture across two organic farms. Nutrient Cycling in Agroecosystems 115, 441-453.

Hooper, D.U., Chapin III, F.S., Ewel, J.J., Hector, A., Inchausti, P., Lavorel, S., Lawton, J.H., Lodge, D.M., Loreau, M., Naeem, S., Schmid, B., Setälä, H., Symstad, A.J., Vandermeer, J., Wardle, D.A. (2005) Effects of biodiversity on ecosystem functioning: A consensus of current knowledge. Ecological Monographs 75, 3-35.

Hoorens, B., Aerts, R., Stroetenga, M. (2003) Does initial litter chemistry explain litter mixture effects on decomposition? Oecologia 137, 578-586.

Hothorn, T., Bretz, F., Westfall, P. (2008) Simultaneous inference in general parametric model. Biometrical Journal 50, 346-363.

Houba, V.J.G., Temminghoff, E.J.M., Gaikhorst, G.A., van Vark, W. (2000a) Soil analysis procedures using 0.01 M calcium chloride as extraction reagent. Communications in Soil Science and Plant Analysis 31, 1299-1396.

Houba, V.J.G., Temminghoff, E.J.M., Gaikhorst, G.A., van Vark, W. (2000b) Soil analysis procedures using 0.01 M Calcium Chloride as extraction reagent. Communications in Soil Science and Plant Analysis 31, 1299-1396.

Houlton, B.Z., Almaraz, M., Aneja, V., Austin, A.T., Bai, E., Cassman, K.G., Compton, J.E., Davidson, E.A., Erisman, J.W., Galloway, J.N., Gu, B., Yao, G., Martinelli, L.A., Scow, K., Schlesinger, W.H., Tomich, T.P., Wang, C., Zhang, X. (2019) A world of cobenefits: Solving the global nitrogen challenge. Earth's Future 7, 865-872.

Huang, Y., Zou, J., Zheng, X., Wang, Y., Xu, X. (2004) Nitrous oxide emissions as influenced by amendment of plant residues with different C:N ratios. Soil Biology and Biochemistry 36, 973-981.

IPCC, (2014) Climate Change 2014: Synthesis report. Contribution of working groups I, II and III to the fifth assessment report of the intergovernmental panel on climate change, in: Core Writing Team, R.K.P.a.L.A.M.e.I., Geneva, Switzerland, 151 pp. (Ed.), IPCC, Geneva, Switzerland, p. 151 pp.

Isbell, F., Adler, P.R., Eisenhauer, N., Fornara, D., Kimmel, K., Kremen, C., Letourneau, D.K., Liebman, M., Polley, H.W., Quijas, S., Scherer-Lorenzen, M. (2017) Benefits of increasing plant diversity in sustainable agroecosystems. Journal of Ecology 105, 871-879.

Isbell, F., Calcagno, V., Hector, A., Connolly, J., Harpole, W.S., Reich, P.B., Scherer-Lorenzen, M., Schmid, B., Tilman, D., Van Ruijven, J., Weigelt, A., Wilsey, B.J., Zavaleta, E.S., Loreau, M. (2011) High plant diversity is needed to maintain ecosystem services. Nature 477, 199-202.

Jahanzad, E., Barker, A.V., Hashemi, M., Eaton, T., Sadeghpour, A., Weis, S.A. (2016) Nitrogen release dynamics and decomposition of buried and surface cover crop residues. Agronomy Journal 108, 1735-1741.

Janzen, H.H. (2006) The soil carbon dilemma: Shall we hoard it or use it? Soil Biology and Biochemistry 38, 419-424.

Jian, J., Du, X., Reiter, M.S., Stewart, R.D. (2020) A meta-analysis of global cropland soil carbon changes due to cover cropping. Soil Biology and Biochemistry 143.

Kallenbach, C.M., Grandy, A.S., Frey, S.D., Diefendorf, A.F. (2015) Microbial physiology and necromass regulate agricultural soil carbon accumulation. Soil Biology and Biochemistry 91, 279-290.

Kampichler, C., Bruckner, A. (2009) The role of microarthropods in terrestrial decomposition: A meta-analysis of 40 years of litterbag studies. Biological Reviews 84, 375-389.

Kaspar, T.C., Bakker, M.G. (2015) Biomass production of 12 winter cereal cover crop cultivars and their effect on subsequent no-till corn yield. Journal of Soil and Water Conservation 70, 353-364.

Kaspar, T.C., Singer, J.W. (2011) The use of cover crops to manage soil. Publications from USDA-ARS / UNL Faculty. 1382.

Kaye, J., Finney, D., White, C., Bradley, B., Schipanski, M., Alonso-Ayuso, M., Hunter, M., Burgess, M., Mejia, C. (2019) Managing nitrogen through cover crop species selection in the U.S. mid-Atlantic. PLoS ONE 14, e0215448.

Kaye, J.P., Quemada, M. (2017) Using cover crops to mitigate and adapt to climate change. A review. Agronomy for Sustainable Development 37, 4.

Klug, M.J., (1999) The determination of microbial biomass. , Standard soil methods for long-term ecological research. Oxfor University Press, pp. 291-317.

Knorr, M., Frey, S.D., Curtis, P.S. (2005) Nitrogen additions and litter decomposition: A meta-analysis. Ecology 86, 3252-3257.

Köppen, W. (1900) Versuch einder Klassifikation der Klimate, vorzugsweise nach ihren Beziehungen zur Pflanzenwelt. Geogr. Zeitschr., 593-611, 657-679.

Kroes, J., Van Dam, J., Groenendijk, P., Hendriks, R., Jacobs, C., (2009) SWAP version 3.2. Theory description and user manual. Alterra.

Kroes, J.G., Dam, J.C.v., Bartholomeus, R.P., Groenendijk, P., Heinen, M., Hendriks, R.F.A., Mulder, H.M., Supit, I., Walsum, P.E.V.v., (2017) SWAP version 4 : theory description and user manual. Wageningen Environmental Research, Wageningen.

Kruger, D.H.M., Fourie, J.C., Malan, A.P. (2013) Cover Crops with Biofumigation Properties for the Suppression of Plant-Parasitic Nematodes: A Review. South African Journal of Enology and Viticulture 34, 287-295.

Kruidhof, H.M., Bastiaans, L., Kropff, M.J. (2009) Cover crop residue management for optimizing weed control. Plant and Soil 318, 169-184.

Kuzyakov, Y. (2010) Priming effects: Interactions between living and dead organic matter. Soil Biology and Biochemistry 42, 1363-1371.

Lal, R. (2004) Soil carbon sequestration impacts on global climate change and food security. Science 304, 1623-1627.

Lange, M., Eisenhauer, N., Sierra, C.A., Bessler, H., Engels, C., Griffiths, R.I., Mellado-Vázquez, P.G., Malik, A.A., Roy, J., Scheu, S., Steinbeiss, S., Thomson, B.C., Trumbore, S.E., Gleixner, G. (2015) Plant diversity increases soil microbial activity and soil carbon storage. Nature Communications 6.

Lavallee, J.M., Conant, R.T., Paul, E.A., Cotrufo, M.F. (2018) Incorporation of shoot versus root-derived 13C and 15N into mineral-associated organic matter fractions: results of a soil slurry incubation with dual-labelled plant material. Biogeochemistry 137, 379-393.

Lavallee, J.M., Soong, J.L., Cotrufo, M.F. (2020) Conceptualizing soil organic matter into particulate and mineral-associated forms to address global change in the 21st century. Global Change Biology 26, 261-273.

Lecerf, A., Marie, G., Kominoski, J.S., Leroy, C.J., Bernadet, C., Swan, C.M. (2011) Incubation time, functional litter diversity, and habitat characteristics predict litter-mixing effects on decomposition. Ecology 92, 160-169.

Lehmann, J., Kleber, M. (2015) The contentious nature of soil organic matter. Nature 528, 60-68.

Lenth, R., Singmann, H., Love, J., Buerkner, P., Huerve, M., (2019) Emmeans: Estimated marginal means, aka least-squares means, 1.3.5.1 ed.

Li, C., Hoffland, E., Kuyper, T.W., Yu, Y., Li, H., Zhang, C., Zhang, F., van der Werf, W. (2020) Yield gain, complementarity and competitive dominance in intercropping in China: A meta-analysis of drivers of yield gain using additive partitioning. European Journal of Agronomy 113, 125987.

Li, D., Peng, S., Chen, B. (2013) The effects of leaf litter evenness on decomposition depend on which plant functional group is dominant. Plant and Soil 365, 255-266.

Li, L., Sun, J., Zhang, F., Guo, T., Bao, X., Smith, F.A., Smith, S.E. (2006) Root distribution and interactions between intercropped species. Oecologia 147, 280-290.

Li, X., Petersen, S.O., Sørensen, P., Olesen, J.E. (2015) Effects of contrasting catch crops on nitrogen availability and nitrous oxide emissions in an organic cropping system. Agriculture, Ecosystems & Environment 199, 382-393.

Li, Y., Chen, X., Veen, G.F.C., Eisenhauer, N., Liang, Y., Zhou, X., Zhang, N., Ma, K. (2018) Negative effects of litter richness on root decomposition in the presence of detritivores. Functional Ecology 32, 1079-1090.

Liang, C., Amelung, W., Lehmann, J., Kästner, M. (2019) Quantitative assessment of microbial necromass contribution to soil organic matter. Global Change Biology 25, 3578-3590.

Liang, C., Schimel, J.P., Jastrow, J.D. (2017) The importance of anabolism in microbial control over soil carbon storage. Nature Microbiology 2, 17105.

Liang, L.L., Eberwein, J.R., Allsman, L.A., Grantz, D.A., Jenerette, G.D. (2015) Regulation of CO_2 and N_2O fluxes by coupled carbon and nitrogen availability. Environmental Research Letters 10, 034008.

Lin, G., Zeng, D.H. (2018) Functional identity rather than functional diversity or species richness controls litter mixture decomposition in a subtropical forest. Plant and Soil 428, 179-193.

Liu, X., Herbert, S.J., Hashemi, A.M., Zhang, X., Ding, G. (2006) Effects of agricultural management on soil organic matter and carbon transformation - A review. Plant, Soil and Environment 52, 531-543.

Loreau, M., Hector, A. (2001) Partitioning selection and complementarity in biodiversity experiments. Nature 412, 72-76.

Lubbers, I.M., Brussaard, L., Otten, W., Van Groenigen, J.W. (2011) Earthworm-induced N mineralization in fertilized grassland increases both N_2O emission and crop-N uptake. European Journal of Soil Science 62, 152-161.

Lummer, D., Scheu, S., Butenschoen, O. (2012) Connecting litter quality, microbial community and nitrogen transfer mechanisms in decomposing litter mixtures. Oikos 121, 1649-1655.

Makkonen, M., Berg, M.P., van Logtestijn, R.S.P., van Hal, J.R., Aerts, R. (2013) Do physical plant litter traits explain non-additivity in litter mixtures? A test of the improved microenvironmental conditions theory. Oikos 122, 987-997.

Maltais-Landry, G., Frossard, E. (2015) Similar phosphorus transfer from cover crop residues and water-soluble mineral fertilizer to soils and a subsequent crop. Plant and Soil 393, 193-205.

Manzoni, S., Jackson, R.B., Trofymow, J.A., Porporato, A. (2008) The global stoichiometry of litter nitrogen mineralization. Science 321, 684.

Marcillo, G.S., Miguez, F.E. (2017) Corn yield response to winter cover crops: An updated meta-analysis. Journal of Soil and Water Conservation 72, 226-239.

MathWorks, I., (2013) MATLAB and statistics toolbox release. Author Natick, MA.

Miguez, F.E., Bollero, G.A. (2005) Review of corn yield response under winter cover cropping systems using meta-analytic methods. Crop Science 45, 2318-2329.

Minasny, B., Malone, B.P., McBratney, A.B., Angers, D.A., Arrouays, D., Chambers, A., Chaplot, V., Chen, Z.S., Cheng, K., Das, B.S., Field, D.J., Gimona, A., Hedley, C.B., Hong, S.Y., Mandal, B., Marchant, B.P., Martin, M., McConkey, B.G., Mulder, V.L., O'Rourke, S., Richer-de-Forges, A.C., Odeh, I., Padarian, J., Paustian, K., Pan, G., Poggio, L., Savin, I., Stolbovoy, V., Stockmann, U., Sulaeman, Y., Tsui, C.C., Vågen, T.G., van Wesemael, B., Winowiecki, L. (2017) Soil carbon 4 per mille. Geoderma 292, 59-86.

Mitchell, E., Scheer, C., Rowlings, D., Conant, R.T., Cotrufo, M.F., Grace, P. (2018) Amount and incorporation of plant residue inputs modify residue stabilisation dynamics in soil organic matter fractions. Agriculture, Ecosystems & Environment 256, 82-91.

Miyazawa, K., Murakami, T., Takeda, M., Murayama, T. (2010) Intercropping green manure crops—effects on rooting patterns. Plant and Soil 331, 231-239.

Moller, K., Reents, H.J. (2009) Effects of various cover crops after peas on nitrate leaching and nitrogen supply to succeeding winter wheat or potato crops. Journal of Plant Nutrition and Soil Science 172, 277-287.

Möller, K., Stinner, W., Leithold, G. (2008) Growth, composition, biological N2 fixation and nutrient uptake of a leguminous cover crop mixture and the effect of their removal on field nitrogen balances and nitrate leaching risk. Nutrient Cycling in Agroecosystems 82, 233.

Montané, F., Romanyà, J., Rovira, P., Casals, P. (2013) Mixtures with grass litter may hasten shrub litter decomposition after shrub encroachment into mountain grasslands. Plant and Soil 368, 459-469.

Morley, N., Baggs, E.M. (2010) Carbon and oxygen controls on N_2O and N_2 production during nitrate reduction. Soil Biology and Biochemistry 42, 1864-1871.

Muhammad, I., Sainju, U.M., Zhao, F., Khan, A., Ghimire, R., Fu, X., Wang, J. (2019) Regulation of soil CO_2 and N_2O emissions by cover crops: A meta-analysis. Soil and Tillage Research 192, 103-112.

Muhammad, W., Vaughan, S.M., Dalal, R.C., Menzies, N.W. (2011) Crop residues and fertilizer nitrogen influence residue decomposition and nitrous oxide emission from a Vertisol. Biology and Fertility of Soils 47, 15-23.

Murrell, E.G., Schipanski, M.E., Finney, D.M., Hunter, M.C., Burgess, M., LaChance, J.C., Baraibar, B., White, C.M., Mortensen, D.A., Kaye, J.P. (2017) Achieving Diverse Cover Crop Mixtures: Effects of Planting Date and Seeding Rate. Agronomy Journal 109, 259-271.

Nevins, C.J., Lacey, C., Armstrong, S. (2020) The synchrony of cover crop decomposition, enzyme activity, and nitrogen availability in a corn agroecosystem in the Midwest United States. Soil and Tillage Research 197, 104518.

Otsing, E., Barantal, S., Anslan, S., Koricheva, J., Tedersoo, L. (2018) Litter species richness and composition effects on fungal richness and community structure in decomposing foliar and root litter. Soil Biology and Biochemistry 125, 328-339.

Pérez Harguindeguy, N., Blundo, C.M., Gurvich, D.E., Díaz, S., Cuevas, E. (2008) More than the sum of its parts? Assessing litter heterogeneity effects on the decomposition of litter mixtures through leaf chemistry. Plant and Soil 303, 151-159.

Petersen, J., Belz, R., Walker, F., Hurle, K. (2001) Weed suppression by release of isothiocyanates from turnip-rape mulch. Agronomy Journal 93, 37-43.

Pimentel, L.G., Weiler, D.A., Pedroso, G.M., Bayer, C. (2015) Soil N_2O emissions following cover-crop residues application under two soil moisture conditions. Journal of Plant Nutrition and Soil Science 178, 631-640.

Pinheiro, J., Bates, D., DebRoy, S., Sarkar, D., Team, R.C., (2019) Linear and nonlinear mixed effects models, R package version 3.1-140 ed.

Poeplau, C., Don, A. (2015) Carbon sequestration in agricultural soils via cultivation of cover crops – A meta-analysis. Agriculture, Ecosystems & Environment 200, 33-41.

Poeplau, C., Don, A., Six, J., Kaiser, M., Benbi, D., Chenu, C., Cotrufo, M.F., Derrien, D., Gioacchini, P., Grand, S., Gregorich, E., Griepentrog, M., Gunina, A., Haddix, M., Kuzyakov, Y., Kühnel, A., Macdonald, L.M., Soong, J., Trigalet, S., Vermeire, M.-L., Rovira, P., van Wesemael, B., Wiesmeier, M., Yeasmin, S., Yevdokimov, I., Nieder, R. (2018) Isolating organic carbon fractions with varying turnover rates in temperate agricultural soils – A comprehensive method comparison. Soil Biology and Biochemistry 125, 10-26.

Poffenbarger, H.J., Mirsky, S.B., Weil, R.R., Kramer, M., Spargo, J.T., Cavigelli, M.A. (2015) Legume proportion, poultry litter, and tillage effects on cover crop decomposition. Agronomy Journal 107, 2083-2096.

Porre, R.J., van der Werf, W., De Deyn, G.B., Stomph, T.J., Hoffland, E. (2020) Is litter decomposition enhanced in species mixtures? A meta-analysis. Soil Biology and Biochemistry 145, 107791.

Preston, C.M., Nault, J.R., Trofymow, J.A., Smyth, C. (2009) Chemical changes during 6 years of decomposition of 11 litters in some Canadian forest sites. Part 1. elemental composition, tannins, phenolics, and proximate fractions. Ecosystems 12, 1053-1077.

Pribyl, D.W. (2010) A critical review of the conventional SOC to SOM conversion factor. Geoderma 156, 75-83.

Prieto, I., Birouste, M., Zamora-Ledezma, E., Gentit, A., Goldin, J., Volaire, F., Roumet, C. (2017) Decomposition rates of fine roots from three herbaceous perennial species: combined effect of root mixture composition and living plant community. Plant and Soil, 1-14.

Puttaso, A., Vityakon, P., Rasche, F., Saenjan, P., Treloges, V., Cadisch, G. (2013) Does organic residue quality influence carbon retention in a tropical sandy soil? Soil Science Society of America Journal 77, 1001-1011.

Quemada, M., Baranski, M., Nobel-de Lange, M.N.J., Vallejo, A., Cooper, J.M. (2013) Meta-analysis of strategies to control nitrate leaching in irrigated agricultural systems and their effects on crop yield. Agriculture, Ecosystems & Environment 174, 1-10.

R Core Team, (2018) R: A language and environment for statistical computing. R Foundation for Statistical Computing, Vienna, Austria.

Reeves, D.W. (1997) The role of soil organic matter in maintaining soil quality in continuous cropping systems. Soil and Tillage Research 43, 131-167.

Rumpel, C., Kögel-Knabner, I. (2011) Deep soil organic matter-a key but poorly understood component of terrestrial C cycle. Plant and Soil 338, 143-158.

RVO, (2015) Gemeenschappelijk landbouwbeleid nieuw GLB.

Santonja, M., Rancon, A., Fromin, N., Baldy, V., Hättenschwiler, S., Fernandez, C., Montès, N., Mirleau, P. (2017) Plant litter diversity increases microbial abundance, fungal diversity, and carbon and nitrogen cycling in a Mediterranean shrubland. Soil Biology and Biochemistry 111, 124-134.

Sariyildiz, T., Anderson, J.M. (2003) Interactions between litter quality, decomposition and soil fertility: a laboratory study. Soil Biology and Biochemistry 35, 391-399.

Scheepens, P., Hoek, H., Molema, G.-J., Bastiaans, L., Groeneveld, R., Schoenmaker, E., Pikaar, P., (2003) Innovatieve onkruidpreventie in biologische landbouw door groenbemesters in het bouwplan, Plant research international b.v. Wageningen.

Schimel, J.P., Hättenschwiler, S. (2007) Nitrogen transfer between decomposing leaves of different N status. Soil Biology and Biochemistry 39, 1428-1436.

Schmatz, R., Recous, S., Aita, C., Tahir, M.M., Schu, A.L., Chaves, B., Giacomini, S.J. (2017) Crop residue quality and soil type influence the priming effect but not the fate of crop residue C. Plant and Soil 414, 229-245.

Schmid, B., Hector, A., Saha, P., Loreau, M. (2008) Biodiversity effects and transgressive overyielding. Journal of Plant Ecology 1, 95-102.

Schmidt-Traub, G., Obersteiner, M., Mosnier, A. (2019) Fix the broken food system in three steps. Nature 569, 181-183.

Schmidt, M.W.I., Torn, M.S., Abiven, S., Dittmar, T., Guggenberger, G., Janssens, I.A., Kleber, M., Kögel-Knabner, I., Lehmann, J., Manning, D.A.C., Nannipieri, P., Rasse, D.P., Weiner, S., Trumbore, S.E. (2011) Persistence of soil organic matter as an ecosystem property. Nature 478, 49-56.

Schnitzer, S.A., Klironomos, J.N., HilleRisLambers, J., Kinkel, L.L., Reich, P.B., Xiao, K., Rillig, M.C., Sikes, B.A., Callaway, R.M., Mangan, S.A., van Nes, E.H., Scheffer, M. (2011) Soil microbes drive the classic plant diversity–productivity pattern. Ecology 92, 296-303.

Schuster, M.J., Dengler, J., Kreyling, J., Berwaers, S., Walter, J., De Boeck, H.J., Nijs, I., Jentsch, A. (2017) Drought inhibits synergistic interactions of native and exotic litter mixtures during decomposition in temperate grasslands. Plant and Soil 415, 257-268.

Sharma, V., Irmak, S., Padhi, J. (2018) Effects of cover crops on soil quality: Part I. Soil chemical properties-organic carbon, total nitrogen, pH, electrical conductivity, organic matter content, nitrate-nitrogen, and phosphorus. Journal of Soil and Water Conservation 73, 637-651.

Six, J., Conant, R.T., Paul, E.A., Paustian, K. (2002) Stabilization mechanisms of soil organic matter: Implications for C-saturation of soils. Plant and Soil 241, 155-176.

Six, J., Paustian, K. (2014) Aggregate-associated soil organic matter as an ecosystem property and a measurement tool. Soil Biology and Biochemistry 68, A4-A9.

Six, J., Paustian, K., Elliott, E.T., Combrink, C. (2000) Soil structure and organic matter I. Distribution of aggregate-size classes and aggregate-associated carbon. Soil Science Society of America Journal 64, 681-689.

Smit, B., Janssens, B., Haagsma, W., Hennen, W., Adrados, J.L., Kathage, J., Dominguez, I.P., (2019) Adoption of cover crops for climate change mitigation in the EU, in: Kathage, J., Perez Dominguez, I. (Eds.), Publications Office of the European Union, Luxembourg, 2019, ISBN 978-92-76-11312-6 (online).

Smith, R.G., Atwood, L.W., Warren, N.D. (2014) Increased productivity of a cover crop mixture is not associated with enhanced agroecosystem services. PLoS ONE 9.

Snyder, C.S., Bruulsema, T.W., Jensen, T.L., Fixen, P.E. (2009) Review of greenhouse gas emissions from crop production systems and fertilizer management effects. Agriculture, Ecosystems and Environment 133, 247-266.

Srivastava, D.S., Cardinale, B.J., Downing, A.L., Duffy, J.E., Jouseau, C., Sankaran, M., Wright, J.P. (2009) Diversity has stronger top-down than bottom-up effects on decomposition. Ecology 90, 1073-1083.

Steele, M.K., Coale, F.J., Hill, R.L. (2012) Winter Annual Cover Crop Impacts on No-Till Soil Physical Properties and Organic Matter. Soil Science Society of America Journal 76, 2164-2173.

Steffen, W., Richardson, K., Rockström, J., Cornell, S.E., Fetzer, I., Bennett, E.M., Biggs, R., Carpenter, S.R., De Vries, W., De Wit, C.A., Folke, C., Gerten, D., Heinke, J., Mace, G.M., Persson, L.M., Ramanathan, V., Reyers, B., Sörlin, S. (2015) Planetary boundaries: Guiding human development on a changing planet. Science 347.

Stewart, C.E., Paustian, K., Conant, R.T., Plante, A.F., Six, J. (2007) Soil carbon saturation: concept, evidence and evaluation. Biogeochemistry 86, 19-31.

Stocker, T.F., D. Qin, G.-K., Plattner, M., Tignor, S.K., Allen, J., Boschung, A., Nauels, Y., Xia, V., Midgley, B., Midgley, P.M., (2013) IPCC, 2013: Climate change 2013: The physical science basis. Contribution of working group I to the fifth assessment report of the Intergovernmental Panel on Climate Change, Cambridge University Press, Cambridge, United Kingdom and New York, NY, USA, 1535 pp.

Stute, J.K., Posner, J.L. (1995) Synchrony between legume nitrogen release and corn demand in the upper midwest. Agronomy Journal 87, 1063-1069.

Syakila, A., Kroeze, C. (2011) The global nitrous oxide budget revisited. Greenhouse Gas Measurement and Management 1, 17-26.

Tardif, A., Shipley, B. (2015) The relationship between functional dispersion of mixed-species leaf litter mixtures and species' interactions during decomposition. Oikos 124, 1050-1057.

Thapa, R., Mirsky, S.B., Tully, K.L. (2018) Cover crops reduce nitrate leaching in agroecosystems: A global meta-analysis. Journal of Environmental Quality 47, 1400-1411.

Thomsen, I.K., Hansen, E.M. (2014) Cover crop growth and impact on N leaching as affected by pre- and postharvest sowing and time of incorporation. Soil Use and Management 30, 48-57.

Thorup-Kristensen, K. (2001) Are differences in root growth of nitrogen catch crops important for their ability to reduce soil nitrate-N content, and how can this be measured? Plant and Soil 230, 185-195.

Thorup-Kristensen, K., Magid, J., Jensen, L.S. (2003) Catch crops and green manures as biological tools in nitrogen management in temperate zones. Advances in Agronomy 79, 227-302.

Tilman, D., Balzer, C., Hill, J., Befort, B.L. (2011) Global food demand and the sustainable intensification of agriculture. Proceedings of the National Academy of Sciences of the United States of America 108, 20260-20264.

Tilman, D., Cassman, K.G., Matson, P.A., Naylor, R., Polasky, S. (2002) Agricultural sustainability and intensive production practices. Nature 418, 671-677.

Tilman, D., Fargione, J., Wolff, B., D'Antonio, C., Dobson, A., Howarth, R., Schindler, D., Schlesinger, W.H., Simberloff, D., Swackhamer, D. (2001) Forecasting agriculturally driven global environmental change. Science 292, 281-284.

Tilman, D., Lehman, C.L., Thomson, K.T. (1997) Plant diversity and ecosystem productivity: Theoretical considerations. Proceedings of the National Academy of Sciences 94, 1857-1861.

Tilman, D., Wedin, D., Knops, J. (1996) Productivity and sustainability influenced by biodiversity in grassland ecosystems. Nature 379, 718-720.

Tonitto, C., David, M.B., Drinkwater, L.E. (2006) Replacing bare fallows with cover crops in fertilizer-intensive cropping systems: A meta-analysis of crop yield and N dynamics. Agriculture, Ecosystems and Environment 112, 58-72.

Tribouillois, H., Cohan, J.P., Justes, E. (2016) Cover crop mixtures including legume produce ecosystem services of nitrate capture and green manuring: assessment combining experimentation and modelling. Plant and Soil 401, 347-364.

Tribouillois, H., Cruz, P., Cohan, J.P., Justes, T. (2015) Modelling agroecosystem nitrogen functions provided by cover crop species in bispecific mixtures using functional traits and environmental factors. Agriculture, Ecosystems and Environment 207, 218-228.

Tscharntke, T., Clough, Y., Wanger, T.C., Jackson, L., Motzke, I., Perfecto, I., Vandermeer, J., Whitbread, A. (2012) Global food security, biodiversity conservation and the future of agricultural intensification. Biological Conservation 151, 53-59.

United Nations General Assembly, (2015) Transforming our world : the 2030 Agenda for Sustainable Development.

Van Dam, J.C., Groenendijk, P., Hendriks, R.F.A., Kroes, J.G. (2008) Advances of modeling water flow in variably saturated soils with SWAP. Vadose Zone Journal 7, 640-653.

Van Leeuwen-Haagsma, W., Hoek, H., Molendijk, L., Mommer, L., Ulen, J., Kroonen-Backbier, B., de Groot, G. (2019) Handboek groenbemesters. Wageningen university & Research.

Van Ruijven, J., Berendse, F. (2005) Diversity-productivity relationships: Initial effects, long-term patterns, and underlying mechanisms. Proceedings of the National Academy of Sciences of the United States of America 102, 695-700.

Vandermeer, J.H. (1989) The ecology of intercropping. Cambridge University Press, Cambridge.

Veen, G.F.C., Freschet, G.T., Ordonez, A., Wardle, D.A. (2015) Litter quality and environmental controls of home-field advantage effects on litter decomposition. Oikos 124, 187-195.

Velthof, G.L., Kuikman, P.J., Oenema, O. (2002) Nitrous oxide emission from soils amended with crop residues. Nutrient Cycling in Agroecosystems 62, 249-261.

Vivanco, L., Austin, A.T. (2011) Nitrogen addition stimulates forest litter decomposition and disrupts species interactions in Patagonia, Argentina. Global Change Biology 17, 1963-1974.

Vogeler, I., Hansen, E.M., Nielsen, S., Labouriau, R., Cichota, R., Olesen, J.E., Thomsen, I.K. (2020) Nitrate leaching from suction cup data: Influence of method of drainage calculation and concentration interpolation. Journal of Environmental Quality 49, 440-449.

Von Lützow, M.V., Kögel-Knabner, I., Ekschmitt, K., Matzner, E., Guggenberger, G., Marschner, B., Flessa, H. (2006) Stabilization of organic matter in temperate soils: Mechanisms and their relevance under different soil conditions - A review. European Journal of Soil Science 57, 426-445.

Wagger, M.G., Cabrera, M.L., Ranells, N.N. (1998) Nitrogen and carbon cycling in relation to cover crop residue quality. Journal of Soil and Water Conservation 53, 214-218.

Wall, D.H., Nielsen, U.N., Six, J. (2015) Soil biodiversity and human health. Nature 528, 69-76.

Wang, X., Butterly, C.R., Baldock, J.A., Tang, C. (2017) Long-term stabilization of crop residues and soil organic carbon affected by residue quality and initial soil pH. Science of the Total Environment 587-588, 502-509.

Wardle, D.A., Bonner, K.I., Nicholson, K.S. (1997) Biodiversity and plant litter: Experimental evidence which does not support the view that enhanced species richness improves ecosystem function. Oikos 79, 247-258.

Wardle, D.A., Nilsson, M.-C., Zackrisson, O., Gallet, C. (2003) Determinants of litter mixing effects in a Swedish boreal forest. Soil Biology and Biochemistry 35, 827-835.

Weidlich, E.W.A., Temperton, V.M., Faget, M. (2018) Neighbourhood stories: role of neighbour identity, spatial location and order of arrival in legume and non-legume initial interactions. Plant and Soil 424, 171-182.

Weil, R.R., Islam, K.R., Stine, M.A., Gruver, J.B., Samson-Liebig, S.E. (2003) Estimating active carbon for soil quality assessment: A simplified method for laboratory and field use. American Journal of Alternative Agriculture 18, 3-17.

Weiler, D.A., Giacomini, S.J., Recous, S., Bastos, L.M., Pilecco, G.E., Dietrich, G., Aita, C. (2018) Trade-off between C and N recycling and N_2O emissions of soils with summer cover crops in subtropical agrosystems. Plant and Soil 433, 213-225.

Wendling, M., Büchi, L., Amossé, C., Jeangros, B., Walter, A., Charles, R. (2017) Specific interactions leading to transgressive overyielding in cover crop mixtures. Agriculture, Ecosystems & Environment 241, 88-99.

Wendling, M., Buchi, L., Amosse, C., Sinaj, S., Walter, A., Charles, R. (2016) Influence of root and leaf traits on the uptake of nutrients in cover crops. Plant and Soil 409, 419-434.

Wendling, M., Charles, R., Herrera, J., Amossé, C., Jeangros, B., Walter, A., Büchi, L. (2019) Effect of species identity and diversity on biomass production and its stability in cover crop mixtures. Agriculture, Ecosystems and Environment 281, 81-91.

White, C.M., DuPont, S.T., Hautau, M., Hartman, D., Finney, D.M., Bradley, B., LaChance, J.C., Kaye, J.P. (2017) Managing the trade off between nitrogen supply and retention with cover crop mixtures. Agriculture, Ecosystems & Environment 237, 121-133.

Wickham, H., (2016) Elegant graphics for data analysis. Springer-Verlag New York.

Wieder, W.R., Bonan, G.B., Allison, S.D. (2013) Global soil carbon projections are improved by modelling microbial processes. Nature Climate Change 3, 909-912.

Wilke, C.O., (2019) Cowplot: Streamlined plot theme and plot annotations for 'ggplot2'.

Wittwer, R.A., Dorn, B., Jossi, W., van der Heijden, M.G.A. (2017) Cover crops support ecological intensification of arable cropping systems. Scientific Reports 7, 41911.

Wittwer, R.A., van der Heijden, M.G.A. (2020) Cover crops as a tool to reduce reliance on intensive tillage and nitrogen fertilization in conventional arable cropping systems. Field Crops Research 249.

Wortman, S.E., Francis, C.A., Bernards, M.L., Drijber, R.A., Lindquist, J.L. (2012) Optimizing cover crop benefits with diverse mixtures and an alternative termination method. Agronomy Journal 104, 1425-1435.

Wu, F., Peng, C., Yang, W., Zhang, J., Han, Y., Mao, T. (2014) Admixture of alder (*Alnus formosana*) litter can improve the decomposition of eucalyptus (*Eucalyptus grandis*) litter. Soil Biology and Biochemistry 73, 115-121.

Yu, Y., Stomph, T.-J., Makowski, D., van der Werf, W. (2015) Temporal niche differentiation increases the land equivalent ratio of annual intercrops: A meta-analysis. Field Crops Research 184, 133-144.

Zhang, D.Q., Hui, D.F., Luo, Y.Q., Zhou, G.Y. (2008) Rates of litter decomposition in terrestrial ecosystems: global patterns and controlling factors. Journal of Plant Ecology 1, 85-93.

Zotarelli, L., Scholberg, J.M., Dukes, M.D., Muñoz-Carpena, R. (2007) Monitoring of nitrate leaching in sandy soils: Comparison of three methods. Journal of Environmental Quality 36, 953-962.

Zuur, A.F., Ieno, E.N., Walker, N.J., Saveliev, A.A., Smith, G.M. (2009) Mixed effects models and extensions in ecology with R. R. Springer.

Summary

Agricultural practices need to become more sustainable in order to keep feeding the global population without causing additional harm to the environment. Incorporating cover crops during the off-season in an agricultural rotation can increase sustainability. During their growth cover crops can reduce N losses and after their growth cover crops are incorporated into the soil and thereby can contribute to the nutrient provisioning of the following cash crop as well as to soil organic matter build-up. From natural systems it is known that mixtures of plant species with complementing traits can exploit resources better and produce more biomass than single species. Thus, cover crop mixtures are expected to increase N and C uptake in the system compared to single species. Cover crop mixtures could therefore be an effective way to increase N use efficiency and C sequestration by agroecosystems. However, the extent to which different cover crop species mixtures can affect N leaching throughout the year, and if they increase soil organic matter is not yet known.

In this thesis, by using two field experiments, a meta-analysis and a greenhouse study, I explored how mixing cover crops affect C and N cycling. I used three common species, radish, oat and vetch, of three plant families with distinct plant traits in agriculture. First I examined how mixing cover crops in pairs of different species and as mixture of all three species using a replacement design affected cover crop growth, plant N content and soil N leaching in autumn as compared to their monocultures and a fallow. I examined how fertilising cover crops at different N rates, affected cover crop growth and N leaching. Subsequently I examined how mixing (cover crop) species affected decomposition and mineralisation rates, and N leaching and N_2O emissions in spring. Third I tested how 3 to 4 years of cover cropping affected the stable and labile soil C fractions of the soil. Lastly, I showed how cover crop mixtures affected the N efficiency in the system as well as cash crop performance.

In the multi-year field experiment I expected that cover crop mixtures produced a higher biomass and N yield as expected from the monocrops. Additionally I expected that this reduced N leaching during cover crop growth, and potentially increased N leaching after cover crop incorporation. I found that the cover crop mixtures consistently produced a similar amount of biomass as the non-leguminous monocrops. The two species mixtures that contained a legume and a non-legume showed overyielding (i.e. higher yields than expected based on their monocultures) in most years; the two-species mixture containing only non-legumes and the three species mixture did not. None of the mixtures produced a higher biomass than the most

productive monocrop. In agreement with my hypothesis, cover crop mixtures reduced leaching losses during their growth as efficiently as the non-leguminous monocultures (**Chapter 2**). Above a certain biomass threshold, which depended on the year (*i.e.* initial soil mineral N, remaining cash crop residues in soil as well as temperature and rainfall), N leaching during crop growth was reduced to below our detection level. Fertilisation of non-leguminous cover crops in order to kick-start their growth and generate additional N uptake from the soil, did not result in a consistent biomass increase (**Chapter 3**). The recovered amounts of N by the single cover crops were, for all species, lower than the applied amounts. This led to a higher risk of N leaching, especially just after fertilisation. Contrary to what was expected high yielding cover crop mixtures with, on average, a low C:N ratio of their biomass did not cause higher leaching losses during the cash crop growing season. However, high soil mineral N levels measured in March of each year indicate that N could have been leached before main crop planting (**Chapter 2**).

In a meta-analysis on 78 peer reviewed published litterbag studies I examined if mixing litters increased mass loss (as a proxy for carbon loss) compared to single litter decomposition. I found that 2-species litter mixtures on average and in large majority of cases had a similar mass loss as expected from that of the single litters (**Chapter 4**). In 15% of the cases mass loss in mixtures was significantly faster (synergistic) or slower (antagonistic) than expected. Yet, contrary to what I expected and what is often stated in literature, litter trait diversity did not explain variations in these litter mixing effects. Thus, in the large majority of cases litter mixtures decompose as the weighted average rate of the two single species. In a lab incubation I tested how mixing cover crop species residues (above- and belowground) alters the decomposition rate of the residues. The results I found were in line with my findings in the meta-analysis. In both cases CO_2 emissions (a proxy of decomposition rate) from the decomposing mixture was as expected compared to the single residues decomposing. So mixing cover crops will most likely not increase litter decomposition.

A greenhouse experiment in which cover crop mixtures were added to soil (based on the ratios of species in the mixture from the field experiment) showed similar results **(Chapter 5)**. No significant interaction effects of mixing were found on any of the parameters measured (soil mineral N, CO_2 and N_2O emissions as well as cash crop biomass and N concentration). In this greenhouse experiment only the effect of quality, not quantity, of residue addition was tested. Radish and vetch both had a similarly low C:N ratio in their aboveground biomass and after they were added to the soil and decomposed they resulted in similar soil mineral N levels. Yet, surprisingly this led to a stark contrast in N_2O emissions, with emissions being higher after incorporation of radish and surprisingly low emissions for vetch (**Chapter 5**). As expected, wheat planted after incorporation of a cover crop with a high C:N ratio benefit from leaving more time between incorporation of the cover crop and planting of the cash crop due to temporal N immobilisation by the soil microorganisms upon decomposition of the high C:N residues. Contrary to this, earlier wheat planting was beneficial after incorporation of vetch and (especially) radish since N became available quickly and might be otherwise lost to the environment through N_2O emissions (or N leaching) (**Chapter 5**).

After 3 and 4 years of incorporating the mono-culture and mixture cover crops into the field soil, SOM levels remained similar to that of the fallow treatment (no cover crop). SOM was only increased after 4 years of the two species mixture radish + vetch and the three species mixture as compared to the levels at the start of the experiment, whereas in the fallow plots SOM levels tended to decline (**Chapter 6**). Since cover crops have a reasonably high quality (low C:N ratio) it was expected that the additional C sequestered would especially be located in the mineral-associated organic matter (MAOM) pool. However, I did not find this, possibly because the low clay content of the soil in the multi-year field experiment caused the MAOM pool to be close to the saturation limit.

After a 4 year rotation with cover crop monocultures and mixtures included we saw no negative effects of cover crops on the cash crop performance compared to a preceding fallow soil (**Chapter 7**). Positive effects of cover crops were only found for the cover crop mixtures. The vetch + radish mixture and the oat + radish mixture increased potato biomass compared to when planted after a fallow soil. And in 2019 all cover crop mixtures caused a higher barley N yield compared to when planted after the fallow whereas the cover crop monocultures did not. The N efficiency of including cover crops (even when fertilised) in the rotation increased over the years with mixtures (and vetch) showing the highest increase in cash crop yield and mixtures alone gave the highest increase in N uptake (**Chapter 7**).

In this thesis I showed that the two-species mixture of cover crops radish and vetch provide most of benefits in terms of C and N cycling as compared to the benefits of individual species of cover crops which are better in one or the other aspect. This mixture reduced N leaching, provides enough N to the following cash crop and (over time) provides more biomass to the soil and thus have a higher potential for C sequestration. Surprisingly litter trait diversities did not affect mass loss, mineralisation rates or N availability in mixtures. None of the mixtures

produced a higher biomass than the most productive monocrop. Future research should examine if species combinations with less interspecific competition can produce higher yields. Additionally research regarding C sequestration in sandy soils should focus on longer timescales. Since only a small selection of cover crops was tested in this thesis additional species should be studied in mixtures as well as species mixtures that include (more than) 3 species. Given that the C sequestration potential on sandy soil is limited I suggest further research on soils with a higher silt and clay content to assess the true potential of cover crop mixtures to increase C sequestration.

Samenvatting

Om de wereld te kunnen blijven voeden zonder de natuur en omgeving verdere schade te berokkenen is een verduurzaming van de landbouw noodzakelijk. Het gebruik van groenbemesters buiten het groeiseizoen kan helpen bij deze verduurzaming: groenbemesters kunnen tijdens hun groei stikstof (N)-verliezen verminderen en, wanneer ze na de groei worden ondergewerkt, kunnen ze nutriënten verschaffen aan het opvolgende hoofdgewas en bijdragen aan verhoging van het organische stof (OS)-gehalte van de bodem. Vanuit natuurlijke ecosystemen weten we dat mengsels van plantensoorten met complementaire eigenschappen grondstoffen beter kunnen benutten dan individuele soorten, en daardoor meer biomassa produceren. Het is dan ook te verwachten dat mengsels van groenbemesters de opname van N en koolstof (C) in het systeem verhogen ten opzichte van een monocultuur. Het gebruik van mengsels van groenbemesters zou daarom een effectieve strategie kunnen vormen om de N-gebruiksefficiëntie en de C-vastlegging te vergroten binnen landbouwecosystemen. Onduidelijk is echter in welke mate verschillende groenbemestermengsels een effect hebben op N-uitspoeling door het jaar heen en op het OS-gehalte van de bodem.

In dit proefschrift heb ik, met behulp van twee veldproeven, een meta-analyse en een kasproef, onderzocht hoe het mengen van groenbemesters de C en N kringlopen beïnvloedt. Ik heb drie soorten gebruikt, bladrammenas, haver en wikke, die veel in de landbouw gebruikt worden en die voortkomen uit families met verschillende eigenschappen. Ten eerste heb ik verschillende soortencombinaties (alle mogelijke paren en een mengsel van alle drie de soorten) vergeleken met monoculturen en met braakliggend terrein. Daarbij heb ik gekeken naar de groei en het N-gehalte van de groenbemesters en naar N-uitspoeling uit de bodem in het najaar. Ook heb ik onderzocht hoe verschillende N-bemestingsgiften de groei en N-uitspoeling onder deze groenbemesters beïnvloedde. Vervolgens heb ik het effect onderzocht van mengen van groenbemesters op de afbraak- en mineralisatiesnelheden, alsook hun effect op N-uitspoeling en lachgas (N_2O)-uitstoot gedurende het voorjaar. Als derde heb ik gekeken hoe 3 tot 4 jarig gebruik van groenbemesters de stabiele en labiele C-fracties in de bodem verandert. Tenslotte heb ik laten zien welke effecten mengsels van groenbemesters hebben op de N-efficiëntie van het landbouwecosysteem en op de groei en prestatie van het hoofdgewas.

In de meerjarige veldproef verwachtte ik voor mengsels van groenbemesters een hogere biomassa en N-opname, dan verwacht zou mogen worden op basis van de resultaten voor monoculturen (overproductie). Daarnaast was er de verwachting dat dit vertaald zou worden naar lagere N-uitspoeling tijdens de groei van de groenbemesters, en wellicht naar een hogere N-uitspoeling na de groei. Door de jaren heen produceerden de mengsels een biomassa die gelijk was aan de niet-vlinderbloemige groenbemesters (bladrammenas en haver). Wanneer het mengsel bestond uit een vlinderbloemige (wikke) en een niet-vlinderbloemige soort was er voor de meeste jaren sprake van overproductie. Voor de mengsels met bladrammenas en haver en met alle drie de soorten werd geen overproductie vastgesteld. Geen van de mengsels had een hogere opbrengst dan de productiefste monocultuur. Zoals verwacht waren mensgels van groenbemesters net zo effectief in het verminderen van N-uitspoeling als monoculturen van niet-vlinderbloemige soorten (**Hoofdstuk 2**). Boven een bepaalde drempelwaarde voor biomassa, die jaarafhankelijk was (d.w.z. varieerde door verschillen in N in de bodem en plantenresidu van het hoofdgewas, maar ook door temperatuurs- en neerslagverschillen), waren N-verliezen door uitspoeling tijdens de groei van de groenbemesters gereduceerd tot onder de detectielimiet. Bemesting van niet-vlinderbloemige soorten om de groei en N-opname te stimuleren resulteerde niet in een hogere biomassa (**Hoofdstuk 3**). De N-opname door monoculturen was voor elk van de soorten lager dan de N-bemesting. Hierdoor nam het risico op N-uitspoeling toe, vooral direct na de bemesting. In tegenstelling tot de verwachting was er bij mengsels van groenbemesters met een hoge opbrengst en een lage C:N-verhouding geen verhoogde N-uitspoeling zichtbaar tijdens de groei van het hoofdgewas. De in maart gemeten hoge minerale N-concentraties in de bodem kunnen echter duiden op verhoogde N-uitspoeling voordat het hoofdgewas werd ingezaaid (**Hoofdstuk 2**).

In een meta-analyse van 78 wetenschappelijke studies naar de afbraak van plantenresten heb ik onderzocht of het mengen van verschillende soorten plantenresten resulteert in een groter massaverlies (als indicator voor C-verlies) dan bij plantenresten van één bron. Ik vond dat het massaverlies bij plantenrestenmengsels van twee verschillende soorten, gemiddeld en in de meeste gevallen, vergelijkbaar was met de verwachte afbraak gebaseerd op verliezen bij individuele soorten (**Hoofdstuk 4**). In 15% van de gevallen verliep de afbraak van mengsels significant sneller (synergetisch) of langzamer (antagonistisch) dan verwacht. Tegen mijn verwachting in, en in tegenstelling tot wat vaak in de literatuur wordt gerapporteerd, kon het effect van plantenrestenmengsels niet verklaard worden door de eigenschappen van de plantenresten. In de meeste gevallen was de afbraak van mengsels dus gelijk aan de gemiddelde afbraak van de individuele soorten. In een incubatiestudie heb ik getest hoe het mengen van (zowel boven- als ondergrondse) residuen van groenbemesters de afbraaksnelheid van deze resten beïnvloedt. De resultaten uit deze proef zijn in overeenstemming met die uit de meta-analyse: in beide studies was de kooldioxide (CO_2)-uitstoot (een indicator voor de afbraaksnelheid) vergelijkbaar met de gemiddelde afbraak van de individuele soorten. Het is daarom onwaarschijnlijk dat mengsels van groenbemesters een effect hebben op de afbraak van plantenresten.

Ik heb vergelijkbare resultaten gevonden in een kasproef waarin ik mengsels van groenbemesters aan bodem heb toegevoegd (gebaseerd op de biomassa-verhouding van de individuele soorten in mengsel uit de veldproef; **Hoofdstuk 5**). Er was geen significant interactie-effect van mengsels op de gemeten parameters (mineraal N in de bodem, CO_2- en N_2O-uitstoot, alsook opbrengst en N-opname van het hoofdgewas). In deze kasproef heb ik alleen het effect van de kwaliteit, niet kwantiteit, van de toegevoegde plantenresten getest. Bladrammenas en wikke hadden beide een lage C:N-verhouding in de bovengrondse biomassa, en hun afbraak na toevoeging aan de bodem resulteerde in een vergelijkbaar mineraal N-gehalte in de bodem. Toch was er een verrassend verschil in N_2O uitstoot te zien, waarbij de uitstoot hoger was voor bladrammenas en opmerkelijk laag voor wikke (**Hoofdstuk 5**). Zoals verwacht had het hoofdgewas (tarwe) dat gezaaid was na groenbemesters met een hoge C:N-verhouding (haver) baat bij een langere tijdsperiode tussen het onderwerken van de groenbemester en het zaaien van het hoofdgewas. Dit komt waarschijnlijk door tijdelijke N-vastlegging die optreedt bij de afbraak van plantenresten met een hoge C:N-verhouding door micro-organismen. Daarentegen was na wikke en (in het bijzonder) na bladrammenas een vroeg zaaitijdstip juist gunstig voor het hoofdgewas, omdat de lagere C:N-verhouding van deze soorten zorgt voor een snellere N-beschikbaarheid die kan leiden tot hogere N_2O-uitstoot en N-uitspoeling als het vrijgekomen N niet wordt benut (**Hoofdstuk 5**).

Na 3 en 4 jaar aan gewasrotaties waarin monoculturen en mengsels van groenbemesters waren ondergewerkt, waren de OS-gehaltes van de bodem vergelijkbaar met die in de controlebehandeling (zonder gebruik van groenbemesters). Alleen het OS-gehalte na 4 jaar in groei van de behandeling bladrammenas + wikke en het 3-soorten mengsel waren verhoogd ten opzichte van die bij de start van de proef, terwijl de gehaltes in de controlebehandeling leken af te nemen (**Hoofdstuk 6**). Omdat de groenbemestersresten van redelijk hoge kwaliteit waren (dus met een lage C:N-verhouding) was de verwachting dat de extra vastgelegde C zich voornamelijk zou bevinden in de mineraal-geassocieerde C-fractie (MAOM). Dit heb ik echter niet teruggevonden in dit experiment, mogelijk omdat deze MAOM-fractie al verzadigd was door het lage kleigehalte van de bodem.

Na een vierjarige gewasrotatie met monoculturen en mengsels waren er geen negatieve effecten van groenbemesters op de groei en prestatie van het hoofdgewas zichtbaar ten opzichte van de controlebehandeling (**Hoofdstuk 7**). Positieve effecten van groenbemesters werden alleen gevonden voor de mengsels. De combinaties bladrammenas + wikke en haver + bladrammenas verhoogden de biomassa van aardappels in vergelijking met de controlebehandeling. In 2019 was er bij alle mengsels een hogere N-opname door gerst, terwijl voor monoculturen dit effect uitbleef. Het meenemen van groenbemesters zorgde voor een verhoging van de N-efficiëntie (ook wanneer er N bemest werd) in de rotatie gedurende de veldproef. De mengsels van groenbemesters (en de wikke-monocultuur) zorgden voor de grootste toenames in opbrengst en N-opname van het hoofdgewas (**Hoofdstuk 7**).

In dit proefschrift heb ik laten zien dat het mengsel van bladrammenas en wikke de meeste voordelen geven met betrekking tot de C en N kringlopen, ten opzichte van de monoculturen. Dit mengsel verminderd verliezen door N-uitspoeling, verschaft genoeg N aan het hoofdgewas, brengt meer biomassa in de bodem (over langere tijd), en legt mogelijkerwijs dus meer C vast. Verrassend genoeg leidden de verschillende eigenschappen van de gewasresten in mengsels niet tot verschillen in massaverlies, mineralisatiesnelheid en N-beschikbaarheid. Geen van de mengsels had een hogere biomassa dan de meest productieve monocultuur. Vervolgonderzoek zou moeten onderzoeken of soortencombinaties met een lagere competitie tussen soorten kunnen leiden tot hogere opbrengsten. Daarnaast zou onderzoek naar C-vastlegging in zandgronden zich moeten richten op effecten over een langere termijn. Aangezien ik in dit proefschrift slechts een klein deel van de groenbemesters heb getest zouden ook andere soorten bestudeerd moeten worden, alsook verdere mengsels met (meer dan) drie soorten. Omdat de opties voor C-vastlegging in zandgronden gering zijn stel ik voor dat toekomstige proeven gedaan worden op bodems met hogere kleigehaltes, zodat de werkelijke potentie van groenbemestermengsels om C vast te leggen in kaart kan worden gebracht.

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Lore from the moment I met you in the bus on our fieldtrip to the coast in England in 2009 I knew we would be great friends! I still can’t help laughing whenever I remember your very proud face that you brought a warm pasta dish in a thermos….and then you realised you forgot your spoon. I know we don’t see each other often by always living in other countries, but every time we meet up it feels as good and happy as before! I hope we will continue being friends for a long time to come.

Laura, thank you for improving my quality of life in Wageningen in the last year! I’m not sure I would have enjoyed it as much without you as a running, drinking and most of all dinner buddy! Finally I meet someone who is as (or more!) excited about Christmas as me! Thank you for being my paranymph! I am so grateful that you and Mees are always up for a drink! I cannot imagine staying in Wageningen if you were not here now to cheer things up.

Mart and Juan, I think you guys know I couldn’t have done this without you! You are my best friends and I consider you my family. Mart, Thank you for everything that we have experienced together, all the drinks, the bad tv…the reminders of correct Dutch spelling (Thank you for translating my summary to Dutch! I even learned a new word while reading it…). I am so grateful that we are still friends after the time you spend away in Cornell. Thank you for being my paranymph. And in the spirit of not saying goodbye, I am excited to be your colleague once more at WEnR ☺. At least this will give ample opportunity for winter camping sometime in the future! Juan-Carlos, you inspired me in terms of your amazing work ethic, you made me stronger by believing in me and most of all, you made me feel happy again and ready to face new challenges and experiences. I will not forget your excited exclamation when you see a cat in the street (Wooaaa!) and the words speciaal, geniaal and taal will never sound the same again… I cannot thank you enough for the time we spent together. Although, I am sure life will be (a little) healthier now you are on the other side of the ocean. I am happy we can stay in touch and I hope to see you soon in Costa Rica. Gracias por todo y pura vida 😉!

Ten slotte mijn familie: Rens en Josiena bedankt voor de sporadische gezellige weekenden in het hoge Noorden, tja… Groningen is ver. Ik heb erg genoten van onze logeerpartijtjes, laten er nog vele komen zodat ik ook Mayla beter leer kennen. Papa en mama bedankt voor jullie onaflatend vertrouwen en support. Papa, heerlijk hoe je mijn werk zo interessant vind! En als we even moeten ontspannen komt het (praten over) hardlopen en een biertje natuurlijk altijd van pas. Mama, bedankt dat je altijd een luisterend oor te bieden hebt. Ik vind het bewonderenswaardig hoe je altijd zo lief, geduldig en gezellig bent en bovendien doet het me altijd goed om eens aan heel wat anders te denken (winkelen, wandelen, peuters…). Zonder zulke lieve en gezellige ouders was ik nooit zover gekomen. En ten slotte, bedankt dat jullie (bijna) altijd mee willen gaan in mijn nieuwe avonturen. Het was fantastisch om tijdens de coronatijd elkaar (nog) beter te leren kennen in Siddeburen. En dat jullie de laatste loodjes van mijn PhD van dichtbij hebben meegemaakt. Bovendien heb ik ontzettend genoten van ons avontuur deze zomer op het Pieterpad!

About the author

Rima Jasmijn Porre was born on the 24th of May, 1989 in Siddeburen, the Netherlands. Here she grew up in between the agricultural fields with a big garden which helped her enthusiasm grow for the outdoors. At the end of secondary school she was fascinated by all things “exciting” in nature such as volcanoes and tsunamis. Equally, she grew a passion for travel and adventure and thus she decided to study the bachelor Environmental Geosciences abroad at the university of Bristol in the United Kingdom. During her bachelor degree, the course which seemed a little boring, “soil sciences” surprisingly interested her the most. She was fascinated by the interplay of chemistry, biology and physics in the soil. This made her chose “Methane emissions from a chrono sequence of restored peatlands” as bachelor topic. After completion of her bachelor she decided to continue her master’s degree at Wageningen university (NL) with a specialisation of soil chemistry and biology. Here she really enjoyed her master thesis on “the effects of mesofauna in soil on nitrous oxide emissions” which resulted in a publication. During her PhD Rima enjoyed working on multiple projects at once, where supervision of students played a strong role. During the “corona lockdown” she discovered how much she really enjoyed data-analysis and writing. Of course, chats with family and friends over beers were also essential. Her happiness is made complete when new goals are set regarding hiking, running and other ambitious feats in nature. Rima is currently working as researcher soil and climate at Wageningen Environment & Research .

PE&RC Training and Education Statement

With the training and education activities listed below the PhD candidate has complied with the requirements set by the C.T. de Wit Graduate School for Production Ecology and Resource Conservation (PE&RC) which comprises of a minimum total of 32 ECTS (= 22 weeks of activities)

Review of literature (4.5 ECTS)
- Nutrient flows in cover crop mixtures

Writing of project proposal (4.5 ECTS)
- Clever cover cropping – Litter trait diversities and elemental flows

Post-graduate courses (7.1 ECTS)
- Soil ecology; PE&RC (2016)
- Root ecology; PE&RC (2016)
- Basic statistics; PE&RC (2017)
- Meta-analysis; PE&RC/SENSE (2018)

Invited review of (unpublished) journal manuscript (7 ECTS)
- Plant and Soil: substrate addition with different C/N ratios (2016)
- Agriculture ecosystem and environment: nitrous oxide emissions from intercrops (2016)
- Geoderma: earthworm, soil structure and N_2O (2016)
- Journal of soils and sediment: litter mixture decomposition (2016)
- OIKOS: nitrogen biochemistry effects on litter decomposition rates (2018)
- Geoderma: N mineralisation in agricultural soils (2019)
- Geoderma: soil structure and N_2O emissions (2019)

Deficiency, refresh, brush-up courses (6 ECTS)
- Ecological modelling and data analysis in R; WUR (2017)

Competence strengthening / skills courses (3.1 ECTS)
- Teaching and supervising master students; WGS (2017)
- Publishing for impact; WUR (2017)
- Scientific integrity; WIAS (2019)
- Marie Curie workshop; EPS (2019)
- Career perspectives; WGS (2020)

Scientific integrity / ethics in science activity (0.4 ECTS)
- Ethics in plant and environmental sciences; SENSE (2018)
- Scientific integrity; SENSE (2020)

PE&RC Annual meetings, seminars and the PE&RC weekend (1.2 ECTS)

- PE&RC Weekend for first years (2016)
- PE&RC Day (2016)

Discussion groups / local seminars / other scientific meetings (8.6 ECTS)

- Soil biology: from ecological theory to land management; WUR, the Netherlands (2016)
- NERN – NAEM; poster presentations; Lunteren, the Netherlands (2016, 2017)
- Plant-soil interactions; WUR, the Netherlands (2016-2018)
- DISQ thesis ring; WUR, the Netherlands (2016-2020)
- Workshop on cover crop mixtures; Kleve, Germany (2017)
- R-users; WUR, the Netherlands (2018)
- Intercropping interdisciplinary discussion group; WUR, the Netherlands (2019)

International symposia, workshops and conferences (6.4 ECTS)

- SOMmic; poster presentation, Leipzig, Germany (2016)
- Soil organic matter conference; Harpenden, England (2017)
- Soil science society of America, annual meeting; poster presentation; San Diego, USA (2019)

Lecturing / supervision of practicals / tutorials (16.2 ECTS)

- Plant soil interactions (2016-2018)
- Functional agricultural resource management (2017)
- Environmental data collection and analysis (2017, 2018)
- Agrobiodiversity (2019)
- Environmental data collection and analysis (2019)

Supervision of MSc students

- Bob Keurentjes : rooting patterns and leaching
- Tshering Zam: litter mixture decomposition
- Ye Tian: cover crop growth and leaching
- Mirza Haque: optimising and synchronising nutrients
- Goncalo Nascimiento: cover crop N mineralisation
- Leon Brooijmans: cover crop mixture effects on labile/stabile carbon
- Tom Klein Obbink: rooting patterns during cover crop legacy
- Sarah Kemp: decomposition affected by cover crop legacy
- Niek Stoel: cover crop performance over time
- Hendrik Holwerda: linking soil N to leaching of mineral N

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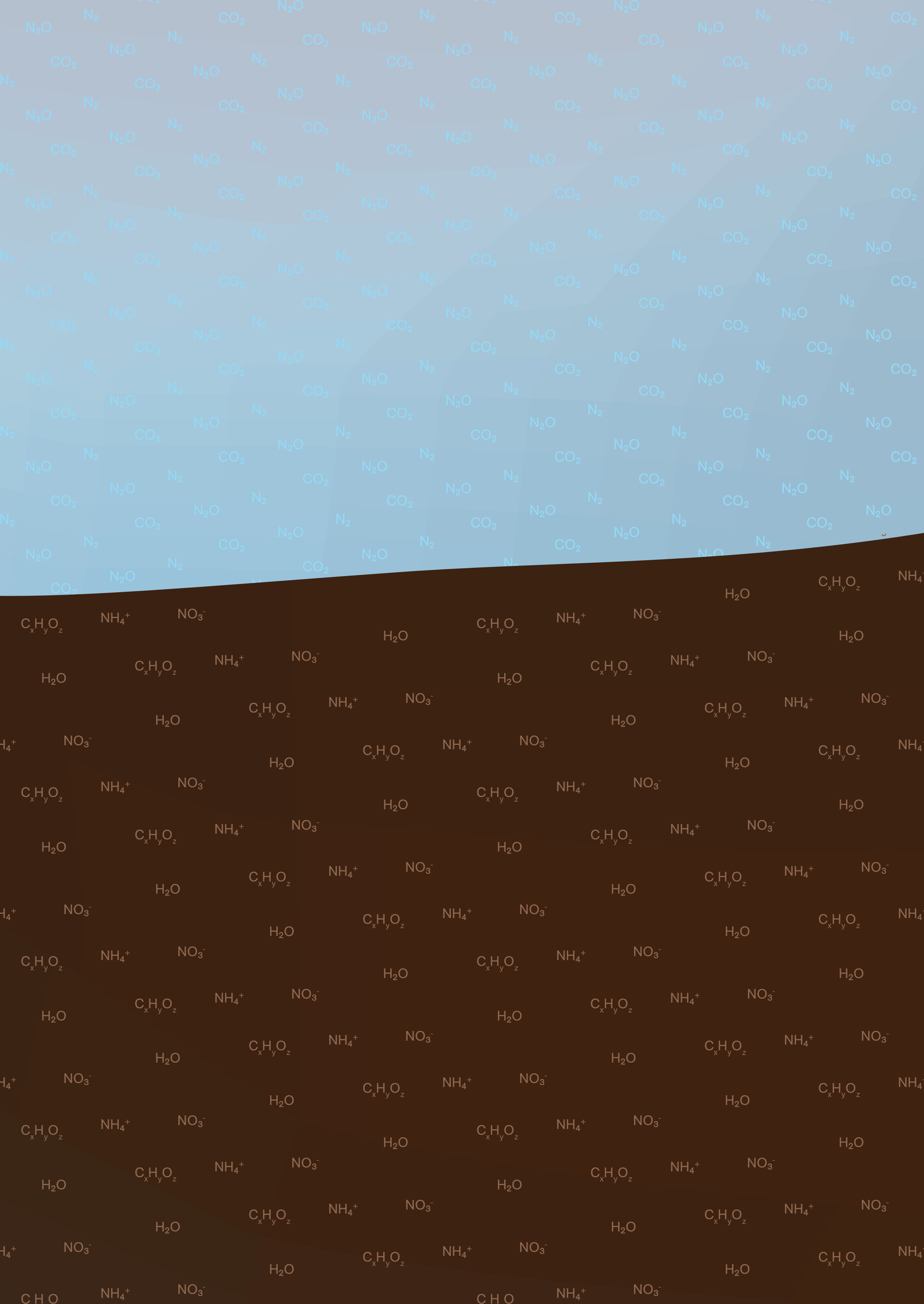
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