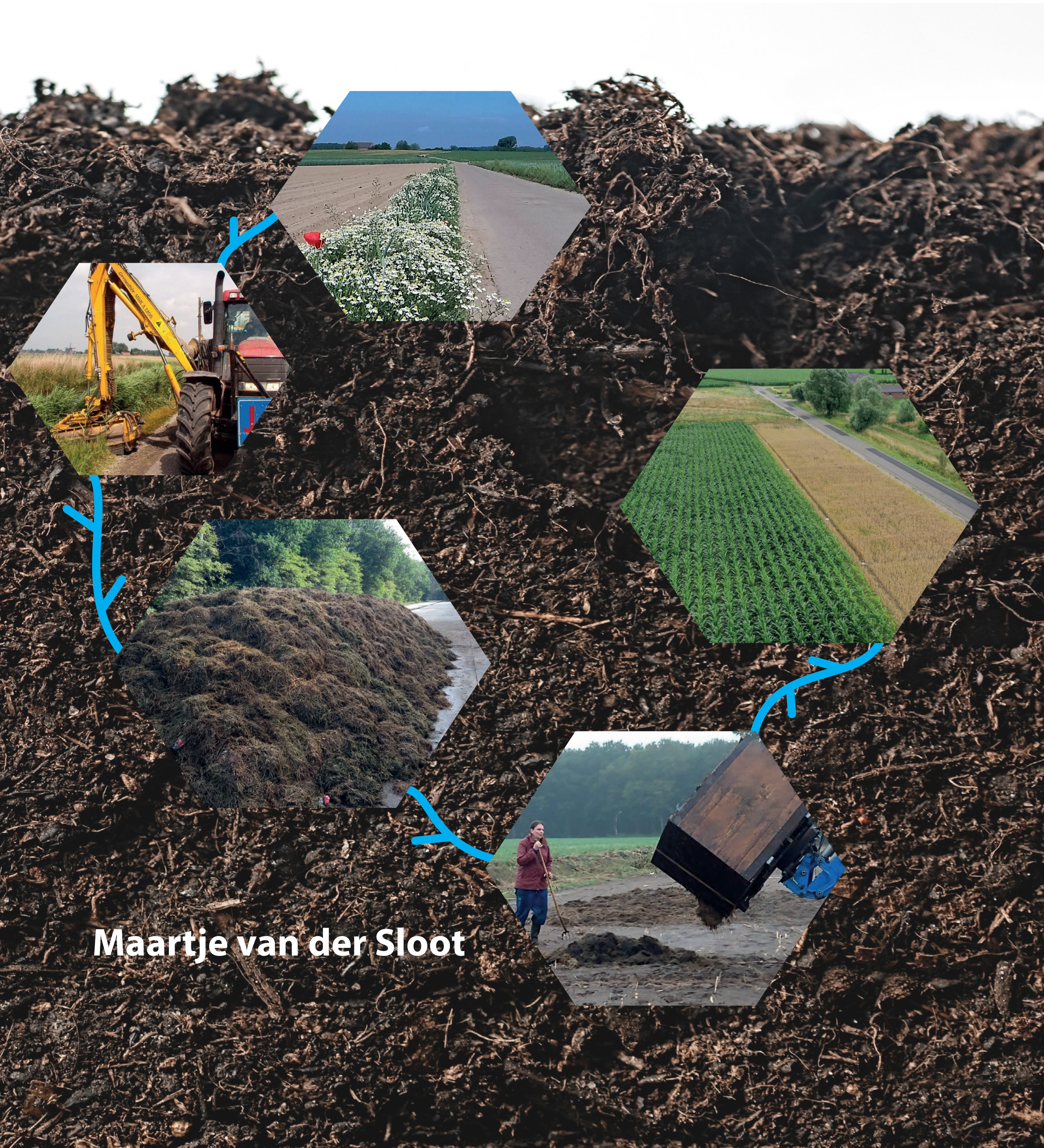
Road verge cuttings as organic amendment on arable fields:

mechanisms, benefits and risks



Maartje van der Sloot

Propositions

1. The benefits outweigh the risks when using road verge cuttings as an organic amendment.
   (this thesis)

2. Fear is the main reason road verge cuttings are currently not used as organic amendment.
   (this thesis)

3. Soil Organic Carbon sequestration without rigorous reduction in global C emissions is insufficient to combat climate change.

4. Scientific studies involving farmers must only be conducted by socially skilled scientists.

5. Making informed choices about which products contribute least to your carbon footprint is too difficult for the average consumer.

6. A musical hobby benefits mindfulness more than stress-management courses.

Propositions belonging to the thesis, entitled

Road verge cuttings as organic amendment on arable fields: mechanisms, benefits and risks

Maartje van der Sloot

Wageningen, 15 November 2023

Road verge cuttings as organic amendment on arable fields: mechanisms, benefits and risks

Maartje van der Sloot

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Road verge cuttings as organic amendment on arable fields: mechanisms, benefits and risks

Maartje van der Sloot

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Chapter 1

General introduction

Maartje van der Sloot

The need for sustainable management of arable soil

The increasing global population is creating a larger demand for food which drives the intensification of agriculture with a single-minded focus to achieve the highest crop productivity possible (Godfray et al., 2010; IPCC, 2019; Lal, 2010). However, these efforts to reach food security also have adverse effects since intensive agriculture practices, such as deep tillage and high usage of (mineral) fertilizers and pesticides, are creating a range of environmental problems including land degradation, biodiversity loss and decreasing soil quality (EEA, 2005; IPCC, 2019; Lal, 2010). These, in turn, are adversely affecting the regulating and supporting ecosystem services on which agriculture relies and may ultimately harm food security. These adverse effects of intensive agricultural practices on soil quality mainly act through their effects on Soil Organic Matter (SOM) content (Knotters et al., 2022; Loveland and Webb, 2003; Matson et al., 1997). The importance of SOM is evidenced by its link to multiple ecosystem functions such as nutrient cycling, water retention, and plant disease suppressiveness (Diacono and Montemurro, 2010; Hoffland et al., 2020; Sullivan et al., 2019; Thiele-Bruhn et al., 2012). Reduction in SOM levels can thereby result in crop yield losses but the relationship between SOM and crop yield is complex since high crop yield is always a combination of sufficient water and nutrients at the right time and protection against plant pathogens and other pests (Hijbeek et al., 2017b, 2018; Lal, 2020).

Outside of negative effects on SOM and related crop yield, intensive farming practices are harming semi-natural habitats bordering arable fields because the high nutrient emissions (especially nitrogen) from agriculture results in flower- and species-poor vegetation in these habitats (Cole et al., 2020). An example of such a semi-natural habitat are road verges which are declining in plant diversity due to eutrophication from both agricultural emissions and cost minimizing practices such as flail mowing without removing the cuttings which reduces their value for pollinators and other invertebrates (Kleijn and Verbeek, 2000; Li et al., 2020). Considering the fact that the total surface area of road verges in The Netherlands is about 60.000 ha (approximately 2% of the land surface area) which can function as connecting habitats between nature areas, it is useful to increase their quality to be a suitable habitat for pollinators and other invertebrates (Raemakers et al., 2001; Schaffers, 2000).

Both the road verge habitat and the declining arable soil can benefit from an interrelated solution. Actively removing cuttings from the road verges will prevent eutrophication and improve species diversity in the road verge (Noordijk et al., 2009) while applying those cuttings in arable soil as organic amendment can simultaneously increase SOM content in arable fields.

A build-up of SOM created by organic amendment addition

Using organic material as soil amendment is an old but useful technique to maintain or increase soil quality via increasing the SOM content. Arable SOM consists for approximately 50 % of carbon (C) (Pribyl, 2010) and can be increased via extra C additions such as organic amendments in agriculture. However, the amount of SOM that increases depends on the amount and type of C applied with the amendment. Organic amendments with easily decomposable C compounds will be decomposed quickly by the soil microbes which results is loss of C as CO^2 after heterotrophic respiration and limits SOM build-up (Ajwa and Tabatabai, 1994; Mohanty et al., 2013). Organic amendments containing larger amounts of recalcitrant C compounds, such as lignin and cellulose, decompose more slowly and tend to increase the SOM build-up more effectively (Ajwa and Tabatabai, 1994; Mohanty et al., 2013). Many different organic materials can be used as organic amendments such as crop residues (Chivenge et al., 2007; Turmel et al., 2015), left-over straw (Xia et al., 2018), green manure (Sharma et al., 2017), or cuttings from semi-natural habitats but their effect varies based on their chemical composition.

Not only the source of the organic material alters the chemical composition and therefore the success in achieving a SOM build-up effect. Organic amendments can also be processed prior to application which affects their composition. Fresh material can be composted, for example, in which aerobic microorganisms decompose the (labile) C compounds of the organic material into CO^2 and water resulting in organic material that consists with higher concentration of recalcitrant C compounds and nutrients (Bernal et al., 1998; Gong et al., 2021; Senesi, 1989; Zmora-Nahum et al., 2005). Next to that, anaerobic fermentation of an organic material via the Bokashi method is thought to break down recalcitrant C compounds which increases the decomposition of the Bokashi when added to soil which might alter the SOM build-up effect as compared to compost (Olle, 2021; Quiroz and Céspedes, 2019).

Additional effects of organic amendments

Apart from influencing SOM build-up, the chemical composition of the used organic amendments also influence soil nitrogen (N) cycling. An amendment with a low carbon: nitrogen (C:N) ratio will supply more immediately available mineral N since the decomposer microbes have N in access. In contrast, amendments with a high C:N ratio result in (temporal) N immobilization as the decomposer microbes are N limited (Bernal et al., 1998; Flavel and Murphy, 2006; Hadas et al., 2004; Kaleeem Abbasi et al., 2015; Mohanty et al., 2013). Organic amendments therefore have the potential to function as slow release fertilizer when the N is mineralized but it can hinder crop production when N is immobilized during periods of crop growth. However, using amendments with a low

C:N ratio to function as fertilizer also has the potential to result in harmful N leaching to groundwater if a large amount of N is mineralized during a period of rainfall without (any) crop N uptake (Malcolm et al., 2019; Steen Jensen and Ambus, 1999). Processing methods (such as composting or fermenting) influence whether N mineralization or immobilization predominates through the effect of the changing C:N ratio of the organic amendment. Achieving a balance between creating a SOM build-up effect while not hindering or even enhancing crop production by releasing N at the right time and preventing N leaching is a main challenge when using organic amendments in agriculture.

An important potential beneficial effect from applying these cuttings as organic amendment is achieving an increase in the soils water retention capacity. The addition of an organic material increases SOM and with that enhances aggregate formation and stability which creates space to hold additional water (Eden et al., 2017). The capacity of the organic amendment to increase SOM is therefore linked to the capacity of the organic amendment to increase the water retention capacity.

Next to that, the addition of an organic amendment is found to improve the general soil suppressiveness which is "the ability of soils to inhibit the growth and activity of soilborne pathogens to some extent, owing to the collective competitive and antagonistic activity of the total soil microbiome competing with the pathogen(s)" according to Schlatter et al. (2017). It improves the soil suppressiveness through enhancing the total soil microbiome in diversity, population size and activity since the soil microbiome decomposes the organic material and uses it as energy source to grow (Bonanomi et al., 2010; Mayerhofer et al., 2021). This effect is, however, highly dependent on the type of material and processing method used since that determines the decomposability of the amendment. It is therefore wise to investigate this relation in realistic circumstances if a new organic amendment is assumed to enhance the soil suppressiveness.

For this application of road verge cuttings, it is important to investigate the potential risks. Especially because the potential users (farmers) do have several fears about this application. Mainly the presence of weed seeds in the material resulting in an increase in weed pressure is according to a study by Kleijn and Verbeek (2000) the main concern. Other contaminations, such as a high concentration of heavy metals due to the close proximity to cars or other anthropogenic litter, could also potentially hamper this application. However, the presence of these risks has not been researched yet, but it is crucial to thoroughly investigate them before implementing this application on a large scale.

General objective of this thesis

In this thesis, I investigate the usage of road verge cuttings as organic amendment in arable soil and provide more knowledge of the underlying mechanisms, potential benefits and risks of this application. Mechanistically understanding the effect of the chemical composition of used road verge cuttings as organic amendments on SOM build-up, the soil microbiome, N cycling and crop production, and potential risks of using road verge cuttings, is necessary to be able to prevent crop production losses in short-term while improving soil quality in longer term. A combination of short-term experiments and a long-term multi-site field experiment (Fig. 1.1) using the same organic amendment treatment allowed me to understand both the mechanistic effects of the organic amendments while also gaining realistic knowledge on the potential benefits and risks.

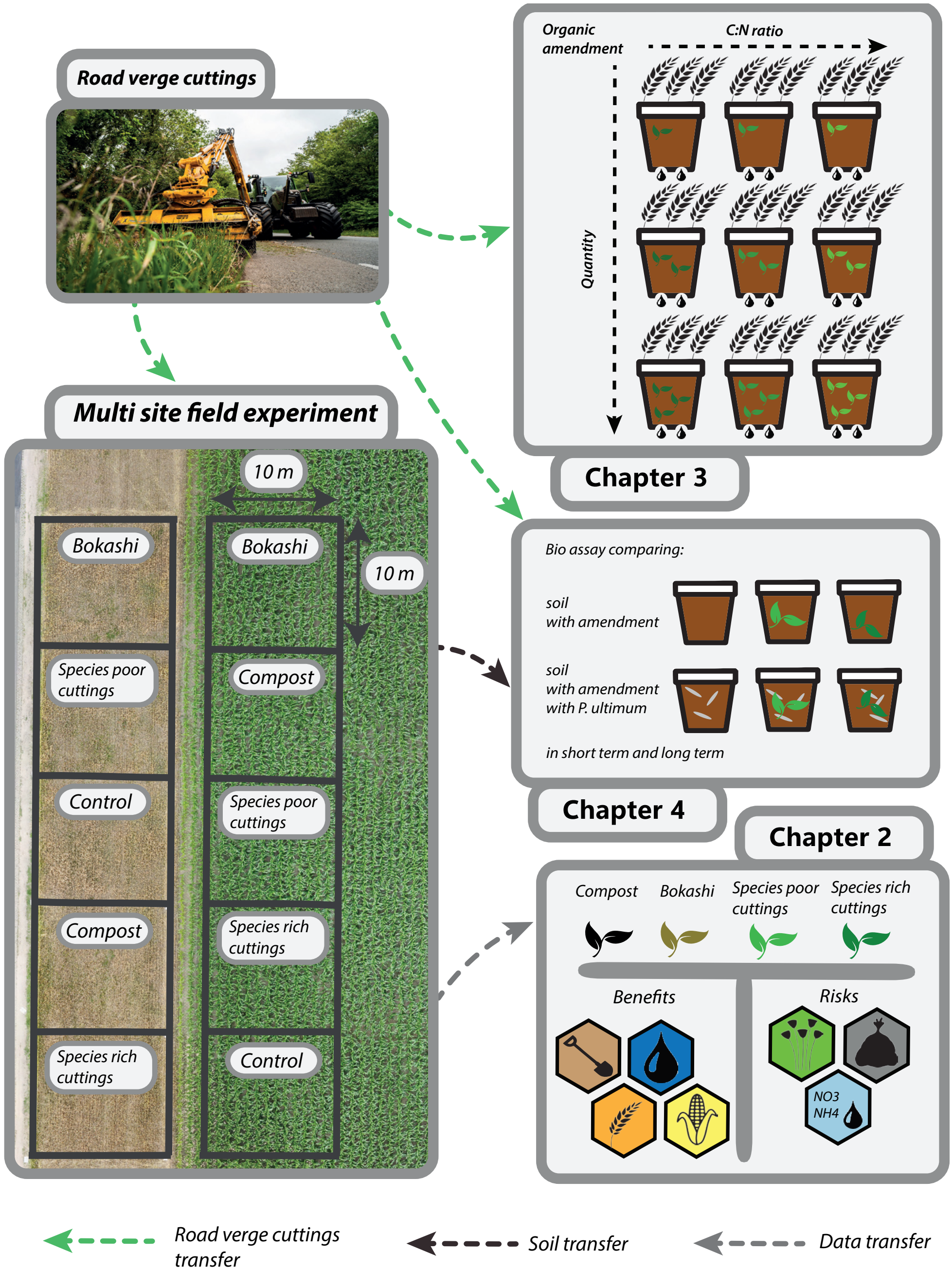


Figure 1.1. Schematic overview of the chapters in this thesis. The same road verge cutting material is used in the mesocosm experiment (chapter 3), the soil disease bioassay (chapter 4) and the multi-site field experiment (green arrows). The soil disease bioassay (chapter 4) uses soil from the multi-site field experiment (brown arrow). Analysis on the overall benefits and risks associated with the application of road verge cuttings as organic amendment (chapter 2) uses all gathered data from the field experiment (grey arrow).

Study system and outline of this thesis

Worldwide, sandy soils cover approximately 900 million ha and an extensive amount of these areas are cultivated (Yost and Hartemink, 2019). However, these soils are known to decline in SOM content which may hamper soil functioning and fertility (Hijbeek et al., 2017b; Johnston et al., 2009; Yost and Hartemink, 2019). These susceptible soils could therefore benefit from extra organic amendment additions. Additionally, the effectiveness of the application of organic amendments to increase SOM has been found to be highest on sandy soils (Chivenge et al., 2007; Hijbeek et al., 2017b; Johnston et al., 2009; Yost and Hartemink, 2019). It therefore makes sense to focus this application on sandy arable soils. To be able to follow an increase in SOM content as a result of organic amendment addition at the soil depth where the amendment was applied (upper 10 cm), no deep tillage was done at the field locations. Minimal soil disturbance was only occurring when cover crops were incorporated in the upper 15 cm in each location which is according to conventional practice and therefore necessary to gain knowledge on the realistic field situation.

All the experiments in this thesis used wheat (*Triticum aestivum*) and maize (*Zea mays* subsp. *mays*) as main crops because these are cultivated extensively on sandy arable soil and especially wheat is sensitive to changes in soil quality (Erekul and Köhn, 2006).

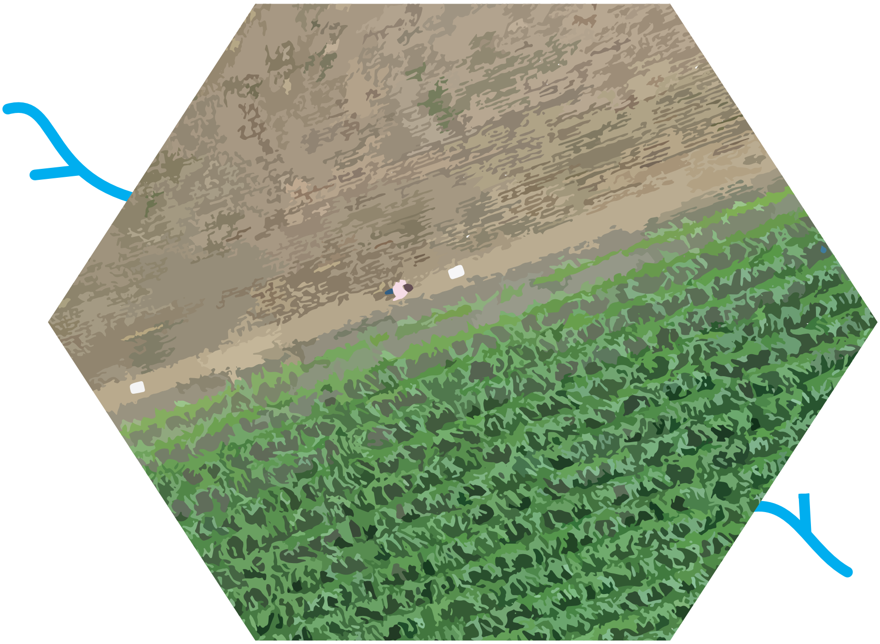
In this thesis, the start material for all the organic amendment treatments were the same, namely road verge cuttings from the municipality of Sint Anthonis (51°37′33″ N, 5°52′52″E; The Netherlands). This allowed me to compare the different studies in this thesis (Fig. 1.1), but it also improved the conclusions I could draw on the processing methods. Since both the compost and Bokashi treatments were derived from the same start material and compared to a fresh amendment treatment and a control without organic addition, this study stands out as the first to assess the impact of processing methods rather than utilizing different materials for each technique.

To further gain knowledge on multiple benefits and risks after application of road verge cuttings as organic amendment, a multi-site field experiment was conducted (**chapter 2**). This experiment contained fifteen field sites, two crops and four different organic amendment treatments and a control treatment. The experiment lasted three years over which SOM content and crop yield were regularly measured. Additional measurements during the experiment allowed us to gain insight of the potential benefits (i.e., an increase in SOM content, water holding capacity, N retention and/or crop yield) and risks (i.e., an increase in N leaching, weed cover and/or heavy metal and litter contamination) of application of road verge cuttings as organic amendments. This realistic field experiment increases the knowledge base on the usage of organic amendments on sandy arable soil greatly.

Chapter 3, explores the mechanisms underlying the effects of applying organic amendments that differ in chemical composition (C:N ratio) and quantity. An open-air mesocosm experiment was conducted mimicking realistic field situations but allowing us to manipulate watering regimes and leachate extraction. The same start material from **chapter 2** was used to create organic amendments that represent a range of different C:N ratios which were applied in different quantities and their combined effect on crop growth, N leaching and soil N retention was investigated. A Structural Equation Model was used to examine the direct and indirect effects of organic amendments C:N ratio and quantity on crop growth, N leaching and soil N retention.

In **chapter 4**, the soil disease suppression capacity of the organic amendments is tested. Organic amendments are known to have the capacity to increase the soils microbiome (in amount and activity) and therefore increase general disease suppressiveness against pathogens (Bailey and Lazarovits, 2003; Bonanomi et al., 2007; Pascual et al., 2000; Scheuerell et al., 2005; Termorshuizen et al., 2006a). However, this is highly dependent on the type of organic amendment and timing after application since the peak in general disease suppressiveness can be expected at the peak of decomposition of the organic amendment. In this study, I used the effect of a *Pythium ultimum* infection on growth of cress (*Lepidium savitum*) seedlings as a bioassay to examine disease suppressiveness of soils from the ongoing multi-site field experiment of **chapter 2**. This design allowed to answer the question if the different organic amendment treatments made from road verge cuttings do increase the soils capacity to withstand the presence of a soil disease.

In **chapter 5**, I synthesize and discuss the results from the previous chapters. The conclusions from the individual chapters can be compared properly because the same organic amendment material is used in all the experiments and chapters (Fig. 1.1). I discuss the sought after effect on SOM build-up as a result of organic amendment application. Also timing effects are discussed because the time period between application of the organic amendments and researched effects, such as N cycling and crop growth, differs between the chapters which allowed us to discuss the short- and long-term effects. The effect of the different organic amendment treatments (compost, Bokashi and fresh species poor and fresh species rich) are compared and conclusions on what treatment to use are explained. Finally, advise for policy changes on the usage road verge cuttings as organic amendment are discussed and ideas for future research are deliberated in this final chapter.



Chapter 2

The potential of using cuttings from semi-natural habitats as organic amendment in arable fields

Maartje van der Sloot,
Juul Limpens,
Gerlinde B. De Deyn,
and David Kleijn

Abstract

Using cuttings from semi-natural habitats as organic amendment on arable fields can potentially enhance soil functioning and improve biodiversity and associated ecosystem services in non-productive landscape elements, such as road verges. Adoption of this management practice is currently low because of lack of quantitative information on both the benefits and risks of these amendments. For three years, we experimentally applied processed (composted or fermented as Bokashi) and fresh road verge cuttings (species poor cuttings and species rich cuttings) in a maize-winter wheat crop rotation on fifteen fields at commercial farms. Plots with organic amendments received half of the conventional mineral fertilizer application rates and control plots without organic amendments received mineral fertilizers at conventional application rates. We investigated the potential beneficial effects on soil organic matter (SOM) content, nutrient cycling, water retention and crop yield and possible risks related to nitrogen (N) leaching and the introduction of weed seeds, heavy metals and anthropogenic litter. The application of composted and fresh road verge cuttings significantly increased SOM content in the upper 10 cm of the soil, while composted cuttings even enhanced SOM in the entire cultivation layer of the soil (0-40 cm). Maize and wheat yields did not differ significantly from the control treatment despite the 50% reduction in applied mineral fertilizer in the organic amendment treatments, proving that equal yield can be achieved with half of the mineral fertilizer when these organic amendments are applied. Application of cuttings did not significantly increase N leaching, weed pressure or exceed heavy metal thresholds that would legally prohibit their use. We did observe significant contamination of the road verge cuttings with anthropogenic litter, primarily beer and soft drink cans and bottles. If litter contamination can be prevented or removed pre- or post-processing, or if cuttings are used from litter-free semi-natural habitats, the application of cuttings on arable fields is promising. It combines benefits for farmers, such as healthier soils and lower fertilizer costs without yield loss, with benefits for society, such as more carbon stored in the soil and a cost-effective way to manage semi-natural habitats in a more wildlife-friendly way.

Keywords: organic amendment, road verge cuttings, Bokashi, compost, soil organic matter content, crop yield, nitrogen leaching, agricultural soil

Introduction

Driven by increased food demands from a growing population, technological advances and world trade, agriculture has rapidly intensified over the past century (Godfray et al., 2010; IPCC, 2019; Lal, 2010). In arable landscapes in North-western Europe, this has resulted in two habitat types that occur side-by-side and suffer from opposite but interrelated problems: arable fields and semi-natural habitats. While arable fields suffer from steadily declining soil organic matter (SOM) stocks (Emerson, 1995; Hoffland et al., 2020; Jarvis et al., 1996; Loveland and Webb, 2003; Thiele-Bruhn et al., 2012), semi-natural habitats bordering these fields suffer from nutrient enrichment from agriculture (especially nitrogen) and cost-minimizing practices (Cole et al., 2020) resulting in flower- and species-poor vegetation that support little biodiversity in these last refuges for farmland biodiversity (Kleijn, 1997; Kleijn and Verbeek, 2000; Li et al., 2020). A solution that potentially enhances biodiversity and ecosystem service delivery on both the arable field and the adjacent semi-natural habitats would be to remove biomass, and thus nutrient excess, from the semi-natural habitats and apply it as organic amendment on arable fields to improve soil functions by increasing SOM (Hoffland et al., 2020; Loveland and Webb, 2003). Adoption of this approach is currently hampered by lack of quantitative knowledge of involved benefits and risks of applying verge cuttings on the arable fields.

Potential benefits of applying cuttings as organic amendment to arable fields are improved soil functions such as water retention and nitrogen (N) cycling, both of which are favourable to crop yield (Malhi et al., 2011; Oldfield et al., 2018). These functions are all tightly connected to the effects of the organic amendments on SOM content which, in turn, depends on the biochemical composition of the organic amendments themselves. For example, organic material containing more soluble organic carbon (C) or a low carbon to nitrogen ratio (C:N ratio < 15) will result in less SOM build-up (Bernal et al., 1998; Mohanty et al., 2013; Tachimoto, 1995). In contrast, organic amendments with a high carbon to nitrogen ratio (C:N ratio > 25) or more recalcitrant C fractions, such as lignin and cellulose, are more suitable for increasing SOM content (Freibauer et al., 2004; Mohanty et al., 2013). Furthermore, organic materials harvested from semi-natural habitats can be composted or fermented prior to their application on arable fields. Because these processes change the chemical composition of the organic amendments, such as C:N ratio, they may moderate the expected SOM increase (Gong et al., 2021; Mohanty et al., 2013; Quiroz and Céspedes, 2019; Zmora-Nahum et al., 2005). An additional benefit of using organic amendments from cuttings of nearby semi-natural habitats is that the nutrients they contain could (partially) replace the use of artificial fertilizers thus resulting in cost savings and reductions in the ecological footprint of farming.

A potential risk of using cuttings from semi-natural habitats as organic amendments on arable fields is related to timing the soil N availability to match crop N demand. Nutrients applied with mineral fertilizer become immediately available to the crop and can be applied just prior to peak crop demands. In contrast, nutrients applied through organic amendments first have to be decomposed and mineralized by the soil microbiome. If cuttings have a high C:N ratio, the available N is immobilized in the soil microbial biomass (Flavel and Murphy, 2006; Hadas et al., 2004; Kaleeem Abbasi et al., 2015; Nicolardot et al., 2001) and less mineral N will be available for crop growth shortly after application (van der Sloot et al., 2022). If amendments are being used with a low C:N ratio, most N will become available shortly after application which, during wet periods, may then possibly leach to the ground water (Malcolm et al., 2019; Steen Jensen and Ambus, 1999).

Besides the risks associated with the N cycle, weeds are the main concern of farmers as approximately half of the farmers interviewed by Kleijn and Verbeek (2000) argued against applying field boundary cuttings in the crop edge on the grounds of increased weed pressure. Such risk can be mitigated by processing cuttings from semi-natural habitats prior to application. Composting, for example, results in high temperatures (about 60 °C) that generally eliminate the germination capacity of the present seeds (Cooperband, 2002; Pace et al., 1995). Fermenting organic amendments may also affect viability of weed seeds, although to what extent this anaerobic process eliminates viable weed seeds is unclear (Merfield, 2012). Apart from weeds, cuttings from semi-natural habitats may contain other contaminations that pose a risk for food or feed production, such as heavy metals or anthropogenic litter (Brus and Jansen, 2004; Dach and Starmans, 2005), especially when these habitats are located in public space with a lot of human activity such as road verges or parks.

In this study, we quantify the benefits and risks of applying cuttings from semi-natural habitats as organic amendments on arable fields with sandy soils. We use an experimental plot-based approach on arable fields of fifteen different farms. The farm fields have different management histories and environmental conditions, enabling us to obtain estimates of the effects of the organic amendments that are representative for commercial farming practice in the south-eastern part of the Netherlands. We use cuttings from road-side verges because it represents an easily accessible source of organic material that at the same time has a relatively high risk of containing heavy metal and litter contaminations which could be considered a worst-case scenario with respect to the use of cuttings from semi-natural habitats in general. We examine two types of processed cuttings (composted and fermented via the Bokashi method) and two types of unprocessed cuttings (species poor, grass-dominated vegetation and species rich more forb dominated vegetation) as this may affect the biochemical composition of the amendments and thus affect both the benefits and risks.

We aim to answer the following specific research questions:
1) What are the benefits (potential increases in SOM content, water holding capacity, N retention, crop yield) and risks (potential increases in N leaching, weed pressure, heavy metal and anthropogenic litter contamination) of applying organic amendments to arable sandy soils?

2) Are the risks and benefits affected by the processing method of the organic amendments (fresh material vs. processed through composting or fermenting)?

Material and methods

Field locations and experimental design

To obtain results that would be representative of Dutch arable fields on sandy soil we made use of fifteen fields from fifteen different farmers scattered across the central and south-eastern parts of the Netherlands (Fig. 2.1A). The field experiment ran from September 2019 until September 2022. The years 2020 and 2022 were exceptionally warm and dry with average temperatures of 11.7 °C and 11.6 °C respectively while 2021 was considered normal with an average temperature of 10.4 °C (average for Netherlands in the period 1991 – 2020:10.5 °C ; (KNMI, n.d.).

A) **B)**

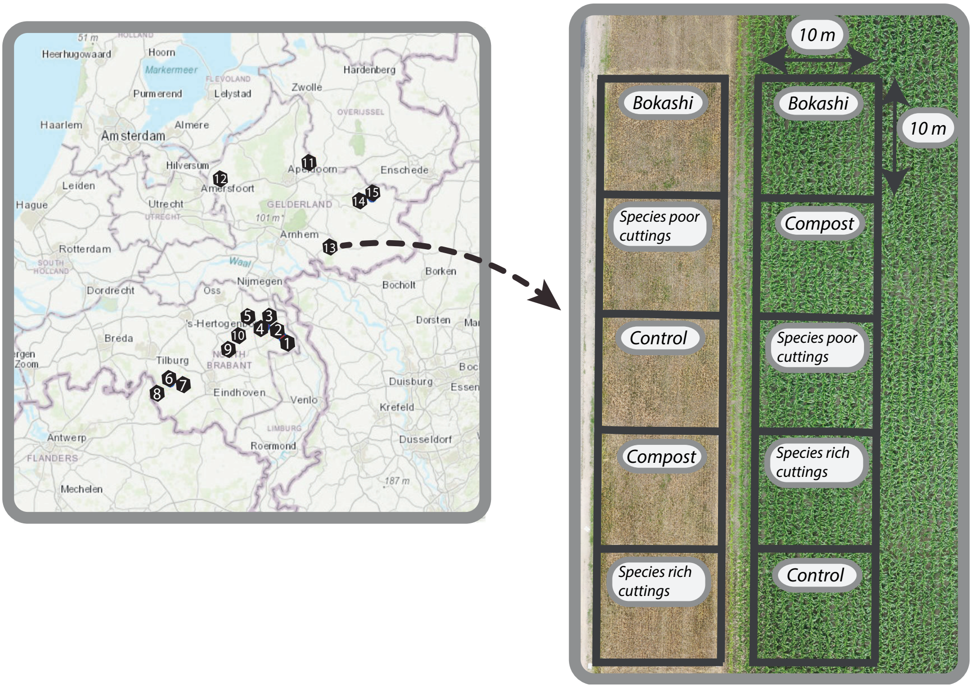


Figure 2.1. A) Locations of the study sites and **B)** Schematic overview and lay-out of the experimental treatments in one of the study sites (nr 13).

Within each field we established a nested design of ten 10 m x 10 m plots organised in two rows. The plots within each row were subjected to the same crop rotation and were randomly assigned one of the five treatments (Fig. 2.1B). The two rotations consisted of the same two crops, however, in one row the rotation started with silage maize (*Zea mays*) and in the other, it started with winter wheat (*Triticum aestivum*). These crops rank first and third in most widely cultivated crops in the Netherlands and are extensively grown worldwide (FAO, 2023). Furthermore, winter wheat is known to be sensitive to changes in soil quality (Erekul and Köhn, 2006) and could therefore be a good indicator of soil quality changes resulting from the organic amendment applications. Winter wheat (hereafter wheat) was sown each year in November and harvested in July. Over the fifteen locations, five different wheat varieties were used of which 'Benchmark' and 'Henrik' were most frequently sown at an average rate of 160 kg seeds/ha. Maize was sown in May and harvested in September. Seven maize varieties were used (mainly 'Pioneer' and 'LG31205') with on average 92.000 seeds/ha. In between the wheat harvest and the sowing of the maize, a cover crop was grown to counteract potential N leaching, in line with agricultural regulations in the Netherlands. Different cover crops were used on the different fields, following farmer preference, but the most frequently used ones were winter wheat (*Triticum aestivum*), winter rye (*Secale cereale*), or radish (*Raphanus sativus* subsp. *oleiferus*). In line with conventional management, the cover crops were incorporated into the soil in April prior to sowing the maize and we assumed that all nutrients fixed by the cover crops were retained in the plant-soil system. All activities that took place in the field experiment are visualized in Fig. 2.2.

Organic amendment treatments

The study compared five treatments: four organic amendment treatments in the form of two different types of unprocessed, fresh cuttings and two different types of processed cuttings (compost and Bokashi), and one control treatment that received no organic additions. The cuttings used for the four organic amendment treatments all came from the same source, roadside verges in the municipality of Sint Anthonis (51°37'33"N, 5°52'52"E), which enabled us to directly compare the effects of the different types of organic amendments. The two processed cutting treatments were both made from cuttings collected in June each year. The cuttings were collected in the first meter of conventionally managed road verges, as part of management to ensure road safety. About 75% of the cuttings were used for composting while 25% were used for fermenting. For composting the material was piled up and turned weekly. Temperature was monitored during the composting process. When high temperatures (roughly above 40 °C) were no longer detected (after approximately eight weeks) we considered the composting process to be completed. By the end of the composting process, the initial biomass of the cuttings was reduced to one third due to the loss of C and water. For Bokashi, the fresh cuttings

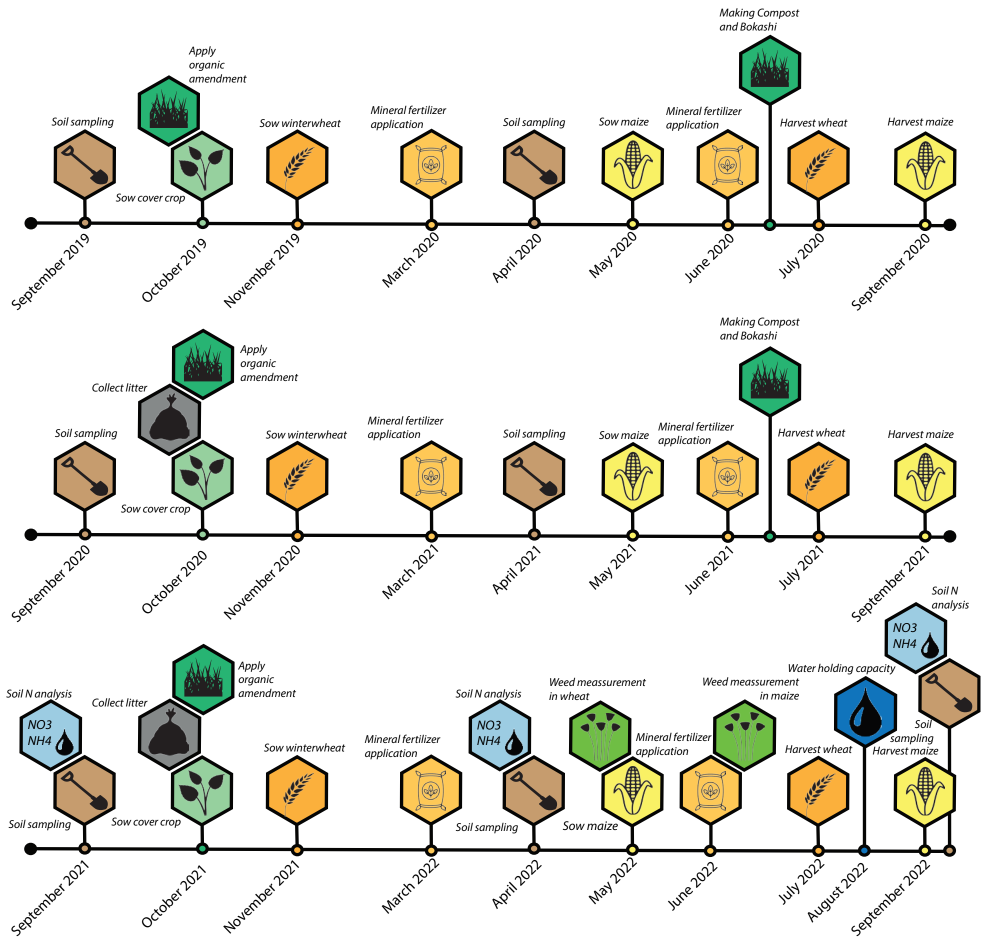


Figure 2.2. Timeline of all practices in the field experiment from September 2019 until September 2022. Cover crops were sown in the cropping cycle between wheat and maize, or prior to the first maize crop.

were fermented under anaerobic conditions (Bij de Oorsprong, 2021; Shin et al., 2017). The fermentation was initiated by adding microorganisms from BB Boden (Multikraft, n.d.) (lactic-acid bacteria) and eMB starter (Multikraft, n.d.) (bacteria that break down cellulose) to the fresh material. Pulverized calcareous shells were added together with the bacteria to prevent acidification due to the fermentation process. The fermentation process also took eight weeks, after which the Bokashi was ready to be applied. Since hardly any C or water is lost during fermentation, the amount of biomass prior to fermentation is very similar to that of the end product. The unprocessed fresh cuttings comprised one from conventionally managed (cutting once a year in fall and leaving the cuttings), grass-dominated herb-poor road verges (species-poor cuttings) and one from verges that had been sown with a wildflower mixture in autumn 2016 and from which cuttings had been removed after mowing ever since (species-rich cuttings). These cuttings were collected in September each year as part of regular road verge management and directly applied to the experimental plots (Fig. 2.2).

All organic amendment treatments were applied annually in the period September-October (Fig. 2.2) at a rate equivalent to 30 tons fresh weight/ha. We chose to standardize the applied amount of the treatments on fresh weight basis because this is most useful in practice as it enables to compare treatment effects of the organic amendments given a certain amount of added mass, instead of using specific chemical concentrations in dried material which are not measurable for most farmers. Each year, just before application of the organic amendments, multiple subsamples were taken from each of the four amendment treatments, pooled in polyethylene bags, and stored at -4 °C prior to analysis following protocols described below for the crops. However, in the first year (2019), organic amendments were stored in paper bags which influenced the moisture percentage needed to calculate amount of C and N applied on each plot. We therefore used only the data from 2020 and 2021 to calculate averages of kg C /plot and kg N/plot (Table 2.1). After spreading out the amendments on the plots, the organic material was incorporated in the upper 10 cm of the soil using a disk harrow. The organic amendment plots were fertilized with half the amounts used in conventional farming (i.e. 90 kg N/ ha). Mineral fertilizers consisted of a combination of NPK fertilizer (12% N which was 50-50 ammonium-nitrate, 10% P, and 18% K) and Limestone Ammonium Nitrate (27% NH_4NO_3 + 6% CaO) during main crop growth. The control treatment received the full conventional load of 180 kg N/ha/year for both crops during main crop growth. Experimental plots with wheat were fertilized every year in April (NPK application) and June (Limestone Ammonium Nitrate application). Experimental plots with maize received all mineral fertilizer in June only. Apart from fertilization and organic amendment application, management of the field sites followed the standard practices of the farmers.

Table 2.1. Chemical composition of the used organic amendments. Data shows average for samples over several years. For C:N ratio, g C/ kg dw, g N / kg dw data was used from 2019, 2020 and 2021 (n=18), while % Lignin (measured in ADL fraction), pH, kg C /plot, kg N / plot used data from 2020 and 2021 (n=12).

Organic amendment treatments	C:N ratio	g C / kg dw	g N / kg dw	% Lignin (ADL)	pH	kg C / plot	kg N / plot
Compost	13.7	252.1	18.3	75.1	8.02	21.8	2.0
Bokashi	30.4	343.8	11.3	34.6	6.17	33.5	1.0
Species poor cuttings	24.4	381.0	15.6	33.9	6.25	42.4	1.6
Species rich cuttings	30.7	378.2	12.3	33.4	6.24	57.5	1.6

Soil measurements

From 2019 to 2022, we sampled the soil annually in September just before the organic amendment applications, and in April right before sowing the maize (Fig. 2.2). Five soil samples were collected from the top 10 cm in the inner 2 m x 2 m of each plot using a 3.5 cm diameter soil auger and pooled in a polyethylene bag. To estimate the effect on the entire crop cultivation layer we additionally sampled the 10-40 cm layer in the last year of the study (September 2021, April 2022, and September 2022). All bags were stored at -4 °C prior to further chemical analysis.

In each of these samples (both samplings depths), soil pH SOM and total N was determined. pH was determined in a demi-water extraction using a WTW inoLAb pH/mV meter. The SOM content was assessed via the loss on ignition method (Hoogsteen et al., 2015). Total N content was analysed by creating a digestate with a mixture of H_2SO_4-Se and salicylic acid according to (Novozamsky et al., 1983). The digestate was then analysed for total N content with a segmented-flow analyser (Skalar San++ system). Mineral N (NO_3-N, NH_4-N) content was analysed for each plot only during the last year of the experiment for both layers according to standard procedures (Temminghoff, 2010) in a 1:10 (w/v) ratio with a 0.01 M $CaCl_2$ solution at 20 °C. Concentration of mineral N in the extracts was analysed with a segmented-flow analyser (Skalar San++ system).

In each soil sampling round, we assessed bulk density and moisture content by taking a single soil sample from the centre of each plot using a metal volumetric cylinder (5 cm high and 5 cm diameter). The soil core was transferred to a polyethylene bag and the fresh soil weight was determined. The soil was subsequently dried at 105 °C for 48 hours and then weighed again. The bulk density was expressed as the ratio of dried soil to the total volume of the cylinder (Al-Shammary et al., 2018) while the soil moisture content was expressed as percentage weight loss.

Soil water holding capacity was measured at the end of the experiment in August 2022 (Fig. 2.2) (Cassel and Nielsen, 1986) using samples from all plots in 10 fields only. A metal volumetric cylinder (5 cm high and 5 cm diameter) was placed into the soil with the top edge to the soil surface level. The cylinder was taken out with an intact soil core and taken to the lab. All cores were completely saturated with water in the lab and were placed on a grid for 48 hours to allow leaching of water that could not be retained by the soil which resulted in soils at field capacity. The cylinders at field capacity were weighed. Then, the cylinders were dried for 24 hours at 105 °C and weighed again and water holding capacity was expressed as the ratio between the cores at field capacity and dried cores:

$$Water\ Holding\ Capacity = \frac{Weight\ of\ wet\ soil\ at\ field\ capacity\ (g)}{Weight\ of\ dried\ soil\ (g)}$$

Crop measurements

Each year before harvest (Fig. 2.2), wheat plants were cut at the soil surface from three sub-plots of 0.5 m x 0.5 m located in the central 4 m x 4 m of each plot, which were pooled for further analysis. Maize plants were cut at the soil surface in three 1 m long subplots that contained a single row of plants, which were pooled for further analysis. Additionally, for maize, we determined between-row distance to be able to calculate crop yield per area. The grains of the wheat and the cobs of the maize were separated from the rest of the plant and weighed. N content of the different yield components were assessed in the last year only. This was done by taking a subsample of 0.5 gram of dried and grinded crop and digest it with a mixture of H_2SO_4-Se and salicylic acid according to (Novozamsky et al., 1983). The digestate was then analysed for total N content with a segmented-flow analyser (Skalar San++ system).

Quantifying contaminations

Weed pressure and weed species richness were assessed in May 2022 in all wheat plots and in June 2022 (Fig. 2.2) in all maize plots by randomly placing two quadrants of 60 cm x 60 cm within each plot and assessing the percentage cover of all plant species.

Heavy metal contamination was determined for a subset of metals with the highest health risk according to Dutch legislation for compost. Heavy metal concentrations in the organic amendment treatments were measured in 2021 by taking six subsamples from each of the organic amendment treatments. These samples were dried at 70 °C for three days and grinded prior to analysing. The concentrations were measured using a HNO_3-HCl-H_2O_2 destruction method (White Jr and Douthit, 1985). Copper, zinc and nickel were measured using optical emission spectrometry (ICP-OES) (Stalović and Đorđević, 2013) and cadmium, chromium and lead were measured using mass spectrometry (ICP-MS) (Thomas, 2013).

The quantity of anthropogenic litter was determined by manually collecting the litter right after application of the amendments on top of the soil and weighing the amount of collected litter. This was done in 2020 and 2021(Fig. 2.2).

Potential N leaching

To explore whether adding organic amendments resulted in an increased loss of N from the system, we calculated a N balance following previous studies (Oenema et al., 2003; Van Beek et al., 2003) using data from the last year only (Fig. 2.2). For each plot, we quantified the main input and output sources in kg N per ha. Input sources represented the N in the organic amendment treatments and mineral fertilizer applications, while output sources represented the N in the harvested crop and the difference in the amount of total N in the soil (0-40 cm) between the start and the end of the cultivation season (September 2021 – September 2022). A negative balance indicates that N is lost from the system while a positive balance indicates that extra N is being retained in the system. An example of a (N) balance calculated for one of the plots can be found in supplementary material, table S2.1.

Statistical analysis

All statistical analyses were performed in R version 4.2.2 (R Core Team, 2013). Prior to the analysis, all response variables were scaled (z-scored) to allow comparison of effects between treatments and response variables. We used linear mixed effect models with the lmertest package version 3.1-3 in R (Kuznetsova et al., 2015) to analyse how SOM content in the upper 10 cm of the soil changed during the seven times it was measured in the different experimental treatments. Explanatory variables were treatment and month since start of the experiment (continuous), and their interaction while location, crop and treatment were included in the model as random factors. SOM content in the entire cultivation layer (upper 0 - 40 cm) and N retention were similarly related to organic amendment treatments, measuring moment, and their interaction. However, because these response variables were only measured three times, we included measuring moment (September 2021, April 2022 and September 2022) as ordinal factor to examine whether trends increased over time. These models included location, crop and treatment as random factors. To analyse whether yields increased in response to the organic amendments over the three years of the study, crop yield (separate for wheat and maize yield) was centred (by dividing every data point by the mean of that year) as well as scaled to correct for the inevitable weather related yield differences between years. After this pre-processing step, crop yield was related to organic amendment treatment, year (ordinal factor) and their interaction with location and treatment as random factors. Weed pressure, N leaching and water holding capacity estimates measured at the end of the experiment were related to organic amendment treatment as independent variable, with location and crop as random factor. Response variables were log transformed where necessary to improve residual scatter and achieve normality. The results were visualised by illustrating the beta coefficients of the four organic amendment treatments relative to that of the control treatment in their relation to the response variables.

The grown maize and wheat varieties, timing of seeding, cover crop species used and weed management techniques created differences between field locations. However, the locations are replicates in this study and are used in all statistical analyses as random factor. This experimental design allowed us to draw conclusions across a range of geographic locations and farming practices on sandy soil.

Results

Fifteen fields were used in this study and in September 2019, at the start of the experiment, average SOM content of these fields was 3.88% (range between 2.81 - 5.03%), pH averaged at 5.80 (range between 4.30 – 7.16), total N concentration averaged at 88.9 g/kg soil dw (range between 71.9 – 126.7 g/kg soil dw) and total P concentration averaged at 22.0 g/kg soil dw (range between 13.1 – 34.3 g/kg soil dw). Detailed averages per location are shown in the supplementary material, table S2.2. Compared to the control that received no organic additions, the addition of compost and fresh cuttings of species poor and species rich road verges significantly increased SOM content in the top 10 cm of the soil (Fig. 2.3 and 2.4A; for details see supplementary material, table S2.3). This increase was most pronounced in the compost treatment with an average increase of 0.81 % over the three years (Fig. 2.4A). During the last year of the study, the SOM content in the cultivation layer (0-40 cm) increased significantly faster in the compost treatment than the control treatment and showed an increasing trend ($p < 0.1$) in the Bokashi treatment (Fig. 2.3 and 2.4B).

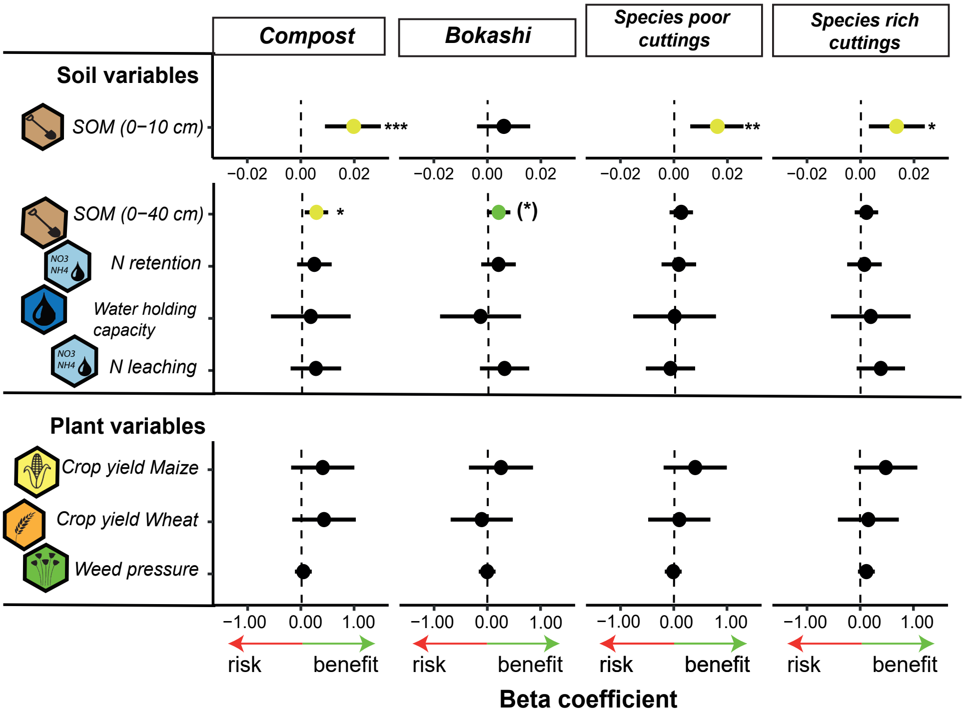


Figure 2.3. Effect of the organic amendment treatments on several soil and plant response variables compared to the control treatments (dashed black line). Data are presented as the parameter estimate (standardized beta coefficient) from the linear mixed effect models with the 95% confidence interval associated with the beta coefficient indicated. A positive beta coefficient illustrates a beneficial effect from the variable while a negative beta coefficient illustrates a harmful risk, thus in case of N leaching and weed pressure the data is transformed (minus becomes positive and vice versa) to follow this form. Black points indicate the parameter coefficients which were not significantly different from the control, green point show a trend towards significance ($p < 0.1$) and yellow point show significant effects ($p < 0.05$). Detailed estimate and p value information is visible in the supplementary material, table S2.3. Significance is indicated according to the p-values of the selected linear mixed effect model according to (*) $p < 0.1$; * $p < 0.05$; ** $p < 0.01$; *** $p < 0.001$.

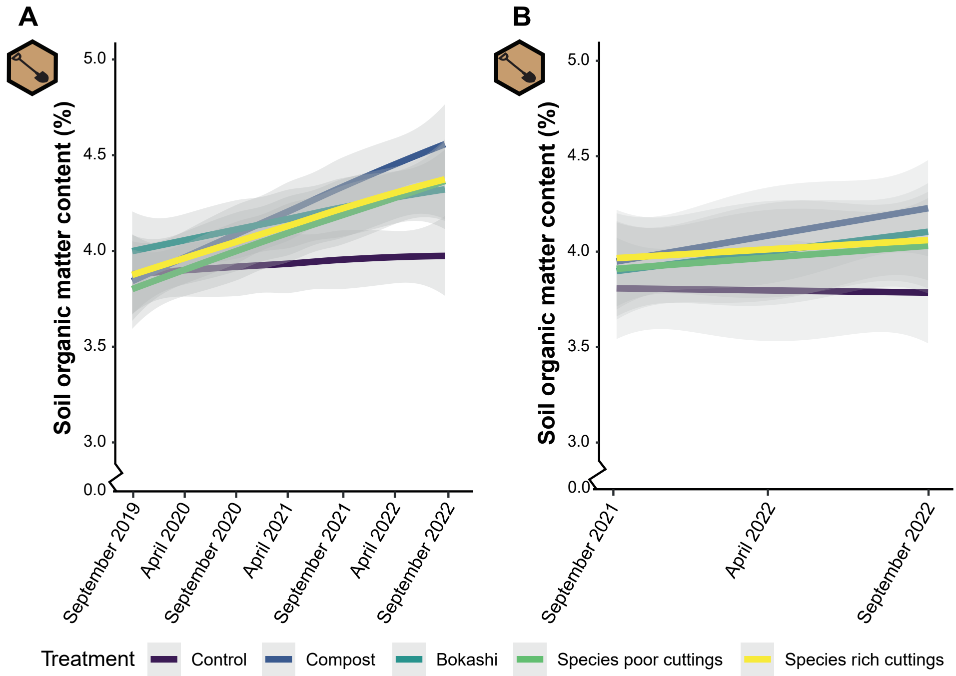


Figure 2.4. Predictions and 95 % confidence intervals of the linear mixed effect models of the **A)** soil organic matter content (%) in the upper 0-10 cm of soil and **B)** soil organic matter content (%) in the 0-40 cm soil layer in relation to time. Per time point and per treatment, the amount of replicates was 30.

Average unstandardized crop yield of each year was 4.4 (2020), 6.8 (2021) and 5.9 (2022) ton per ha for wheat and 16.2 (2020), 27.2 (2021), 16.2 (2022) ton per ha for maize. Standardized wheat yield showed no significant increase in the organic amendment treatments with 50 % fertilization compared to the control (Fig. 2.3 and 2.5A). Maize yield was also not significantly affected by the organic amendment treatment (Fig. 2.3). However, our model did indicate a small negative trend in maize yield in the control treatment over the three years (Fig. 2.5B), whereas, in plots that received organic amendments, maize yields remained stable. N retention, leaching and water holding capacity were not significantly affected by the organic amendment treatments (Fig. 2.3).

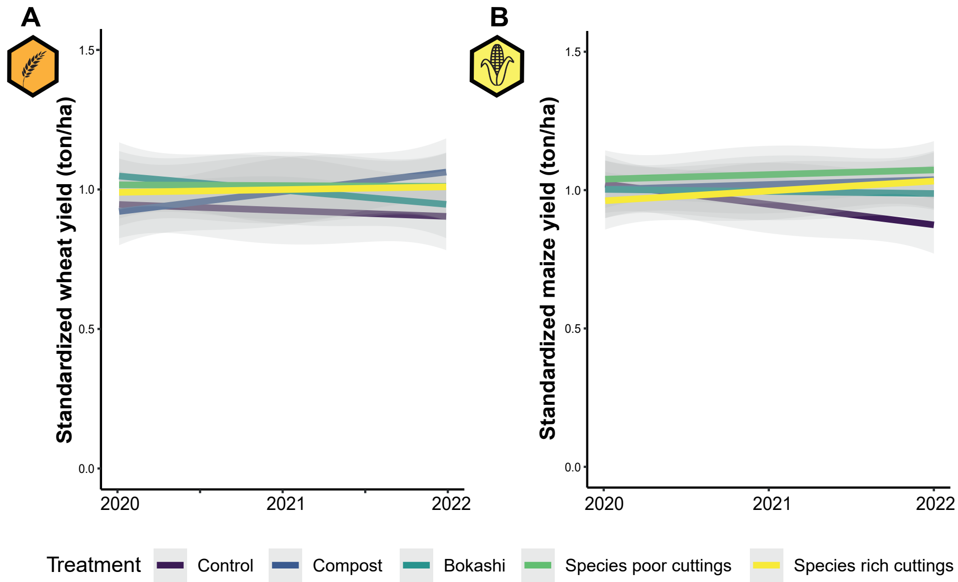


Figure 2.5. Predictions and 95 % confidence intervals of the linear mixed effect models of the **A)** standardized wheat yield (ton/ha) and **B)** standardized maize yield (ton/ha) in relation to time. Yield data is standardized by dividing the data by the mean (n = 75) of the yield of the year.

Weed cover in the field was not significantly affected by any of the organic amendment treatments (Fig. 2.3) and was primarily determined by location rather than treatment. The concentrations of heavy metals in the organic amendments were below the maximum permitted levels for all measured heavy metals, in most cases even less than half the permitted concentration according to Dutch legislation for compost (Table 2.2).

Table 2.2. Average heavy metal concentrations (in mg/kg dw) of the organic amendment treatments and cuttings before processing (n= 6). Legal threshold concentration (in mg/kg dw) according to Dutch legislation for compost is also indicated. After the average, the percentage of the legal threshold is indicated between brackets in italic.

	Cd (Cadmium)	Cr (Chromium)	Cu (Copper)	Ni (Nickel)	Pb (Lead)	Zn (Zinc)
Legal threshold concentration	1	50	90	20	100	290
Organic amendment treatments						
Compost	0.54 *(54 %)*	7.15 *(14 %)*	17.38 *(19 %)*	3.83 *(19 %)*	6.12 *(6 %)*	138 *(48 %)*
Bokashi	0.23 *(23 %)*	11.46 *(23 %)*	8.52 *(9 %)*	8.26 *(41 %)*	3.26 *(3 %)*	65 *(22 %)*
Species poor cuttings	0.44 *(44 %)*	2.55 *(5 %)*	9.1 *(10 %)*	1.62 *(8 %)*	4.74 *(5 %)*	81.8 *(28 %)*
Species rich cuttings	0.64 *(64 %)*	1.06 *(2 %)*	6.32 *(7 %)*	0.84 *(4 %)*	1.09 *(1 %)*	72.5 *(25 %)*
Cuttings before processing	0.53 *(53 %)*	1.34 *(3 %)*	9.48 *(11 %)*	1.14 *(6 %)*	2.23 *(2 %)*	98.8 *(34 %)*

A total amount of 11.2 kilo and 7.3 kilo litter was removed prior to application of amendments in 2020 and 2021 respectively which translates to 9.34 kg per ha and 6.10 kg per ha in 2020 and 2021 respectively, when applying 30 tons of amendment per ha. There were no differences in litter load between the amendment treatments. The main items found in the litter were food packaging and metal drink cans (Fig. 2.6).

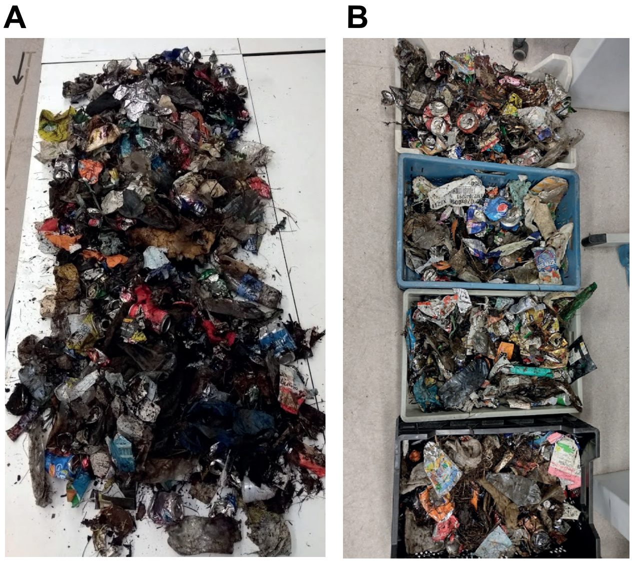


Figure 2.6. Pictures showing the amount of litter collected in **A)** 2020 and **B)** 2021.

Discussion

This study quantified the benefits and risks of applying cuttings from semi-natural habitats as organic amendment on arable fields with sandy soils. Our results suggest significant benefits of applying cuttings as organic amendments for build-up of SOM. Crop yield was maintained, despite the fact that only half the amount of mineral fertilizer was applied relative to the control plots. We found no evidence for further benefits within the time-frame of the study. Effects of the type of organic amendment were small compared to the effect of applying organic amendments. The only significant risk of applying cuttings from semi-natural habitats that we identified is the introduction of significant amounts of anthropogenic litter onto arable fields. In this respect, our results probably represent a worst-case scenario because we used cuttings from road verges which are prone to contain more contaminants than cuttings from other type of semi-natural habitats.

Potential benefits of applying cuttings as organic amendments

Our results show that only three years of applying road verge cuttings can result in a significant and substantial increase in the SOM content in arable fields (Fig. 2.3 and 2.4A). Adding composted cuttings resulted in the most rapid increase in both the top 10 cm as well as the entire cultivation layer. However, with the other types of organic amendments, the increase in SOM content was slower (with application of fresh species poor and species rich cuttings) or not significantly different (with application of Bokashi) from the control. These differences are not due to the total amount of C that was applied to the soil as the compost treatment introduced the lowest quantity of C (21.8 kg C/plot) compared to 33.5, 42.4 and 57.5 kg C/plot for Bokashi, species poor and species rich cuttings respectively (Table 2.1). Rather the results can be explained by the differences in biochemical composition of these amendments, differences that came about due to different processing methods as the same raw materials were used to produce the amendments.

Our results are in line with the hypothesis that application of organic amendments with a large recalcitrant: labile ratio should result in the most rapid increase in SOM (Freibauer et al., 2004; Mohanty et al., 2013). In our study, compost had the lowest C:N ratio, but the highest lignin fraction of all amendments, because composting lowers the C:N ratio and increases the more recalcitrant C fractions as the more labile C fractions are consumed by the decomposing microorganisms during the composting process (Gong et al., 2021; Mohanty et al., 2013; Zmora-Nahum et al., 2005). Fermenting for Bokashi is believed to break down the more recalcitrant C compounds but without C respiration (Olle, 2021), which could explain why it did not affect the C:N ratio but resulted in a significantly lower lignin fraction when compared to compost (Table 2.1). When Bokashi is added to soil, it is subjected to aerobic decomposition of the easy decomposable C compounds, which would result in higher soil respiration as compared to soil respiration from soils amended with compost. This is highly probable but not yet confirmed in academic research.

Our results suggest that if the objective is to enhance SOM content to improve soil functioning (Emerson, 1995; Hoffland et al., 2020; Loveland and Webb, 2003; Thiele-Bruhn et al., 2012), composting is most suitable and fermenting via the Bokashi method is least suitable with fresh cuttings being intermediately effective. The rates of increase observed in our study when using the compost treatment (from an average 3.7 % SOM to 4.5 % over three years in the compost plots) are exceeding to the 4 per mille target (0.4 % rate increase which means from a 3.7% SOM to 3.744 % in three years) formulated in the Paris Climate Agreement (Minasny et al., 2017). This suggests that applying cuttings from semi-natural habitats could contribute to counteracting climate change, but it should be noted that the availability of cuttings is insufficient to apply the rates investigated in this study throughout the Netherlands and SOM levels will stabilize at a new equilibrium.

The trends in wheat and maize yield did not differ significantly between the organic amendment treatments and the control (Fig. 2.3 and 2.5) despite the 50% lower mineral fertilizer rate applied in the organic amendment treatments. This indicates that the crops could obtain enough nutrients from the road verge cuttings to achieve a similar growth as under conventional fertilization practices. Interestingly, there were no significant differences between the four types of organic amendments in their impact on crop yield despite the markedly different C:N ratio's. This was partly unexpected because earlier research showed significant yield losses after application of organic amendments with C:N ratio's higher than 25 (Beri et al., 1995; van der Sloot et al., 2022). The Bokashi and species rich cuttings were well above this threshold (Table 2.1). However, N immobilization occurs in the first half year after application of organic material (Flavel and Murphy, 2006; Hadas et al., 2004; Kaleeem Abbasi et al., 2015; Nicolardot et al., 2001) after which N in spring is released again by the soil microbiome to become available for crop growth. In this study we applied the organic amendments at least 9 months before harvest of the main crop which most likely explains why the different types of organic amendments produced similar yields despite the differences in biochemical composition.

Other studies have found positive effects of organic amendments on crop yield (Wei et al., 2016) and in our study the total amount of N applied per plot (mineral fertilizer and nutrient content of the organic material combined) was generally higher in the organic amendment treatments than in the controls (equivalent of 190-290 vs 180 kg N/ha/year). The fact that the higher N application rates did not translate into higher crop yields may be caused by a number of mutually non-exclusive processes. First, more N could be lost from the cropping system either through denitrification as N^2 loss to the atmosphere, or through leaching as nitrate or dissolved organic N to the ground water. Second, N from the organic amendment treatments is very likely to be fixed in the generally larger amounts of SOM meaning that more N is retained in the soil that is therefore not yet accessible to the crop plants (but can become available later upon mineralization). Third, N availability was not limiting crop growth because the mineral N applied if the minimum fertilization rates applied were already sufficient to obtain maximum crop growth or because other factors, such as water availability, were more limiting during our study. However, in this study, N leaching probably did not play a major role as the N balance calculations indicated no significant loss of N. Moreover, there was even a slightly positive trend in the N balance for three of the four organic amendment treatments suggesting that, if anything, N was accumulating in the cropping system (Fig. 2.3). The amount of N that was retained in the soil did not increase significantly faster in the organic amendment treatments than in the controls, but the mean beta coefficients were positive (Fig. 2.3). Although this means that there were no statistically meaningful differences in N retention, given the large volume of the cultivation layer, this could nevertheless result in ecologically meaningful amounts of extra N being retained in the soil following organic amendment application. Finally, 2020 and 2022 were particularly dry years (KNMI, n.d.) with crop yields that were on average 25% (wheat) and 40% (maize) lower than in the year 2022 that had normal rainfall. This indicates that in at least two out of three study years water availability was probably more limiting than N availability.

The increases in SOM we observed after adding organic amendments did not result in an increase in the water holding capacity (Fig. 2.3). This was probably caused by the relatively high SOM content of the soils of our study sites at the start of the experiment (average of 3.88% with a range between 2.81 - 5.03%). This resulted in a relatively small increase in SOM content caused by the organic amendments after three years, although substantial in absolute terms. Studies that showed a positive effect of organic amendments on water holding capacity of soils are often based on soils that have a lower SOM content at the start (e.g. around 1.5 %, Eden et al., 2017). Furthermore, the relation of SOM and water holding capacity is non-linear with the largest increase in water holding capacity between 1.5 and 3% SOM content (Franzluebbers, 2002).

Potential risks of applying cuttings as organic amendments

Weed pressure was not significantly affected by any of the organic amendment treatments (Fig. 2.3). There was considerable variation in weed pressure between locations, most likely linked to differences in weed management, but weed pressure was not significantly higher in plots that had received cuttings in three consecutive years than plots that had only received mineral fertilizers. This is likely because most plant species that were growing in the road verges are poorly adapted to establish and maintain themselves under the conditions experienced in intensively managed arable fields (Kleijn, 2003). Furthermore conventional weed management of wheat and maize crop was likely sufficient to suppress the growth of any plants that did establish from seeds introduced with the cuttings. With low inherent load of potential arable weeds, the impact of composting or fermenting on weed viability (Cooperband, 2002; Merfield, 2012) is only of minor relevance. Our results therefore indicate that in conventional farming systems, the application of cuttings from semi-natural habitats does not increase weed pressure.

The concentrations of heavy metals in the cuttings turned out to be far below the maximum permitted concentrations in Dutch legislation for composts (Table 2.2). This is in line with several reports from the Netherlands and elsewhere in Europe that reported low concentrations of heavy metals in road verge cuttings (Amlinger et al., 2004; Ehlert et al., 2010; Romkens et al., 2020; Ros and Termorshuizen, 2012; Saveyn and Eder, 2014). While road verges may be enriched with heavy metals from traffic exhausts, the limited bio-availability of the heavy metals likely prevents accumulation in the vegetation and the risks regarding heavy metal accumulation in the arable soil and crop are, therefore, insignificant (Romkens et al., 2020; Ros and Termorshuizen, 2012). Dutch regulations currently constrain the use of road verge cuttings as organic amendments on arable fields because of presumed risks that heavy metals will end up in the food chain. The results of this and other studies point out that this is highly unlikely and call for a review of these regulations to be able to capitalize on the benefits of integrating road verge cuttings into farm management.

The only persistent risk associated with the use of the road verge cuttings was the presence of anthropogenic litter. Especially the large numbers of metal drink cans (Fig. 2.6) were considered problematic as in the Netherlands wheat and maize are used as animal feed and metal fragments can damage the digestive tracts of livestock. The introduction in the Netherlands of deposit schemes for small bottles and metal cans on the first of July 2021 and the first of April 2023 respectively is expected to reduce this risk (Infinitum, 2021) but will most probably not remove it entirely. The use of cuttings from other types of semi-natural habitats, such as on-farm hedgerows and ditch banks or nature reserves, could effectively remove the risk of using cuttings from semi-natural habitats as organic amendment.

Conclusion

Our study showed that application of composted and fresh road verge cuttings can be used to increase SOM content of arable field soils while maintaining crop yield with half of the mineral fertilizer rate. When organic amendments are used at rates similar to those investigated in this study with comparable soil types, this could already result in significant increases in SOM within a few years surpassing the 4 per mille target formulated in the Paris Climate Agreement. Application of cuttings did not significantly increase risks such as N leaching or weed pressure. Nor did the cuttings exceed heavy metal threshold concentrations that would prohibit their use. The large presence of anthropogenic litter, however, poses a substantial risk for the application of road verge cuttings as organic amendment. If litter contamination can be prevented or eliminated before or after processing, or if cuttings are used from litter-free semi-natural habitats, the application of cuttings on arable fields is very promising. This approach offers advantages to farmers, including improved soil quality and reduced fertilizer expenses without yield loss. Moreover, it benefits society by increasing soil C content instead of releasing it into the atmosphere as a greenhouse gas and it also provides a cost-effective way to manage semi-natural habitats in a more wildlife-friendly way. This study can effectively support changes in policies supporting the usage of this valuable resource instead of treating it as a waste stream which is currently the state in most European policies.

Acknowledgements

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Supplementary material

Table S2.1. Example of the N surplus balance calculations from plot 103 where species rich fresh cuttings were applied and winter wheat was grown the sampled year. The table indicates the input and output sources of the balance calculations.

Inputs		
Mineral fertilizer	$N_f=Q_f*C_f$	80
Cutting treatment	$N_{cu}=W_{cu}*C_{cu}*A_p^{-1}$	160.0
Total	$N_{input}=N_f+N_{cu}$	250.0kg/ha

Outputs		
Harvested crop (maize or wheat)	$N_c=Q_c*C_c$	61.3
Difference in the amount of N in the soil	$N_s=(C_{s22}*D_{s22}*A_p^{-1})-(C_{s21}*D_{s21}*A_p^{-1})$	94.6
Total	$N_{output}=N_c+N_s$	155.9kg/ha
Difference in nitrogen	$N_{surplus}=N_{output}-N_{input}$	-94.1kg/ha

Symbol		Unit
N	Amount of nitrogen	kg/ha
Q	Quantity product	kg/ha
C	Concentration nitrogen in product	kg/kg
W	Weight of product	kg
A	Area	ha
D	Density of product	kg

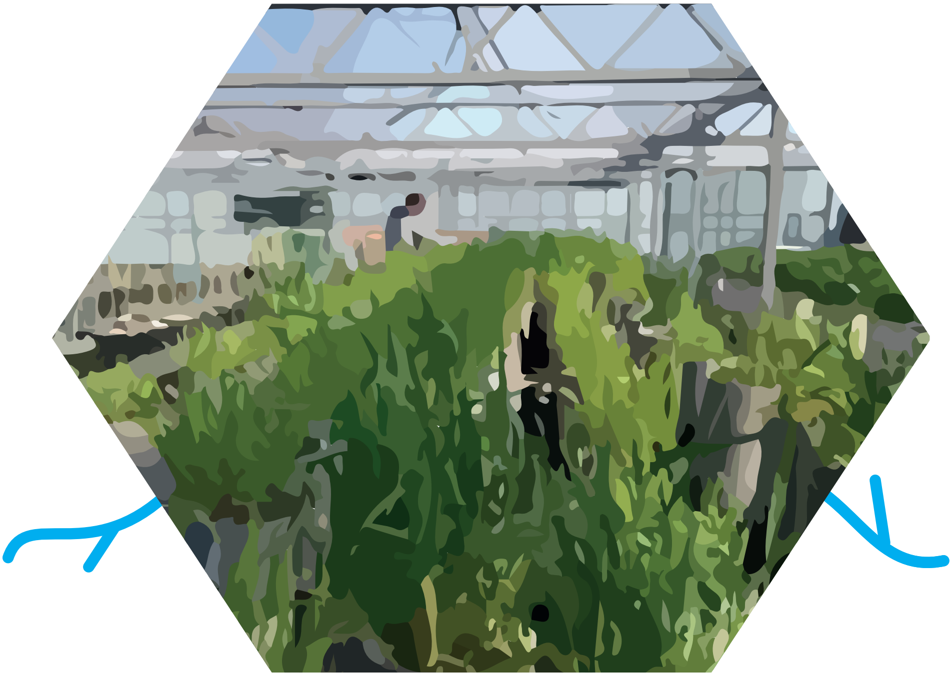
Subscript	
f	mineral fertilizer
cu	cutting treatment
p	Plot
c	crop (maize or winterwheat)
S21	soil September 2021
s22	soil September 2022

Table S2.2. Soil chemical characteristics for every field location at the start of the field experiment (September 2019). Average of 10 replicates is shown.

Locatie nr	SOM content (%)	Moister content (%)	Bulk Density (g/cm3)	pH	Total N content (mg/ kg soil dw)	Total P content (g/ kg soil dw)
1	3.10	5.29	1.10	5.37	71.9	14.1
2	3.13	7.38	1.46	5.10	83.8	24.8
3	4.72	8.20	1.19	5.39	99.5	20.3
4	3.82	6.24	1.26	5.01	126.7	14.7
5	3.55	12.61	1.07	5.36	78.5	17.5
6	3.56	12.01	1.07	6.27	86.7	20.3
7	2.81	17.09	1.08	6.78	73.9	13.1
8	4.55	16.01	0.99	5.87	89.5	16.0
9	3.81	16.91	1.08	6.26	82.6	34.3
10	4.60	16.07	1.09	6.25	107.8	22.8
11	3.61	15.26	1.04	7.16	77.2	22.0
12	4.00	14.53	1.00	4.30	77.5	32.0
13	3.81	15.42	1.05	5.94	73.2	31.8
14	5.03	17.72	0.97	6.30	96.7	25.2
15	4.19	17.27	1.08	5.63	108.0	21.0

Table S2.3. Effect of the organic amendment treatments on several response variables compared to the control treatments. Data are presented as the parameter estimate (beta coefficient) from the linear mixed effect models with associated standard deviation, t-value and P value. Significance is indicated according to the P-values following (*) p < 0.1; * p < 0.05; ** p < 0.01; *** p < 0.001; ns = non-significant.

		Estimate (Beta coefficient)	Std. deviation	t-value	P-value
Compost	SOM (0-10 cm) - log	0.0198	0.0053	3.776	0.0002***
	SOM (0-40 cm)	0.2662	0.1126	2.364	0.0187*
	Crop yield Maize	0.3969	0.3036	1.307	0.193ns
	Crop yield Wheat	0.4206	0.3052	1.378	0.17ns
	N retention - log	0.2273	0.1661	1.369	0.172ns
	Water holding capacity - log	0.1602	0.3823	0.419	0.678ns
	Weed	0.031	0.0809	0.395	0.694ns
	N leaching	0.2558	0.2427	1.054	0.295ns
Bokashi	SOM (0-10 cm) - log	0.0061	0.0052	1.160	0.246ns
	SOM (0-40 cm)	0.2016	0.1126	1.79	0.0745(*)
	Crop yield Maize	0.2574	0.3077	0.895	0.372ns
	Crop yield Wheat	-0.1058	0.2983	-0.355	0.723ns
	N retention - log	0.1989	0.1661	1.198	0.232ns
	Water holding capacity - log	-0.1395	0.3887	-0.359	0.722ns
	Weed	-0.0031	0.0809	-0.038	0.969ns
	N leaching	0.313	0.2366	1.323	0.189ns
Species poor cuttings	SOM (0-10 cm) - log	0.0162	0.0052	3.096	0.002**
	SOM (0-40 cm)	0.1263	0.1126	1.122	0.2627ns
	Crop yield Maize	0.4009	0.3036	1.32	0.189ns
	Crop yield Wheat	0.1019	0.2987	0.341	0.733ns
	N retention - log	0.0802	0.1661	0.483	0.629ns
	Water holding capacity - log	0.0013	0.3974	0.003	0.997ns
	Weed	-0.0129	0.0809	-0.159	0.874ns
	N leaching	-0.0767	0.2366	-0.324	0.747ns
Species rich cuttings	SOM (0-10 cm) - log	0.0134	0.0052	2.569	0.01*
	SOM (0-40 cm)	0.1031	0.1126	0.916	0.3604ns
	Crop yield Maize	0.4783	0.3036	1.575	0.117ns
	Crop yield Wheat	0.1509	0.2923	0.516	0.606ns
	N retention - log	0.0653	0.1661	0.394	0.694ns
	Water holding capacity - log	0.1844	0.3823	0.482	0.633ns
	Weed	0.1135	0.0809	1.404	0.163ns
	N leaching	0.3735	0.2322	1.609	0.111ns



Chapter 3

Carbon (C) to nitrogen (N) ratio and quantity of organic amendment interactively affect crop growth and soil mineral N retention

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Abstract

Using organic amendments to improve arable soils in the long-term is a careful balancing act of applying amendments with the right carbon to nitrogen (C:N) ratio at adequate quantity to avoid nitrogen (N) leaching while promoting or retaining crop growth in the short-term. So far, most studies examining the relationship between C:N ratio and N mineralization and immobilization were done without plants. In this study we explored how crop biomass and N leaching change with increasing C:N ratio and quantity of organic amendments to arable soil. We conducted an open-air mesocosm experiment with organic amendment application across a range in C:N ratio (ten to sixty) and quantity (ten to fifty ton per ha) to sandy arable soil using a full-factorial design. Spring wheat was planted and grown for six months during which three rainfall events were simulated to test treatment effects on N leaching. Applying amendments with a C:N ratio of twenty and higher decreased crop biomass and increased mineral soil N, while organic amendments with a C:N ratio of ten had the opposite effect. Applying larger quantities of amendments reinforced the effect of the C:N ratio on crop biomass. N leaching remained unaffected by either amendment C:N ratio or quantity or even mineral fertilizer as N leaching only occurred in the control treatment without plants. Our results suggests that growing a crop is adequate to prevent N leaching. Applying organic amendments do not pose a different risk regarding N leaching when compared to mineral fertilizer and slurry.

Keywords: agricultural soil, carbon to nitrogen ratio, organic amendment, nitrogen leaching, soil nitrogen retention

Introduction

Meeting the increasing global food demand while minimizing the impact on the environment is a major challenge in crop production in the next decades (Debonne, 2019; Godfray et al., 2010). Sustainable soil management is an important factor when reaching these future food production goals (Lal, 2010; Tilman et al., 2011). Increasing Soil Organic Matter (SOM) content can alleviate environmental problems in sandy arable soil such as nitrogen (N) leaching while promoting crop growth and the soil microbiome (Debonne, 2019; Diacono and Montemurro, 2010; EEA, 2005; Lal, 2006; Tester, 1990; Wei et al., 2016). To maintain and even increase SOM content, annually amending arable soil with organic material is a time-tested technique (Freibauer et al., 2004; Lal, 2006; Wei et al., 2016). This has inspired studies on the impact of organic amendments on arable soil quality and associated soil functions over the last decade (Diacono and Montemurro, 2010; Eden et al., 2017; Wei et al., 2016). These studies suggest that major effects on production related processes, primarily crop growth and mineral N leaching, depend on the quantity of the organic material that is being added but also the quality.

The quality of the organic amendments is largely determined by the carbon to nitrogen ratio (C:N ratio) as it relates to how fast the applied organic N will become plant available as mineral N. Upon decomposition of the organic amendments, soil microorganisms use N for enzyme production and to grow which can lead to temporal N immobilization in microbial biomass when the C:N ratio of the organic material is too high. To be able to break down low quality organic amendments, such as straw that has a C:N ratio of approximately 100, the soil microbes need all the N that is present in the organic material. Additionally the soil microbes will scavenge N from the soil solution that becomes mineralized from the soil inherent SOM, leaving little or no freely available N in the soil (i.e. net N immobilization (Flavel and Murphy, 2006; Hadas et al., 2004; Nicolardot et al., 2001; Sikora and Szmidt, 2001)). High quality amendments, such as composts that have a C:N ratio of approximately ten, contain relatively more N than the soil microbes need during decomposition and will therefore increase soil mineral N availability (i.e. net N mineralization (Flavel and Murphy, 2006; Hadas et al., 2004; Lazicki et al., 2020; Nicolardot et al., 2001; Sikora and Szmidt, 2001)).

How amendment C:N ratio is related to mineralization or immobilization has been explored by Nicolardot et al. (2001) using a simple dynamic model that predicts N availability in the soil after application of crop residue with different C:N ratios. The model was validated using several crop residues that ranged in quality from rye plants (C:N ratio of 9.5) to wheat straw (C:N ratio of 139). The model predicted net N immobilization at C:N ratios above 25 and net N mineralization at C:N ratios below 15. These predictions were subsequently corroborated empirically by Kaleeem Abbasi et al. (2015) who found immediate net N mineralization for residues with C:N ratios of 12.7, 14.4 and 26.4, immediate net N immobilization for residues with C:N ratios of 36.4, 49.2 and 121.5, and initial N immobilization followed by N mineralization after 120 days of incubation with residues with C:N ratios of 12.1 and 20.9.

Enhancing SOM content by means of application of organic amendments to soil such that it does not reduce crop growth or increase N leaching requires a better understanding of how the quantity and quality of organic amendments influence these processes (Andersen and Jensen, 2001; Murphy et al., 1998; Steen Jensen and Ambus, 1999). Applying large quantities of organic material with a low C:N ratio would ensure the mineralization of sufficient N to maximize crop growth (Flavel and Murphy, 2006; Steen Jensen and Ambus, 1999) but may increase harmful N leaching (Malcolm et al., 2019; Steen Jensen and Ambus, 1999). Whether leaching occurs depends on how much of the mobile N (mainly nitrate) is being taken up by the roots of the growing crop which would prevent it from flushing to the groundwater during heavy rainfall. Organic amendments with a high C:N ratio can generally reduce the risk of N leaching due to N immobilization (Bergström and Kirchmann, 2004; Malcolm et al., 2019). However, this can trade-off with reduced crop growth. Using organic amendments to improve arable soils in the short- and long-term is therefore a careful balancing act of applying amendments with the right C:N ratio at adequate quantity to avoid N leaching while supporting crop growth. So far, most studies examining the relationship between C:N ratio and N mineralization and immobilization were done without crop growth (Kaleeem Abbasi et al., 2015; Nicolardot et al., 2001; M. Vigil and Kissel, 1991). Therefore, it is still warranted to test the impact of organic amendment C:N ratio and quantity on N leaching and crop biomass when the crop is seeded shortly after incorporation of the organic amendments into the soil.

In this study we set-up an open-air mesocosm experiment to examine the relationship between organic amendments quality (C:N ratio) and quantity on crop biomass, N leaching and soil N retention. Our main objective was to investigate how crop biomass, N leaching and soil N retention change with increasing quantity and C:N ratio of organic amendments on sandy arable soil. We hypothesized that: (1) with increasing C:N ratio, crop biomass and N leaching would be lower and soil N retention will be higher; (2) applying a higher quantity of organic amendment would partly offset the effect of C:N ratio; and (3) crop biomass, N leaching and soil N retention would be more affected by organic amendment C:N ratio than by its quantity.

Material and methods

To examine the effects of C:N ratio and quantity of organic amendments on crop biomass, N leaching and soil N retention we performed a mesocosm experiment from January 2020 until June 2020. To avoid the confounding effect of C:N ratio and type of organic material applied (Flavel and Murphy, 2006; Hadas et al., 2004; Huang et al., 2004), we created organic amendments with different C:N ratios using a single source of organic material (plant cuttings) in combination with cow slurry (C:N ratio 7.3) to lower the C:N ratio or mixed with dried straw (C:N ratio 310.7) to raise the C:N ratio. We used the C:N ratio rather than lignin: N ratio as proxy for organic amendment quality (Becker et al., 1994; Kumar and Goh, 2003; Taylor et al., 1989) for practical reasons. The C:N ratio is easier to measure for most labs and, given our very different source materials (road verge cuttings, straw and slurry) sufficient for creating a range in degradability (Nicolardot et al., 2001; Taylor et al., 1989) wide enough to test our hypotheses. The experiment comprised a full factorial combination of six C:N ratios (10, 20, 30, 40, 50, 60) and three amendment quantities (the equivalent of 10, 30 and 50 ton fresh weight per ha) with each treatment being replicated five times. All mesocosms were placed in a randomized block design (five blocks of 26 mesocosms each). Mesocosms were placed outside under a transparent roof with open sides. Climate conditions during the experiment were similar to other years in this period in the Netherlands (KNMI, n.d.). The water regime entailed watering twice a week by adding roughly 250 ml of water to all mesocosms during the whole duration of the experiment. This was less than average rainfall in these months in the Netherlands (equivalent of 1,5 litre per mesocosm) but this was necessary to prevent intermediate N leaching outside the rainfall events. The regular watering together with the rainfall events were estimated to be sufficient for crop growth.

Soil collection

Sandy arable soil was collected from an arable field in the vicinity of Wageningen (51°59’28.9”N 5°39’31.5”E; The Netherlands). The soil had 3.16 ± 0.03 % SOM, a pH of 7.39 ± 0.05 and mineral N and phosphorous (P) content were 6.81 ± 0.46 and 1.33 ± 0.09 mg.kg-1, respectively. This soil was air dried and mixed in a cement mixer with water to gravimetrically result with an average moisture level of 16.75 ± 0.06 % in fresh weight. Cylindric mesocosms (40 cm high, diameter 20 cm) were filled with soil (bulk density of 1.22 ± 0.01 g.cm-3 based on dry weight). The open bottom of the mesocosms was covered with root cloth and were standing on individual trays to allow both free leaching of excess water and collection of leachate.

Treatments

Organic amendment treatments were prepared from herbaceous road verge cuttings collected in September 2019 from public road verges in the municipality of Sint Anthonis (51°37′33″N, 5°52′52″E; The Netherlands). After storing the material at 4 °C for four months, these cuttings were subsequently mixed with either cow slurry (C:N 7.3) or wheat straw mulch (C:N 310.7) to create the six C:N ratio treatments. All C:N ratio calculations are based on amount of C and N in fresh weight of the material. Moisture percentages of the start materials are provided in the supplementary material table S3.1. Each C:N ratio treatment was applied at three quantities resulting in eighteen organic amendment treatments (supplementary material table S3.1). Each organic amendment treatment was mixed in with the upper ten cm of the soil, in line with agricultural practice. Ten days after mixing in the organic amendments, the mesocosms were seeded with spring wheat (*Triticum aestivum*, Harenda variety Agrifirm). Fifteen seeds were placed in each mesocosm and just after sprouting, the plant density was reduced to ten viable plants per mesocosm, in line with regular density in arable fields (275 to 300 seedlings per m^2).

To compare the effect of organic amendments to conventional fertilizer application practices, we included a cow slurry-only treatment in our experimental design comparable to regular amounts of fertilization during this six-months period (80 kg N per ha) for spring wheat in the Netherlands. We also applied three levels of mineral fertilizer (Limestone Ammonium Nitrate; 24% N of 50-50% nitrate-ammonium at the levels 21, 80 and 164 of kg N/ha). Slurry and mineral fertilizer treatments were added directly on top of the mesocosms just after crop emergence to mimic conventional farming methods. To assess the crop nutrient uptake from N mineralized from the SOM during the experiment, a control treatment was added without fertilization. To quantify N leaching from the soil in absence of a crop a last control without plants and without fertilization was added. Chemical composition of all treatments are included in the supplementary material table S3.1. 250 ml of a nutrient solution (2.61 g per L K_2SO_4; 2.46 g per L $MgSO_4.7H_2O$; 1.47 g per L $CaCl_2.2H_2O$) was applied to all mesocosms (except the no fertilizer control) three weeks after crop emergence to prevent effects from micro nutrients deficiency during crop growth.

N leaching

Three heavy rainfall events were simulated during the experiment. The simulations were six weeks apart and took place the day after regular watering to have sufficient soil moisture for leaching. The amount of water applied was based on data from a nearby weather station (KNMI weather station 583 (KNMI, n.d.); Wageningen, The Netherlands). Data from 1989-2019 showed a monthly rainfall of 20 mm (which is 20 L rain over 1 m^2).

For our mesocosms experiment this is the equivalent of 750 ml water per mesocosm, which we applied at the top of the mesocosms at a rate of 250 ml water per hour over a period of three hours. The day after a rainfall simulation the leachate was collected by taking the water out the collection trays with a syringe. Total amounts of the leachate was weighed to calculate leachate volume. The total weight of the leached water was used to calculate the percentage of water retention, i.e. the percentage of rainwater that can be retained by arable soil in the crop-soil layer. Percentage of water retention in this study was calculated as the amount of water not leached out (thus retained) as a percentage of the total amount of water applied (i.e. 750 ml per rainfall event). A subsample of 20 ml leachate was stored overnight in a freezer at -20 °C prior to chemical analysis the next day.

Data collection

Total aboveground biomass of the wheat plants was determined just before seed maturation after six months of growth when the plants were at stage GS61 according to Zadok's scale (Zadoks et al., 1974). Shoots were cut off at the soil surface and were dried at 70 °C for three days and then weighed to determine the shoot biomass dry weight.

Five soil cores per mesocosm were taken at the end of the experiment using a 2-cm diameter soil auger. Per core the 0-10 and 20-30 cm layer were collected separately, and per mesocosm the soil of the five cores was pooled per layer and stored in zip-lock bags at -4 °C until further analysis. Roots were sieved out over a 2 mm sieve and soil was dried at 40 °C prior to the soil chemical analysis. To assess the plant available N, mineral N (NO_3-N, NH_4-N) concentration in the soil was analysed for both soil layers. Soil mineral N extraction was performed according to standard procedures (Temminghoff, 2010) using a 1:10 (w:v) ratio of dried soil: 0.01 mol*L-1 $CaCl_2$ solution at 20 °C. The concentration of mineral N in the extracts was analysed with a segmented-flow analyser (Skalar San++ system). Separate soil layers did not show significant differences in soil mineral N concentration and were therefore pooled for further analyses. These pooled mineral N measurements were treated as the legacy soil mineral N retention.

All leachate samples were analysed the day after collection. Mineral N (NO_3-N, NH_4-N) concentration was determined with a continuous flow analyser (Temminghoff, 2010) (Skalar San++ system). The total amount of mineral N leached was calculated by multiplying the concentrations of both NO_3-N and NH_4-N by the volume leached. The cumulative N leached per mesocosm over three leaching events was summed to create the parameter 'N leached'.

Statistical analysis

All statistical analyses were performed in R studio version 2021.09.2+382 with core R version 4.1.2 (R Core Team, 2013). To explore relative importance of direct and indirect effects of organic amendment C:N ratio and quantity on crop biomass, N leaching and soil N retention we used Structural Equation Modelling (SEM; R package 'piecewiseSEM' (Lefcheck, 2016; R Core Team, 2013)) according to an a priori conceptual SEM model we developed (Fig. 3.1). To test this SEM model, we only used data from the organic amendment treatments (so excluding the control, slurry and mineral fertilizer addition treatments). The C:N ratio and quantity of the organic amendments were considered the exogenous variables. The endogenous dependent variables included in the model were crop biomass (crop biomass; total aboveground crop biomass in g dry weight per mesocosm), percentage moisture retained in the soil after the rainfall events (percentage of water retained; calculated by the percentage of water volume retained after a rainfall event), cumulative amount of mineral N leached over all three rainfall simulations (N leached; in mg mineral N per mesocosm) and the concentration of mineral N in the whole soil column at the end of the experiment (legacy soil mineral N; in mg N per kg soil) after log transformations to achieve normality and standardization. Block location was added as a random factor. The Fisher's Chi-square test was used to test goodness of fit of the model (Lefcheck, 2016). The goodness of fit of this SEM entails $0 \leq \chi^2$, d.f. ≤ 2 and $p \geq 0.05$.

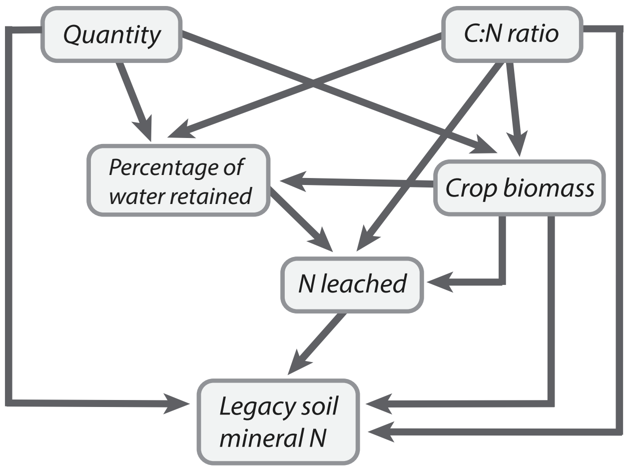

Figure 3.1. Conceptual Structural Equation Model (SEM) showing the expected effects of organic amendment C:N ratio and quantity on crop biomass, N leaching and soil N retention (legacy soil mineral N).

To compare the effect of organic amendment application on crop biomass, N leaching and soil N retention with mineral and slurry fertilization methods we used linear mixed effects models (R package 'lmerTest' (Kuznetsova et al., 2015)). The model tested the effect of the different fertilization methods (organic amendment, slurry, mineral fertilizer low, regular and high level) and controls without fertilization (control with and without plants) as fixed independent variables on the dependent variables crop biomass, N leaching and soil N retention which were log transformed to meet the requirement of a normal distribution. Block was added as random factor. Subsequently, one-way ANOVA's and Post-hoc Tukey tests were used to determine the relative effects of the treatments. All significance levels were assessed at $p < 0.05$.

Finally, linear mixed effects models were used to examine whether the C:N ratio influences the relationship between the quantity of the applied N and crop biomass, N leaching and soil N retention. For these analyses, data from the organic amendment treatments (C:N ratio as numeric variable) and the mineral fertilizer treatments (C:N ratio as zero) were used. The amount of N applied and the C:N ratio, as well as their interaction were included as independent variables and log transformed crop biomass, N leaching and soil N retention data as dependent variables and block was added as random factor. Relations between dependent and independent variables (and their interaction) were tested with t-statistics using use Satterthwaite's method (Satterthwaite, 1946) to calculate the degrees of freedom. Significant relations ($p \leq 0.05$) are reported in the results section.

Results

Effects of C:N ratio and quantity of organic amendments

Crop biomass was negatively related with both C:N ratio and quantity of the organic amendments, but these amendment properties had limited impact on N leaching (Fig. 3.2). The percentage of water that was retained in the soil after the rainfall events was lower with higher crop biomass. The amount of mineral N left in the soil at the end of the experiment increased with increasing amount of organic amendments applied and was not directly affected by the C:N ratio of the organic amendments, only indirectly through its impact on crop biomass.

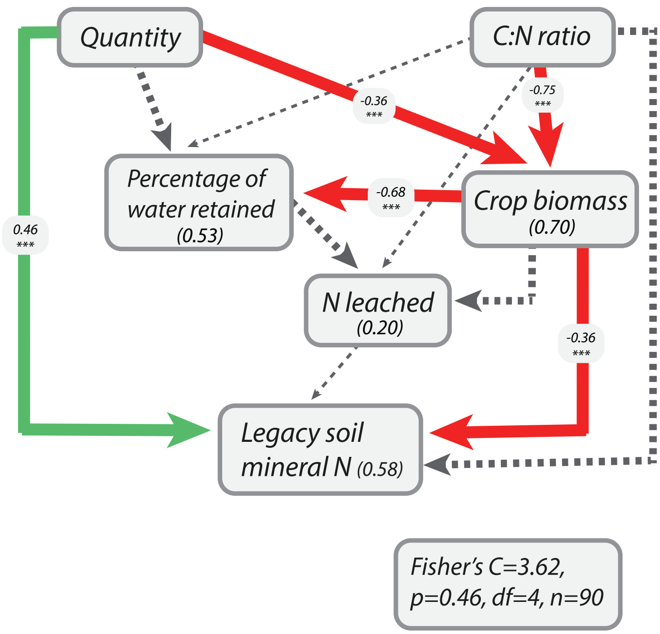

Figure 3.2. Piecewise Structural Equation Model (SEM) of the effect of the C:N ratio and quantity of an organic amendment on crop biomass, the percentage of water retained, N leached and legacy soil mineral N. Green arrows show a significant positive path, red arrows show a significant negative path and grey and dashed arrows show a non-significant path. Numbers on the significant arrows indicate standardized path coefficients and the strength of the path coefficient is shown proportionally to the width of the arrow. The proportion of the variation explained by the fixed and random predictor variables (conditional R^2) is shown as the number between brackets in the box of each response variable. The grey box in the bottom of the figure shows the result of the Fisher's exact test (Fisher's C), p value (p) of the test, degrees of freedom (Df) of the model and the number of observations (n) used for the analysis. Significance is indicated according to the P-values following (*) p < 0.1; * p < 0.05; ** p < 0.01; *** p < 0.001.

Organic amendment versus slurry and mineral fertilization methods

On average, the addition of organic amendments resulted in a significantly lower crop biomass compared to all mineral fertilizer and slurry treatments (Fig. 3.3A; Numerator DF= 5, Denominator DF= 116, F value= 24.4, p value < 0.01). On average, and across all organic amendment treatments with different C:N ratios, even the unfertilized plants (control +) produced more crop biomass than those growing in mesocosms with organic amendments. N leaching was not significantly different between the application of organic amendments (cuttings) or all levels of mineral fertilizer and slurry (Fig. 3.3B; Numerator DF= 6, Denominator DF= 119, F value= 9.0, p value < 0.01). Only the control treatment without plants (control -) differed significantly in N leaching with approximately ten times more N leached than the average of all other treatments. Despite the significant N leaching losses, there was a large amount of mineral N left in the soil at the end of the experiment in the mesocosms with the control treatment without plants (Fig. 3.3C; Numerator DF= 6, Denominator DF= 119, F value= 10.5, p value < 0.01). Adding organic amendments resulted in significantly higher amounts of legacy mineral N at the end of the experiment compared to the high and regular mineral fertilization treatments and the slurry treatment.

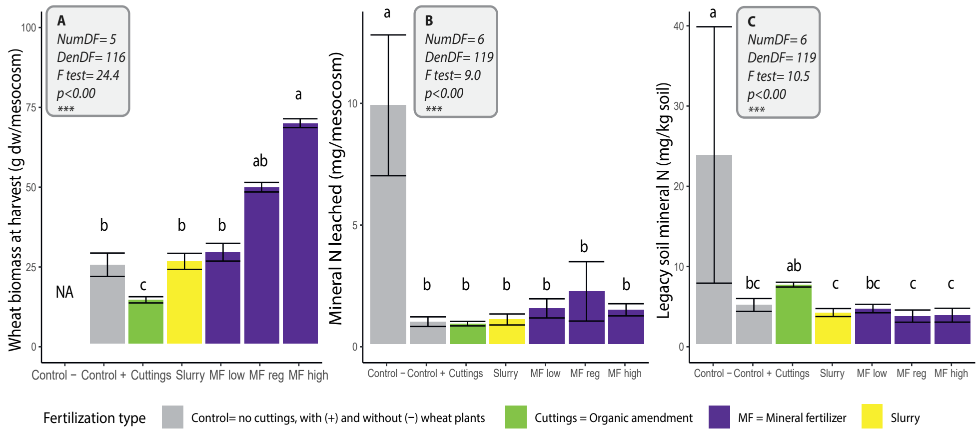

Figure 3.3. Fertilization treatment effects on crop biomass (**A**), amount N leached (**B**) and legacy soil mineral N (**C**). Error bars indicate standard error of the mean (Control - n= 6; Control + n= 6; Cuttings n= 90; Slurry n= 7; MF low n= 7; MF reg n= 7; MF high n= 7). Letters indicate significant differences between treatments at p ≤ 0.05 tested with a one-way ANOVA and post-hoc Tukey HSD test. The grey box shows the result of the F test and corresponding p value (p) of the ANOVA. Significance is indicated according to the P-values following (*) p < 0.1; * p < 0.05; ** p < 0.01; *** p < 0.001; ns = non-significant.

The relationship between the quantity of the applied N and crop biomass was strongly dependent on the C:N ratio of the amendment (Fig. 3.4A; significant interaction between C:N ratio and the amount of N applied). Application of mineral fertilizers or organic material with a C:N ratio of ten generally resulted in a positive relationship between the amount of N applied and crop biomass. Application of organic amendments with C:N ratios of twenty or higher resulted in negative relationships between the amount of N applied and crop biomass. The amount of N applied or the C:N ratio did not influence N leaching in any of the treatments (Fig. 3.4B). The relationship between the quantity of the applied N and amount of mineral N remaining in the soil after crop harvest was influenced by the C:N ratio of the applied treatments (Fig. 3.4C; significant interaction between C:N ratio and the amount for N applied). In the mesocosms with mineral fertilizer treatments, the mineral N that was left in the soil decreased with increasing N application. Applying more N through organic amendments generally resulted in higher legacy mineral N, which furthermore consistently increased with increasing C:N ratio.

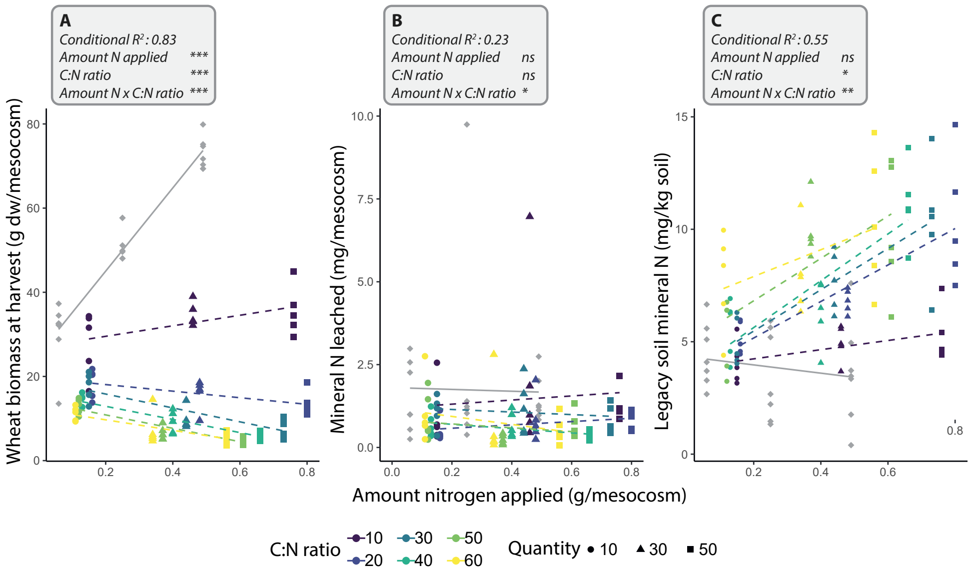

Figure 3.4. Crop biomass (**A**), amount N leached (**B**) and legacy soil mineral N (**C**) in relation to the amount of nitrogen applied with the organic amendment treatments (coloured points) and mineral fertilizer treatments (grey points). Correlations significance is analysed using linear mixed effect models including C:N ratio and amount of nitrogen applied as independent variables and their interaction ($p \leq 0.05$). Results of conditional R^2 values and correlations of the models are presented in grey boxes. Significance is indicated according to the P-values following (*) p < 0.1; * p < 0.05; ** p < 0.01; *** p < 0.001; ns = non-significant.

Discussion

The objective of this study was to investigate how crop biomass, N leaching and soil N retention change with increasing C:N ratio and quantity of organic amendments on sandy arable soil. We found that application of organic amendments with a C:N ratio of twenty or higher decreased crop biomass (Fig. 3.2, Fig. 3.3A, Fig. 3.4A) and increased mineral N retention in the soil (Fig. 3.3C, Fig. 3.4C). The amount of mineral N retained was directly and positively affected by the quantity of organic amendment applied and indirectly and positively affected by the C:N ratio through crop biomass (Fig. 3.2). Contrary to our hypotheses, the cumulative amount of mineral N that leached after simulations of heavy rainfalls was not affected by the organic amendments C:N ratio, quantity applied or by separate mineral fertilizer or slurry application (Fig. 3.2, Fig. 3.3B, Fig. 3.4B). Significant N leaching was only observed when there were no crop present to take up mineral N (Fig. 3.3B).

Crop biomass growth

Crop biomass decreased with increasing C:N ratio and quantity of the organic amendment (Fig. 3.2), as hypothesised. Specifically we observed a decline in crop biomass compared to mineral fertilizer when the C:N ratio was twenty or higher (Fig. 3.4A) probably because mineral N was immobilized. N immobilization is likely, given that the crop in the unfertilized control grew significantly more biomass than the mean crop biomass across all amendment treatments (Fig. 3.3A). When there was an organic amendment applied with a C:N ratio of ten, the N in the amendment was readily available, and crop biomass did not significantly differ from adding a similar amount of N as mineral fertilizer. Increasing the amount of organic amendment applied reinforced the impact of C:N ratio on crop biomass, decreasing crop biomass when applying amendments with a C:N ratio above 20 and increasing crop biomass when applying a C:N ratio of 10. Previous studies (Kaleeem Abbasi et al., 2015; Nicolardot et al., 2001) found similar tipping points between net N mineralization and immobilization when measuring and modelling N dynamics in the soil. However, these studies did not include the effect on crop biomass. Our study shows that the crop biomass response closely follows the moment when N mineralization starts to dominate over N immobilization, illustrating the strong microbial control on crop growth in N limited conditions.

N leaching

N leaching was not affected by the organic amendments application quantity or C:N ratios (Fig. 3.2, Fig. 3.3B, Fig. 3.4B). Even the highest amount of N applied via mineral fertilizer did not result in mineral N leaching concluding no effect from the fertilization treatments

(Fig. 3.3B, Fig. 3.4B). Leaching was, however, significantly higher in the control treatment without crop growth. This suggests that N uptake associated with growth of the wheat plants was sufficient to prevent N leaching during our experiment. Our results are in line with the N capture capacity of cover crops during fallow periods: it has been shown that cover crops can reduce nitrate leaching on average by 56% during the main crop growth (Thapa et al., 2018). While the presence of a crop prevented N leaching from our experimental mesocosms even in the mineral fertilizer treatments, the scavenging for N by crops is possibly not strong enough to prevent N leaching during periods of low crop cover in winter during heavy rainfall events or during fallow periods between crops. While organic amendments with high C:N (above twenty) may help preventing leaching in the short-term during the winter period by microbial N immobilization, they could also prevent leaching by improving water retention in the longer term as the SOM content increases with organic amendment application (Tester, 1990). Extrapolating these results to the field situation comes with a note of caution. Actual N leaching in the field strongly depends on both local soil, crop and weather conditions which are not captured in our experiment. Next to that, the timing of N availability, as has been shown to be difficult to forecast when applying organic amendments, is important when extrapolating the findings from this experiment regarding N leaching to field level. Measuring N leaching in a field experiment over a whole growing season including a fallow period with and without organic amendment application will help in further understanding the potential of organic amendments to decreasing excess N leaching.

Soil mineral N retention

The amount of mineral N retained at the end of the experiment was directly driven by the quantity of the organic amendment and indirectly by the C:N ratio. Applying a larger quantity of organic amendment increased the amount of mineral N retained at the end of the experiment and this was most apparent when applying organic amendments with a C:N ratio of twenty or more (Fig. 3.2, Fig. 3.3C, Fig. 3.4C). There results suggest that the N released from the organic amendment was initially immobilized, while being mineralized towards the end of the experiment. Kaleeem et al. (2015) also saw this result after applying organic amendments with C:N ratios of 12.1 and 20.9 with initial immobilization and mineralization afterwards. Lazicki et al. (2020) showed in an incubation experiment of 84 days at 23 °C that 0% N had mineralized from organic amendments with a C:N ratio of twenty whereas from amendments with C:N ratio of six about 30% of the added N became mineralized. However, in our SEM analysis (Fig. 3.2) we observed that leftover mineral N in the soil was directly affected by the quantity applied but not directly by the C:N ratio as this parameter only worked indirectly through effects on crop biomass. The studies of Kaleeem et al. (2015) and Lazicki et al. (2020) both did not include the effect of crop growth in their experiments which can explain this difference. Despite adding high quantities of amendment, N availability did not line up with N requirement from the wheat during our experiment. This result implies that the timing between the organic amendment application and the peak N demand of the crop was not optimally aligned in our experiment and illustrates the difficulty of timing soil processes to crop growth.

Implications of this study

In this study we confirmed already known tipping points of N mineralization and immobilization (Flavel and Murphy, 2006; Hadas et al., 2004; Kaleeem Abbasi et al., 2015; Nicolardot et al., 2001; Sikora and Szmidt, 2001; M. F. Vigil and Kissel, 1991). However, our study provided more understanding in linking the results of these mechanisms towards practical measures such as crop growth and N leaching. Overall we confirmed part of our first hypothesis that increasing C:N ratio results in low crop biomass and indirectly increased soil mineral N retention. Especially C:N ratios above twenty will result in N immobilization which limits the crop growth when an organic amendment is applied directly before sowing. This is a problem when convincing farmers to use organic amendments as soil improver since decreased crop yield will result in decreased income. Applying more N with the organic amendments would not fix this problem, unless amendments with C:N ratio of 10 or lower are used, because we showed that applying a larger quantity will merely decrease crop growth even more when the crop is seeded shortly after the application of the organic amendment to the soil. In our second hypothesis we suspected that adding a larger quantity will offset the effect of C:N ratio, however this was contradicted by the results discussed. Our third hypothesis is therefore true for crop growth since the C:N ratio is largely dictating the effect on crop biomass. N leaching was not affected by the different organic amendment treatments or fertilization treatments and therefore we cannot confirm our hypothesis of the effect of different C:N ratio or quantity on N leaching. In practice this entails that, according to our study, the addition of organic amendments (even with a low C:N ratio) has a similar low risk of N leaching as mineral fertilisers when measured during the cropping season. The next step in safely using organic amendments in agriculture is taking previous knowledge of the underlying mechanisms combined with our findings and perform a large scale field experiment over multiple crop cycles where one can investigate if the results regarding crop growth, N leaching and soil N retention remain similar.

Conclusion

We investigated the effect of differing C:N ratio and quantity of organic amendment application on crop biomass, N leaching an soil N retention and compared this with conventional fertilizers such as mineral fertilizers and slurry. Our results show a decrease in crop biomass and an increase in mineral soil N retention after application of an organic amendment with a C:N ratio 20 and higher. This shows the importance of balancing the timing of application of an organic amendment and the C:N ratio to meet the goal in providing sufficient crop growth while over the long-term improving agricultural soil. N leaching was only observed when there were no plants present indicating no severe risk of N leaching during crop growth for both organic amendment, mineral fertilizer and slurry application. A large scale field study is the next step that should test if field impact of organic amendments on crop biomass and soil N leaching match the results of our pot experiment.

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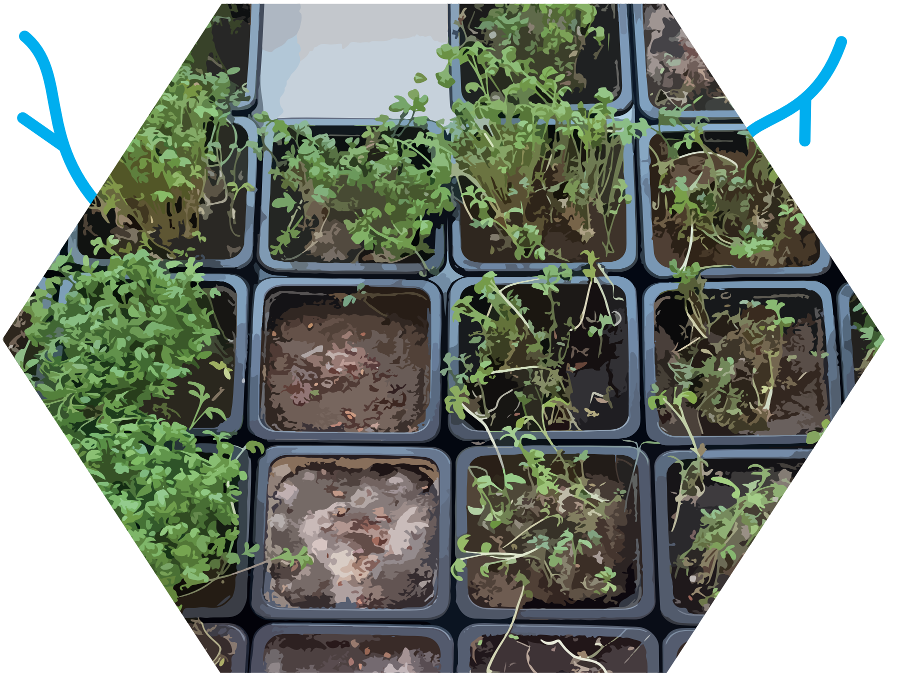
Supplementary material

Table S3.1. Chemical composition of the start materials (n=5) and the amount of carbon and nitrogen applied to the mesocosms, C:N ratio and replicates of all the organic amendments and controls. Amount of P and K of start material road verge cuttings was measured. Amount of P and K of slurry and straw were added as reference values (*) based on data by Eurofins and Agrifirm.

Start material						
	g C / kg fw	*g N / kg fw*	*g P / kg dw*	*g K / kg dw*	*C:N*	*Moisture (%)*
Road verge cuttings	67.19	5.62	1.7	10.8	11.96	76.58
Slurry	29.42	4.04	1.3*	4.5*	7.28	92.61
Straw	431.87	1.39	1.0*	12.8*	310.70	9.81

Organic amendment treatments				
Quantity (ton/ha)	*g C per mesocosm*	*g N per mesocosm*	*C:N*	*Replicates*
10	1.52	0.15	10	5
30	4.55	0.46	10	5
50	7.58	0.76	10	5
10	3.28	0.16	20	5
30	9.83	0.48	20	5
50	16.38	0.80	20	5
10	4.35	0.15	30	5
30	13.04	0.44	30	5
50	21.73	0.73	30	5
10	5.31	0.13	40	5
30	15.95	0.40	40	5
50	26.59	0.66	40	5
10	6.10	0.12	50	5
30	18.29	0.37	50	5
50	30.48	0.61	50	5
10	6.78	0.11	60	5
30	20.33	0.34	60	5
50	33.88	0.56	60	5

Control treatments				
	g C per mesocosm	*g N per mesocosm*	*C:N*	*Replicates*
Slurry	1.25	0.25	7	7
Mineral fertilizer low (MF low)	0.00	0.06	0	7
Mineral fertilizer regular (MF reg)	0.00	0.25	0	7
Mineral fertilizer high (MF high)	0.00	0.49	0	7
Control with plants (Control +)	0.00	0.00	0	6
Control without plants (Control -)	0.00	0.00	0	6



Chapter 4

Bokashi promotes general arable soil disease suppressiveness in short-term but not in long-term

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Abstract

Soil-borne diseases can cause significant crop losses and should be tackled sustainably in current and future agroecosystems. Increasing the capacity of soils to suppress the effects of soil-borne diseases (soil suppressiveness) is an important goal in sustainable crop production to achieve. Soil suppressiveness can be improved by adding organic amendments to the soil for multiple years, but the effects vary greatly dependent on the type of organic amendment (composted, fermented, or fresh material) and the timing since application. The objective of this study was therefore to test the disease suppression capacity of sandy arable soil from a realistic multi-year field experiment where fresh plant cuttings, compost made from the same cuttings, or Bokashi (fermented amendment) from the same cuttings have been applied across 10 field sites. Next to that, the effect of short-term application of the same organic amendment treatments on soil suppressiveness was tested using control arable sandy soil from 2 sites from the field experiment. Soil-borne disease suppressiveness was measured with a bioassay using the *Lepidium savitum* (cress) – *Pythium ultimum* model system. Field sites strongly differed in cress growth independent of the organic amendment treatments. Absence of field effects in the sterilised treatment and additional measurements on soil chemical and biological (PLFA) characteristics suggested differences in inherent pathogen load between field sites. Focussing on sites with low inherent pathogen load we found no significant impact of long-term organic amendment application on either cress weight or soil suppressiveness. However, short-term application of Bokashi did significantly promote soil suppressiveness. This effect can likely be attributed to the increased metabolic activity of the soil's inherent microorganisms in response to Bokashi, which contains more easily decomposable compounds as compared to the other soil amendments. Our results suggest that Bokashi could promote the suppression of soil-borne diseases by stimulating the locally adapted soil microbiome but the longevity of this effect requires further field tests.

Keywords: soil suppressiveness, disease suppression, *Pythium ultimum*, cress bioassay, organic amendment, Bokashi

Introduction

Soil-borne plant pathogens can cause significant losses in agricultural crop production. Pathogens such as *Fusarium* spp., *Rhizoctonia* spp., and *Pythium* spp. can reduce crop yield in maize, wheat, vegetables, and fruits by 50 – 70% (Mihajlović et al., 2017; Panth et al., 2020) due to damping off effects in the seedling phase (Lamichhane et al., 2017). Conventional strategies to tackle these soil-borne pathogens such as the use of synthetic fungicides can be harmful to the environment (Mihajlović et al., 2017; Panth et al., 2020). Alternative methods aimed at slowing down the build-up of populations of harmful soil biota, such as using a diverse crop rotation, intercropping, or growing specific resistant cultivars, can be difficult due to practical limitations (Mihajlović et al., 2017; Panth et al., 2020). Suppressing soil-borne diseases by stimulating the locally adapted soil microbiome may offer a third alternative that is less harmful to the environment and is easier applicable in the field (Schlatter et al., 2017).

The soil suppressiveness in this study is focused on general suppression. "General suppression is the ability of soils to inhibit the growth and activity of soilborne pathogens to some extent, owing to the collective competitive and antagonistic activity of the total soil microbiome competing with the pathogen(s)" according to Schlatter et al., (2017). General suppression can be enhanced by increasing the diversity, population size, and/or activity of the soil microbiome (Baker and Cook, 1974; Chen et al., 1988; Pérez-Piqueres et al., 2006; Schlatter et al., 2017; Termorshuizen et al., 2006a).

To improve general disease suppression in the soil, organic amendments such as crop residues, composts, or plant cuttings can be used (Bailey and Lazarovits, 2003; Baker and Cook, 1974; Garbeva et al., 2004; Pascual et al., 2000; Scheuerell et al., 2005). However, the effect caused by the organic amendment applications on soil disease suppression differs greatly. For example, Bonanomi et al. 2007 reviewed multiple disease suppression studies and found an increase in soil suppressiveness in 45% of the studies. A non-significant effect of the organic amendments was found in 35% of the studies and even an increase in disease incidence was found in 20% of the studies. The type of organic amendment was a major cause explaining these differences, with compost being the most suppressive organic amendment used (more than 50% of the compost studies showed effective disease control) while crop residues had a more unpredictable effect. Termorshuizen et al. 2006 also confirmed that a variety of compost treatments were most successful in increasing soil suppressiveness compared to varying results from other organic amendments.

The relationship between the type of organic amendment and soil suppressiveness is likely a combined result of organic amendment quality and the local composition of the microbiome (Clocchiatti et al., 2021, 2020; Luo et al., 2018; Mayerhofer et al., 2021). For example, when making compost, the aerobic process leads to an end product with a more recalcitrant stabilized organic fraction compared to fresh material because of the release of labile carbon (C) during composting (Luo et al., 2022; Mondini et al., 2003; Neher et al., 2013). This could create different effects on soil suppressiveness between fresh organic material and compost because the decomposability of an organic material is a major factor influencing the soil microbes (Bonanomi et al., 2010; Clocchiatti et al., 2020; Mayerhofer et al., 2021; Stone et al., 2001; Widmer et al., 1998). The organic material serves as an energy source for the soil microbiome and the easier the material can be decomposed, the quicker the boost in the activity in the soil microbiome occurs. Bokashi is mentioned to be a more easily decomposable organic amendment due to the fermentation process the organic material has been through (Luo et al., 2022; Shin et al., 2017). These different organic amendment treatments (fresh, compost, or Bokashi) were not directly compared to each other in former studies or realistic field situation. It might therefore be interesting to compare these different organic amendments made with the same start material and investigate if these different processing methods (either fresh, composting, or fermenting) change the effect on general soil suppressiveness.

Apart from the chemical quality, also the time since application of the organic amendment co-determines the size and direction of its effect on soil suppressiveness since decomposability is a timed process (Bonanomi et al., 2010). Compost for example consisting of more recalcitrant C might decompose over a longer time frame and therefore have a better effect in the longer term after multiple applications. Bokashi might decompose faster and have a more short-term effect. Clocchiatti et al. (2021) also noted this time-scale effect showing that application of different materials can initially increase the disease incidence but after four weeks increase soil suppressiveness. Furthermore, repeated organic amendment application, which can be desired to achieve higher arable soil quality, can improve the soil microbiome over time (Bonanomi et al., 2020, 2018; Pérez-Piqueres et al., 2006) and therefore the general disease suppression capacity of the soil (Bonanomi et al., 2018; Schlatter et al., 2017). Nevertheless, very few studies investigated the short- and longer term effects of different organic amendments in a realistic situation produced from the same starting material but with different qualities due to diverging microbial activity during their preparation. Therefore, it is relevant to investigate the disease suppression capacity in a realistic field situation and compare the effects of multiple additions of organic amendments in the field to the direct short-term effects of these organic amendments.

This study aimed to answer the following research questions:
i) Does soil disease suppressiveness increase after applying composted, fermented (Bokashi), or fresh organic amendments of the same source on sandy arable field soil?

ii) Does the impact on soil disease suppressiveness differ between short-term and multi-year applications of these organic amendments?

We hypothesized that multiple applications of organic amendments in a field situation increases the disease suppressiveness of the soil compared to soil without organic amendment application. The effect on the disease suppressiveness shortly after the application of the organic amendment would also be increased, but this might depend on the type of organic amendment. We expect Bokashi to increase soil suppressiveness in the short-term since this method creates easily decomposable material that might boost the microbes in the short-term. Compost on the other hand consists of more recalcitrant material that might promote soil suppressiveness after long-term application for multiple years. Fresh material may create a more variable response in the short-term but mainly promotes soil disease suppression in the long-term when the material is sufficiently decomposed. To test these hypotheses we used soil collected from a realistic multi-site field experiment that received organic amendments for multiple years. The soil was used in complementing bioassay experiments.

Material and methods

Two bioassays using the plant-pathogen *Lepidium sativum* (cress) – *Pythium ultimum* model system were conducted to answer both research questions. The first bioassay used soil from a multi-site field experiment where organic amendments were added to the soil for two consecutive years and compared with soil without any organic amendment to test the long-term effectiveness of the amendments. To test the short-term effect of the amendments, a second bioassay was conducted using soil without organic amendment addition from the multi-site field experiment to gain realistic applied knowledge and the different organic amendments were added shortly before the bioassay.

Multi-site field experiment

10 field locations were chosen from the multi-site field experiment (detailed information presented in the supplementary material S4.1). To accommodate a good comparison within this thesis, the numbering from the field experiment is the identical in this chapter. The field experiment was set up in September 2019. At every site, ten-by-ten meter plots (100 m^2) were created where five different treatments were applied in September 2019 and September 2020, with an equivalent quantity of 30 tons/ha, and were mixed in with the upper 15 cm of the soil. All treatments were present at all sites and were placed randomly within a site to create a randomized block design. The five treatments consisted of a control (no amendment) and four different organic amendments for which organic material from road verge cuttings from the municipality of Sint Anthonis (51°37’33” N, 5°52’52”E) was used and processed in different ways. The fresh cuttings were collected in

September 2021 right before the first bioassay and cuttings from June 2021 were used to create the processed organic amendment treatment. The four organic amendments were: i) compost, ii) Bokashi, iii) fresh cuttings from road verges with low plant diversity (species poor cuttings), and iv) fresh cuttings from road verges with high plant diversity (species rich cuttings).

The compost was derived from spring (June 2021) cuttings of road verges with low plant diversity by turning the material weekly to enable aerobic decomposition. The temperature of the compost was checked (peaking at 70 °C before turning) and when high temperatures were no longer detected (roughly above 40 °C) after eight weeks the compost was finished. Bokashi was derived from the same species poor road verge cuttings as the compost by fermenting the fresh cuttings under anaerobic conditions and covering the material under a plastic sheet in accordance with the method followed by Bokashi-making companies in the Netherlands (Bij de Oorsprong, 2021). The fermentation was initiated by adding microorganisms from BB Boden (Multikraft, n.d.) (lactic-acid bacteria) and eMB starter (Multikraft, n.d.) (bacteria that break down cellulose) to the fresh material. Pulverized calcareous shells were added together with the bacteria to prevent acidification due to the fermentation process. After eight weeks the plastic sheet was opened to use the Bokashi underneath. The two fresh road verge cuttings treatments consisted of September 2021 cuttings, that were stored maximally five days before application to the arable fields. The chemical composition of these organic amendment treatments are presented in table 4.1. At the start of the field experiment in September 2019 winter wheat (*Triticum aestivum*) was grown as the main crop which was harvested in July 2020. The crop rotation alternated to a cover crop - maize (*Zea mays* subsp. *mays*) system in September 2020 and maize was harvested in September 2021. During this field experiment, mineral fertilizer was applied in spring to fertilize the main crop, in line with common agricultural practice. The mineral fertilizer consisted of a combination of NPK fertilizer (12% N which was 50-50 ammonium-nitrate, 10% P, and 18% K), with Limestone Ammonium Nitrate (27% NH_4NO_3 + 6% CaO) addition during main crop growth which is a regular practice in the Netherlands. In total, an equivalent of 180 kg N/ha, 75 kg P/ha, and 135 kg K/ha was applied each year in the control plots, which is according to normal fertilization practices in the Netherlands. The organic amendment plots received half of this mineral fertilization (90 kg N/ha, 37.5 kg P/ha, and 67.5 kg K/ha) to allow the investigation of the fertilization capacity of the organic amendments. Fertilization took place every year in April (only NPK was applied) and June (only Limestone Ammonium Nitrate was applied) in the wheat plots and for the maize plots in June (all fertilization).

Table 4.1. Chemical composition of the organic amendment treatments used in the multi-site field experiment in 2021 and the second bioassay. Mean of 5 replicates per treatment and ± standard error.

Treatment	C:N ratio		g C/kg dw		g N/kg dw		Moisture (%)		Organic matter (%)		pH – H_2O	
Compost	12.2	±0.25	177.6	±14.0	14.6	±1.0	62.29	±0.70	37.71	±0.70	8.1	±0.04
Bokashi	31.8	±0.56	267.4	±55.2	8.4	±1.8	68.70	±2.34	31.30	±2.34	6.8	±0.04
Species rich cuttings	29.1	±1.87	410.0	±7.0	14.1	±1.0	68.47	±0.35	31.53	±0.35	7.0	±0.09
Species poor cuttings	28.5	±1.73	436.4	±7.6	15.3	±0.9	82.17	±5.76	17.83	±5.76	7.5	±0.02

Soil collection

After two consecutive years of amending the soil with organic amendment treatments, the soil was collected from the field experiment after crop harvest in September 2021. The soil collection took place one year after the last time of applying the organic amendment. We collected soil at 10 sites (serving as replicates) in plots with five different treatments per site, totalling 50 plots. Soil was collected from the 15 cm topsoil per plot with a small shovel by taking multiple samples in the inner two-by-two meters of each plot and approximately two kg of soil was collected from each plot. Soil samples were homogenized, sieved at 5 mm mesh for soil parameter analysis and 8 mm for the bioassays. The soil was stored in polyethylene bags at -4 °C prior to soil parameter analysis and at 20 °C in the dark prior to the bioassay. Bulk density and a moisture percentage of the field soil were determined by taking a separate soil sample per plot using a 100 ml ring of soil that was weighed fresh, subsequently dried at 105 °C for 48 hours, and then weighed again.

Several soil chemical characteristics were determined per soil sample. Soil Organic Matter (SOM) content was assessed via the loss on ignition method (Hoogsteen et al., 2015). Soil pH was determined in a water extract using a soil subsample of 20 g of fresh soil and 50 ml of demi-water mixed in a 100 ml Teflon tube. Samples were shaken for two hours and afterwards, the pH was measured with a pH/mV scale using a WTW inoLAb pH/mV meter. Mineral N (NO_3-N, NH_4-N) was analysed according to standard procedures (Temminghoff, 2010) in a 1:10 (w/v) ratio with a 0.01 M $CaCl_2$ solution at 20 °C. Concentration of mineral N in the extracts was analysed with a segmented-flow analyser (Skalar San++ system). The total amount of N in the soil was analysed in 0.5 g dried soil samples by creating a digestate with a mixture of H_2SO_4-Se and salicylic acid according to (Novozamsky et al., 1983). The digestate was then analysed for total N content with a segmented-flow analyser (Skalar San++ system).

To explore potential differences in the soil microbiome between field sites, Phospholipid fatty acid (PLFA) extraction and analysis took place in samples of every control soil of the 10 field locations. PLFA extraction was performed on 3 g freeze-dried soil according to well-known protocols (Frostegård and Bååth, 1996; Hedlund, 2002) based on the Bligh and Dyer method (Bligh and Dyer, 1959). Used biomarkers were based on previous research by Zelles (1999), Hedlund (2002) and Buyer and Sasser (2012); Gram-positive bacteria markers were iso and anteiso-saturated branched fatty acids (Zelles, 1999); Gram-negative bacteria, mono-unsaturated fatty acids and cyclopropyl 17:0 and 19:0 (Zelles, 1999); actinomycetes bacteria, 10-methyl fatty acids (Buyer and Sasser, 2012); methanotroph bacteria, 16:1 w8; saprotrophic acids, 18:2w6; arbuscular mycorrhizal fungi, 16:1w5. Total amount of microbial biomass was quantified as the sum of all detected PLFAs biomarkers.

Soil suppressiveness bioassays

The plant-pathogen system *Lepidium sativum* (garden cress) – *Pythium ultimum* was used as a model system to test soil disease suppressiveness. This system shows consistent results for disease suppression in agricultural studies and the mechanisms found can be used as a parameter for general disease suppression capacity of a soil (Bongiorno et al., 2019; Mayerhofer et al., 2021; Tamm et al., 2010; Thuerig et al., 2009). The protocol used in this study is based on the protocol by Tamm et al. (2010) and Bongiorno et al. (2019). The bioassay consisted of sowing cress on the soil surface that either had been inoculated with *Pythium* prior or not. Prior to the bioassay, *Pythium ultimum* (culture code: Py1, 2005) originally isolated from tomato (provided and stored by Biointeraction and Plant Health, Wageningen Plant Research, The Netherlands) was grown on Potato Dextrose Agar (PDA) and used to inoculate previously sterilized millet seeds (24 g millet + 20 ml demineralized water). Millet seeds were incubated in the dark at 20 °C and after eight days the mycelium together with the millet seeds were homogenized using a sterilized blender. The homogenized *Pythium*/millet culture was mixed with sand (1:80 (w/w)) to allow for a homogenous distribution of *Pythium* in the soil. Subsequently, 10 g of the *Pythium*/millet/sand mixture was mixed per liter of soil to obtain a final concentration of 0.125 g of *Pythium*/millet culture per liter of soil. The concentration was based on a preliminary experiment where a range of concentrations was tested to produce a 50-75% reduction in fresh weight of the cress plants compared to uninfected soil. After seven days, the bioassay was finished and the cress was harvested by clipping the plants at soil surface level and the fresh weight of the shoots was weighed. According to common practice in this bioassay (Bongiorno et al., 2019; Tamm et al., 2010) and because of the short experimental period, fertilization is not necessary since the garden cress uses all the required nutrients from the seed and does not use mineral N in the soil solution during this period.

The first bioassay used soil from ten experimental field sites with five treatments per site, in total fifty samples. The five treatments per site comprised i) compost, ii) Bokashi, iii) high plant diversity fresh cuttings, iv) low plant diversity fresh cuttings, and v) control without organic amendment addition. Half of each soil sample was autoclaved at 121 °C for twenty minutes to kill soil microorganisms and this soil was then considered sterilized

(Trevors, 1996). Half of the sterilized and non-sterilized soil was then inoculated with the plant pathogen by adding the *Pythium*/millet/sand mixture to the soil in polyethylene bags and shaken to ensure homogenous distribution of the *Pythium*. This created four subsamples for each sample, namely sterilized without *Pythium*, sterilized with *Pythium*, non-sterilized without *Pythium*, non-sterilized with *Pythium* resulting in 200 samples for this assay (Fig. 4.1). A bulk density measurement of each field site was used to determine the amount of soil used in each pot and therefore the amount of soil used per pot was the equivalent to a realistic arable field situation. This resulted in an average amount of soil of 283 grams per pot. Due to limited space in the incubator, this experiment was split into two batches based on site number. Soil from sites 1, 2, 3, 4, and 10 were in the first batch, and soil from sites 5, 6, 7, 8, and 9 in the second batch. Pots (6x6x7 cm) were filled according to field bulk density, watered to field capacity, and sown on top with 500 mg of cress seeds (*Lepidium sativum* untreated organic seeds from De Bolster, Epe, The Netherlands). To avoid cross-contamination but allow water evaporation the bottom of the pots were individually wrapped in aluminium foil. Pots were completely randomized and placed into

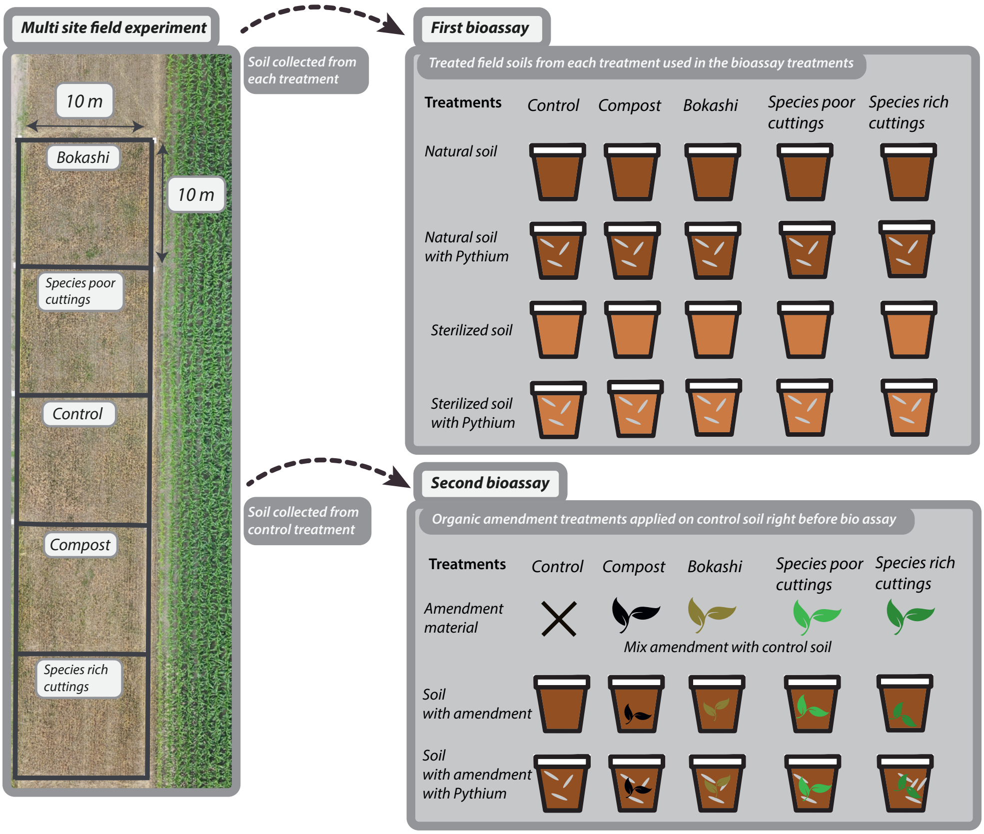


Figure 4.1. Overview of the experimental design showing the treatments of the multi-site field experiment (left), the first bioassay (above) and second bioassay (below).

an incubator (Tollabtech, type VTL 650 KB) at 20.5 °C with a day-length of 16 h and 80% relative humidity. For the first two days, pots were covered with transparent plastic bags to ensure 100% humidity to promote seed germination. After removal of the transparent bags, pots were watered when needed and seven days after sowing, shoot fresh weight was assessed by cutting the shoots with scissors directly above the soil surface.

The second bioassay was performed to investigate the effects of organic amendment application on soil disease suppressiveness shortly after the application of the amendments. The experimental setup consisted of soil from 2 field sites, four amendments mixed with field soil, and a control of soil without amendment (thus totalling five treatments) with three replicates per combination resulting in 30 samples (Fig. 4.1). Soil from control plots from 2 field sites from the field experiment was used, site nr 9 and 13. These 2 sites were selected based on low natural pathogen pressure which, as shown in the first bioassay. To test the impact of the organic amendments on disease suppressiveness we used the same four amendments (compost, Bokashi, fresh species poor, and fresh species rich cuttings) as were used in our field experiment. The chemical composition of these amendments are presented in table 4.1. After their preparation, the amendments were stored at 4 °C for two weeks. One week before the start of the assay, the amendments and the soil were placed in an incubation room at 20 °C to permit stabilization of the microbial communities. 10.8 g of amendment was added to each pot which is equivalent to the amount of amendment applied in the field (30 tons/ha). All samples were split and half was inoculated with *Pythium* according to the same concentration as with the previous bioassay while the other half was not inoculated. Both the amendment and *Pythium* was mixed through the soil by mixing the soil in polyethylene bags right before the bioassay. The same protocol and conditions were used for the inoculation, incubation, and harvest as in the previous bioassay.

For both bioassays, the amount of cress grown on the pots was used as an indicator for soil suppressiveness because the *Pythium* decreases the germination and growth of the cress. Therefore the difference in cress weight between *Pythium* versus without *Pythium* addition indicates the soil's capacity to withstand such pathogen (Bongiorno et al., 2019; Tamm et al., 2010). Fresh cress weight was used because it was more accurate than drying and weighing very small amounts of dried cress material. Next to that, the cress was grown in the same humidity and watering conditions in a short period of time and therefore the moisture content of the plants are very likely to be similar between all pots. This was confirmed by drying, weighing, and plotting the fresh weight of the cress to their dry weight which resulted in a linear relationship with an R^2 of 0.96. It was therefore verified to use the fresh weight for further analysis.

Statistical analysis

All statistical analyses were performed with R version 4.2.2 (R Core Team, 2013). The effect on garden cress fresh weight was in every analysis assessed by Generalized Linear Mixed Models (GLMM) using the package *glmmTMB* (Brooks et al., 2017) to correct for zero-inflation in the data. Selected models were tested for overdispersion, goodness-of-fit, outliers, and non-correlation of residuals using the package *DHARMa* (Hartig and Hartig, 2017). Next to that, Akaike Information Criterion (AIC) scores were evaluated to estimate the robustness of the models and to select the appropriate distribution (Burnham and Anderson, 2004). For all analysis, the Tweedie distribution, which is a family of exponential dispersion models with power variance functions $V(\mu) = \varphi\mu^{power}$ with $1 < power < 2$ (Dunn and Smyth, 2008), was selected for all analyses (Brooks et al., 2017). The effect of the amendment, site nr, and *Pythium* was assessed by ANOVA on the generalized linear mixed effect models. When the ANOVA indicated a significant interaction at p-value $\leq$ 0.05, Tukey's HSD post-hoc test was used to assess significant differences between the treatments.

When assessing the effect of the different locations on cress fresh weight, site nr was the independent variable, cress weight grown on non-sterilized soil was the dependent variable and both amendment and incubator batch were added as a random factor. When checking the effectiveness of the bioassay and the effect of perceived prior pathogen load, the cress weight was the dependent variable while the pathogen load, *Pythium* addition, and their interaction were the independent variables, and the incubator batch was added as random factor. This analysis was done twice for cress grown on non-sterilized and sterilized soil. To investigate if prior pathogen load affected the amendment addition, the interactive effect of pathogen load, *Pythium* addition, and amendment addition was tested as independent variables on the cress weight grown on non-sterilized soil as dependent variable with batch as random factor. For both the short- and long-term effects of the amendments on soil suppressiveness, the cress weight grown on non-sterilized soil was the dependent variable and the organic amendment treatment, *Pythium* addition and their interaction were the independent variables with site nr as random factor in both analyses.

Results

Soil suppressiveness after long-term application of organic amendment in the field

Soil suppressiveness after two years of application of the different organic amendments was tested using a bioassay where garden cress was grown with and without the addition of soil-borne pathogen *Pythium ultimum*. The weight of garden cress grown on non-sterilized soils without *Pythium* addition differed strongly between field locations (Fig. 4.2A, DF= 9, Wald Chi^2= 234.18, p< 0.0001), suggesting that the different field locations varied in their inherent soil-borne pathogen load. We assigned the sites a parameter

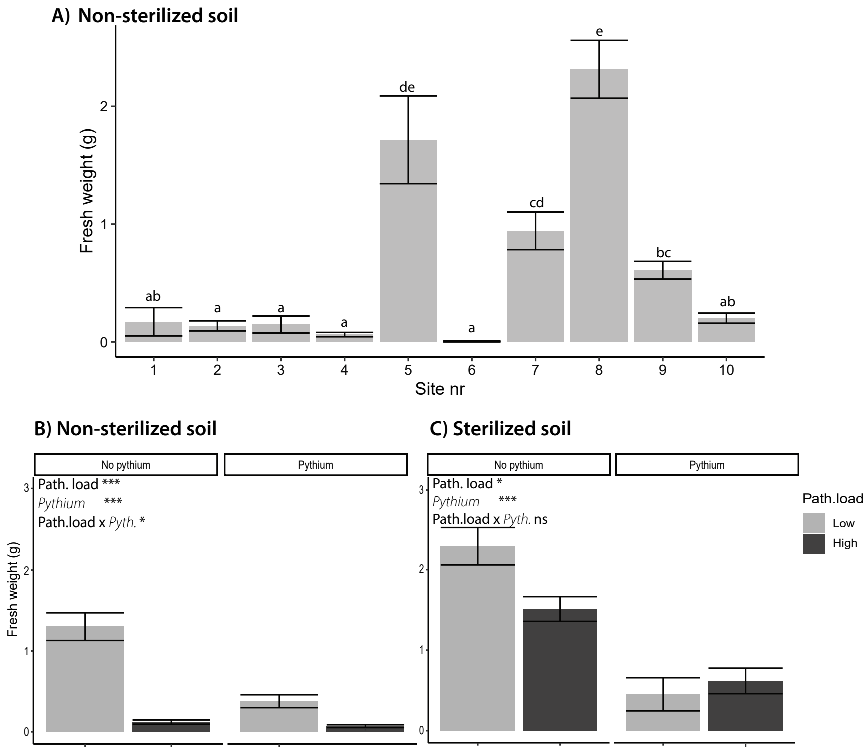


Figure 4.2. A) Mean cress shoot fresh weight in grams across non-sterilized field soils and across all amendments from 10 experimental field sites without *Pythium* addition. Error bars represent the mean ± standard error (n= 5). Different letters indicate significant differences at p≤ 0.05 with ANOVA followed by Tukey's HSD post-hoc comparison. **B)** Mean cress shoot fresh weight in grams grown in non-sterilized field soil across all amendments affected by the *Pythium* addition and perceived prior pathogen load. The error bars represent the mean ± standard error (High path load, n= 30, Low path load, n= 20). The effect of the perceived pathogen load, *Pythium* addition, and the interaction are shown in the upper right corner in the graph. **C)** Mean cress shoot fresh weight in grams grown in sterilized field soil across all amendments affected by the perceived prior pathogen load and *Pythium* addition. The error bars represent the mean ± standard error (High path load, n= 30, Low path load, n= 20). The effect of the perceived pathogen load, *Pythium* addition, and the interaction are shown in the upper right corner in the graph. Significance is indicated according to the P-values indicating (*) p < 0.1; * p < 0.05; ** p < 0.01; *** p < 0.001; ns = non-significant.

of either a high perceived inherent pathogen load (site nr 2, 5, 6, 7, 10, and 15) or low perceived inherent pathogen load (site nr 9, 12, 13, and 14) based on the amount of cress weight grown in non-sterilized soil without *Pythium* addition (Fig. 4.2A). The effect of *Pythium* addition on cress weight significantly interacted with the perceived inherent pathogen load in not sterilized soil (Fig. 4.2B), but not in the sterilized soil (Fig. 4.2C). Since soil sterilization removes the effect of the pathogen(s) present in non-sterilized soil, this suggests that the low cress weight in non-sterilized soil of sites nr 2, 5, 6, 7, 10 and 15 was caused by a biological factor. This was further supported by absence of significant soil chemical differences (in SOM, pH, plant available N and total amount of N: Table 4.2) between the groups with suspected high and low pathogen load (Table 4.2). Additional information on the general soil microbiome in the control plots of the 10 locations via PLFA analysis (supplementary material, Fig. S4.2) showed that the amount of microbial biomass (both in fungi and bacteria) and the specific microbial species composition in the locations showed no clear difference with locations with suspected high (location nr. 2, 5, 6, 7, 10 and 15) versus low (location nr. 9, 12, 13 and 14) pathogen load. The results

Table 4.2. Chemical characteristics of the soil used for the first bioassay from the multi-site field experiment. Mean of 5 replicates per location and ± standard error is shown. The average of the high suspected pathogen load is compared to the average of the low suspected pathogen load using a linear mixed effect model. Significance per soil chemical characteristic is indicated in the lowest row and is according to (*) p < 0.1; * p < 0.05; ** p < 0.01; *** p < 0.001; ns = non-significant.

Location	Organic matter (%)	Moisture percentage (%)	pH - H2O	Bulk Density	Plant available N (mg/kg soil dw)	Total amount of N (mg/ kg soil dw)
High suspected pathogen load						
2	3.48 ± 0.21	13.5 ± 0.4	5.3 ± 0.2	1.24 ± 0.04	36.0 ± 3.5	89.8 ± 6.9
5	4.13 ± 0.20	13.1 ± 0.6	5.7 ± 0.1	1.31 ± 0.03	32.1 ± 3.4	96.8 ±5.1
6	3.53 ± 0.12	9.7 ± 0.3	6.1 ± 0.1	1.26 ± 0.07	18.8 ± 1.7	80.2 ± 3.0
7	3.16 ± 0.08	6.8 ± 0.6	6.4 ± 0.1	1.13 ± 0.13	28.0 ± 2.2	86.2 ± 3.0
10	5.14 ± 0.24	14.5 ± 0.3	5.6 ± 0.1	1.17 ± 0.04	53.1 ± 8.3	147.8 ± 6.9
15	4.26 ± 0.07	14.3 ± 0.5	5.1 ± 0.1	1.26 ± 0.03	45.6 ± 1.9	114.2 ± 3.3
Average	3.49 ± 0.14	12.0 ± 0.5	5.70 ± 0.1	1.23 ± 0.03	35.6 ± 2.6	102.5 ± 4.6
Low suspected pathogen load						
9	4.24 ± 0.23	15.3 ± 0.6	5.9 ± 0.2	1.14 ± 0.08	59.1 ± 11.3	101.8 ± 6.3
12	5.98 ± 0.36	12.7 ± 0.9	4.8 ± 0.1	1.02 ± 0.04	57.7 ± 7.0	125.6 ± 8.5
13	3.61 ± 0.05	11.8 ± 0.2	6.2 ± 0.1	1.24 ± 0.03	38.0 ± 7.6	91.8 ± 1.8
14	5.31 ± 0.17	19.2 ± 0.7	6.6 ± 0.1	1.34 ± 0.01	21.3 ± 2.0	124.8 ± 4.7
Average	4.79 ± 0.24	14.7 ± 0.7	5.9 ± 0.2	1.18 ± 0.04	44.0 ± 5.0	111.0 ± 4.3
Significance	ns	ns	ns	ns	ns	ns

in sterilized vs unsterilized soil (Fig. 4.2) and the extra information on soil chemical (Table 4.2) and biological (supplementary material Fig. S4.2) data support our idea that the differences in cress growth between field sites was strongly driven by variation in natural disease presence. Consequently, further analyses incorporated the distinction between field sites with low versus high inherent pathogen load.

The effect of the *Pythium* addition in the bioassay differed significantly between field sites with low versus high inherent pathogen load, as evidenced by the significant interaction of *Pythium* and inherent pathogen load in the mixed effect model (Table 4.3). In the bioassay, *Pythium* suppressed cress weight only in the field sites with low inherent pathogen load. Also, the effect of amendment addition on cress weight depended significantly on the field soil's inherent pathogen load (Table 4.3).

Table 4.3. Results of a mixed effect model where cress weight grown in non-sterilized field soil was the dependent variable and amendment addition, Pythium addition, and perceived inherent pathogen load as independent variables. The Chi-square value (degrees of freedom in parenthesis), p value, and significance level are shown. Differences are considered significant at p ≤ at 0.05 (values ≤ are given in bold). Significance is indicated according to the P-values indicating (*) p < 0.1; * p < 0.05; ** p < 0.01; *** p < 0.001; ns = non-significant.

	Wald Chisq (df)	p value	Sig level
Amendment	5.75 (4)	0.22	ns
Pythium	56.99 (1)	**<0.001**	***
Path. load	46.78 (1)	**<0.001**	***
Amendment x Pythium	6.12 (4)	0.19	ns
Amendment x Path.load	12.80 (4)	**0.01**	*
Pythium x Path.load	8.43 (1)	**0.004**	**
Amendment x Pythium x Path.load	2.71 (4)	0.61	ns

The organic amendment effect on disease suppression was altered by the prior pathogen load and therefore we split the analysis in two based on the same division in sites of figure 4.2B and C. The sites with a high inherent level of pathogen load showed overall low cress weight (Fig. 4.3A). Here adding compost slightly increases cress weight relative to the control treatment without organic amendments. However, because of the low overall cress weight in all treatments, there was no effect of the *Pythium* addition or the interaction of amendment with *Pythium*. The sites with low inherent pathogen load showed overall higher cress weight than sites with high inherent pathogen load (Fig. 4.3). *Pythium* addition in these sites significantly reduced cress weight, with a trend of amelioration of this *Pythium* effect by some of the organic amendments.

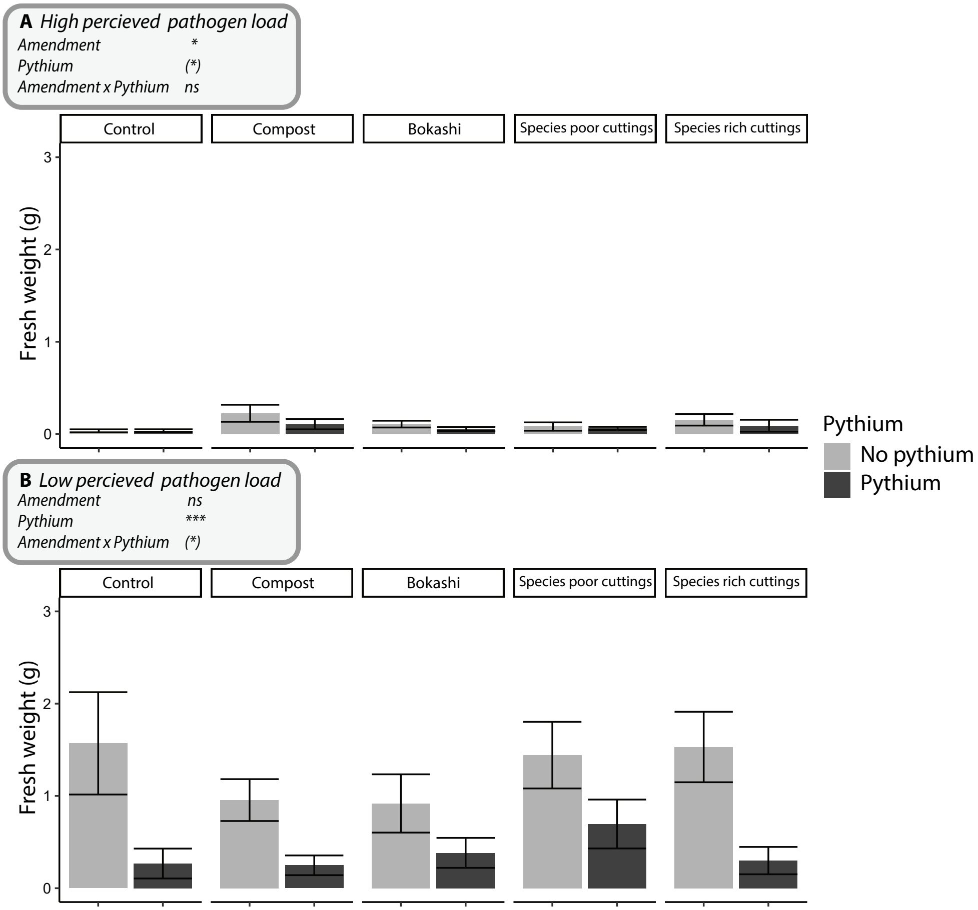


Figure 4.3. A) Mean cress shoot fresh weight in grams of sites with high perceived pathogen load (nr 2, 5, 6, 7, 10 and 15) effected by the different amendment treatments and *Pythium* addition. The error bars represent the mean ± standard error (n= 6). The effect of the amendment, *Pythium* addition and the interaction are shown in the upper right corner in the graph. **B)** Mean cress shoot fresh weight in grams of sites with low perceived pathogen load (nr 9, 12, 13 and 14) effected by the different amendment treatments and *Pythium* addition. The error bars represent the mean ± standard error (n=4). The effect of the amendment, *Pythium* addition and the interaction are shown in the upper right corner in the graph. Significance is indicated according to the P-values following (*) p < 0.1; * p < 0.05; ** p < 0.01; *** p < 0.001; ns = non-significant.

Soil suppressiveness shortly after organic amendment application

The effects on soil suppressiveness shortly after application of the organic amendments were tested in a second bioassay, for which we applied the different amendments to soil of two field locations (fields nr 9 and 13), selected for their low inherent pathogen load, and explored cress weight with and without the addition of *Pythium*. We found that adding organic amendments generally increased cress weight relative to the control without organic amendments, in particular for the treatment with species rich cuttings (Fig. 4.4A). Adding *Pythium* effectively reduced cress weight in most treatments. The strength of the *Pythium* effect differed between treatments, being lowest for the treatment with Bokashi and highest for the treatment with species rich cuttings (Fig. 4.4A). The *Pythium* suppressive effect of Bokashi was further confirmed with a t-test within each treatment: *Pythium* significantly decreased cress weight in the control, compost, species poor and species rich cuttings but not in the Bokashi treatment, suggesting high disease suppression for Bokashi (Fig. 4.4A and B).

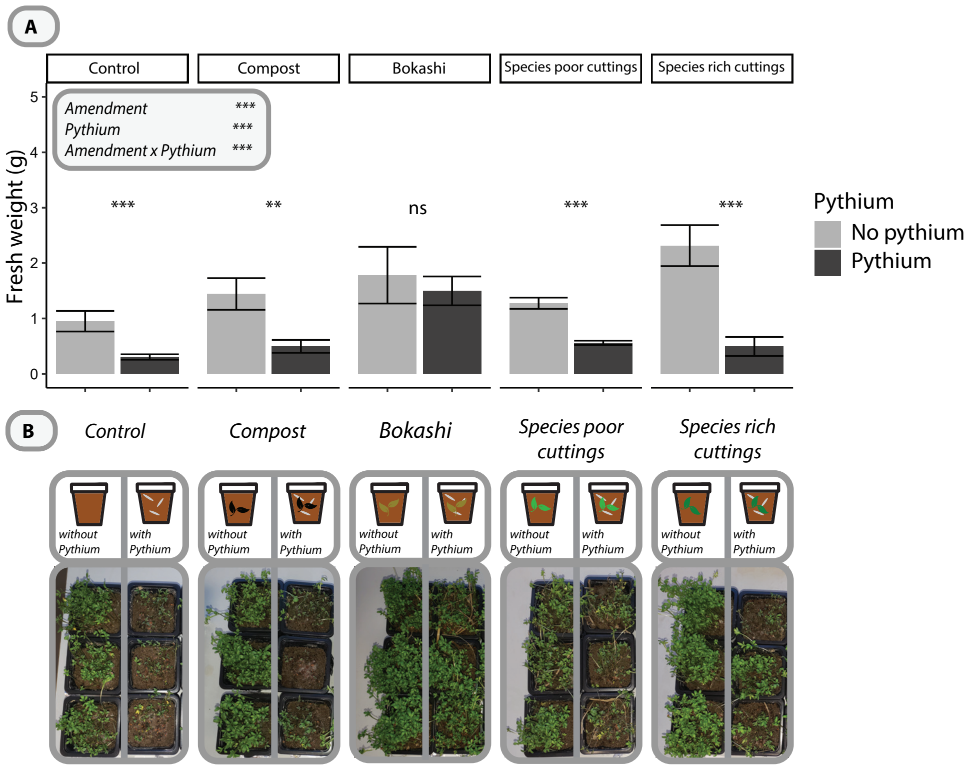


Figure 4.4. A) Mean cress shoot fresh weight in grams effected by the different amendment treatments and *Pythium* addition on non-sterilised soil from two field locations with low pathogen load (site nr 9 and 13). The error bars represent the mean ± standard error (n= 6). The effect of the amendment, *Pythium* addition and the interaction are shown in the upper right corner in the graph. A Student t test was performed within each treatment to test if the *Pythium* addition had an effect. Significance is indicated according to the P-values following (*) p < 0.1; * p < 0.05; ** p < 0.01; *** p < 0.001; ns = non-significant. **B)** Visualization of three random replicates are shown of every treatment both with and without inoculation of *Pythium*.

Discussion

To investigate whether different organic amendments (compost, Bokashi, or fresh road verge cuttings) increased soil suppressiveness after short-term or multi-year application we conducted two bioassays. The bioassay made use of the plant-pathogen *Lepidium sativum* (cress) – *Pythium ultimum* model system which can provide conclusions regarding general soil suppressiveness of a specific soil (Bongiorno et al., 2019; Mayerhofer et al., 2021; Tamm et al., 2010; Thuerig et al., 2009). To be relevant in the field we made use of a multi-year field experiment across 10 different field sites which functioned as replicates for the study. However, across the sites we found a large difference in cress weight in the non-sterilized soil (Fig. 4.2A). It is known that different locations can differ widely in their level of inherent soil-borne diseases and therefore sites can generate different bioassay outcomes (Bongiorno et al., 2019; Löbmann et al., 2016; Tamm et al., 2010). We concluded that the difference between locations in how *Pythium* affected cress weight is not a chemical (Table 2) but a biological effect shown with evidence from the sterilized treatments (Fig. 4.2B and C) and is likely due to a high inherent pathogen load in some of the locations. Methodological differences are unlikely as the bioassays used the same plant species, genotype and isolate of *Pythium* at the same concentration across all soils. Given that the field sites did not show a clear pattern in fungal and microbial biomass and general composition across microbial groups between suspected low and high inherent pathogen load (supplementary material Fig. S4.2), while they did differ in their disease suppressiveness, we argue that differences in inherent pathogen load between fields best explained our results. Therefore, we do have strong, albeit indirect, evidence that the differences between field sites were biological in origin and not related to soil chemical differences. The effect of the organic amendments on soil-borne disease suppression was only significant in fields with a low inherent pathogen load (Table 3 and Fig. 4.3). The latter suggests that the capacity of an amendment to improve soil suppressiveness is more preventative than curative. Very few studies investigated this further and these studies did find similar results where the effect of an organic amendment did not "cure" soil from a plant pathogen (Wim J. Blok et al., 2000).

The organic amendment treatments showed different effects on soil suppressiveness in the bioassays in general. We hypothesized that the decomposability of an organic amendment would influence the soil microbiome the most and therefore influence the soil suppressiveness. Matured compost is expected to decompose the slowest after addition to the soil and therefore have the smallest effect on soil suppressiveness in the short-term but a larger one in the longer term. We found no effect of the compost treatment on soil suppressiveness in both short- and long-term (Fig. 4.3B and 4.4). However, compost is mentioned by Bonanomi et al. (2007) to be the most suppressive organic amendment. This discrepancy may be due to differences in the materials used as Bonanomi et al. (2010)

concluded that a wide range of start materials is used in disease suppression studies and that the maturity of the compost might interfere with this effect, as well as the use of higher compost dosages applied to potting soil substrates when horticultural systems were taken into account. Next to that, Mayerhofer et al. (2021) detected a negative relationship between compost age and soil suppressiveness. The maturity of our used compost was not tested extensively but the composting process took eight weeks and was believed to be finished when the compost did not have an elevated temperature above 40 °C. This practice ensured a matured compost which could explain the lack of soil suppressiveness after application since the availability of easily degradable C sources (Mondini et al., 2003) is low which does not activate or increase the soil microbiome in the short- and long-term as well other amendments (Bonanomi et al., 2010; Luo et al., 2022; Neher et al., 2013).

The fresh organic amendment treatments were hypothesized to be highly variable since the decomposability is difficult to estimate (Bonanomi et al., 2007, 2010). In our research, the fresh organic amendment treatments did not increase soil suppressiveness in the short- or long-term (Fig. 4.3B and 4.4), nor did they promote the effect of the disease. Perhaps, the peak of decomposition of these fresh organic amendment treatments did not line up with the timing of bioassays. Studies that compared fresh and composted organic amendments from the same start material are rare, but Pascual et al. (2000) did include this aspect and noticed a disease suppressiveness effect on *Pythium* from both composted and fresh municipal solid waste 24 months after application of the amendments. In our long-term experiment, the first application was also 24 months prior to the bioassay which is in the same timeframe as Pascual et al. (2000). However, the soil used for the experiment of Pascual et al. (2000) had a significant lower SOM content (0.3 %) than in our study (average of our sites 4.2 % SOM). Adding an organic amendment to a very poor soil (as indicated by very low % SOM) can boost the soil microbiome more drastically than in our study, and therefore create more significant results regarding the effect on soil suppressiveness.

In our study, Bokashi was the only organic amendment that achieved an increase in soil suppressiveness in the short-term bioassay (Fig. 4.4). We hypothesized that the Bokashi treatment would indeed increase soil suppressiveness due to the fermenting process which increases the decomposability of the organic material once added to the soil. This short-term boost of available resources was expected to increase the soil microbiome's activity and with that the competition against the pathogen (Luo et al., 2022; Shin et al., 2017). Since the positive effect of the Bokashi application can only be found in our short-term application, it could also be argued that the microbes added to the soil with the Bokashi material would create extra competition for the plant pathogen. Shin et al. (2017) tested this hypothesis by applying both sterilized and non-sterilized Bokashi, made with the same microbial products as the Bokashi in our study, and compared the effect in a similar bioassay. They found no consistent suppression effect of Bokashi with live micro-organisms compared to sterilized Bokashi. Nor did the micro-organisms added with the Bokashi change the total microbial activity and bacterial community composition in the soil. The soil suppressiveness effect of the Bokashi treatment was therefore mostly attributed to a boost in microbial activity of the soil inhabiting microbes and triggered by the high decomposability of Bokashi material. With that reasoning, it also makes sense that the long-term effect of Bokashi was not present in our bioassay (Fig. 4.3B).

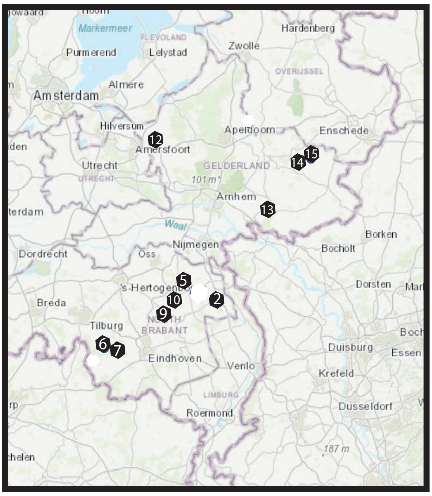
Overall the potential of organic amendments to increase soil suppressiveness in arable fields is present, at least in the short time after addition and as a preventative measure. However, the type of organic amendment and timing of application is very important to establish an increase in soil suppressiveness. With this study, we can conclude that fermenting organic material via the Bokashi method does increase soil suppressiveness right after application when compared to compost or fresh amendments from the same starting material. Long-term effects however were more difficult to detect. A multi-year field experiment where soil is collected for bioassays multiple times per year from short to longer after the last application might show a soil suppressiveness effect of the different organic amendment treatments. Next to that, this study focused on applied effects in realistic situations but in depth sequencing of microbial communities both in the soil and amendment might increase the mechanistic knowledge surrounding this topic further. We recommend that future studies use the same start material when comparing compost and Bokashi since this is very important to prevent confounding effects of different materials.

Acknowledgements

We would like to thank Tamas Salanki for helping out in the logistical parts of the bioassay experiments and overall help in the lab. Thanks to Paolo Di Lonardo for helping with the PLFA measurements and Jan van Walsem for the soil chemical measurements. This study has been made possible through financial support from the Soil Biology Group (Wageningen University) and the province of Noord-Brabant, the province of Gelderland, the water boards Brabantse Delta, De Dommel and Aa en Maas and the municipalities of Sint Anthonis and Gilze en Rijen.

Supplementary material

A)



B)

Site nr	Street	Municipality	Province	Coordinates
1	Zandkant	Sint Anthonis	Brabant	51.616403, 5.924533
2	Wethouders Lindersstraat	Landhorst	Brabant	51.651374, 5.778837
3	Westelbeersedijk	Diessen	Brabant	51.451638, 5.200192
4	Heikesestraat/Broekdijk	Vessem	Brabant	51.449660, 5.275588
5	Gerecht	Erp	Brabant	51.569613, 5.585742
6	Meerkensweg	Volkel	Brabant	51.628341, 5.631983
7	Barneveldseweg	Nijkerk	Gelderland	52.206065, 5.51 9975
8	Broekstraat	Wehl	Gelderland	51.950962, 6.239565
9	Zwiepseweg	Barchem	Gelderland	52.129573, 6.446915
10	Nettelhortseweg	Geesteren	Gelderland	52.159798, 6.464713

Figure S4.1. A) All field sites indicated on a map of the Netherlands. **B)** Geographic information of all the field sites.

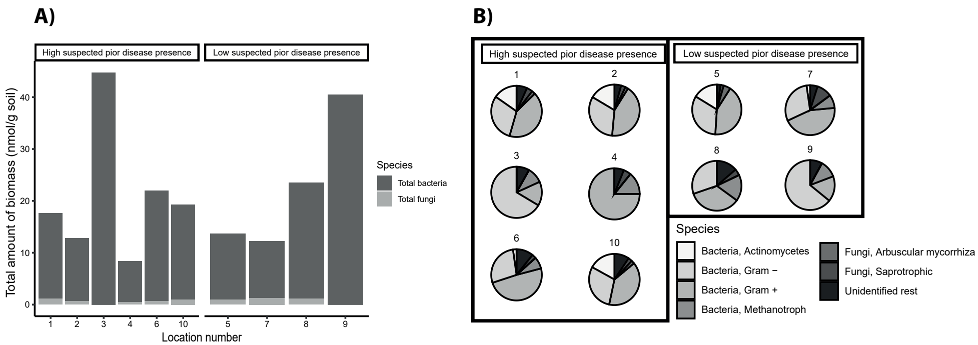


Figure S4.2. A) Total amount of microbial biomass in every control plot per location split into the field sites with suspected high prior disease presence (left) and suspected low prior disease presence (right). The cumulative amount of bacterial and fungal biomass is indicated in the bar graph (n=1). **B)** Species composition according to the groups indicated with PLFA analysis of the control plots (n=1) of the different locations split into the field sites with suspected high prior disease presence (left) and suspected low prior disease presence (right).

Chapter 5

General discussion

Maartje van der Sloot

Increasing global food demands to feed the growing world population in combination with limited arable space are forcing farmers to intensify production to reach the highest possible crop yield per hectare (Godfray et al., 2010; IPCC, 2019; Lal, 2010). However, these intensive farming practices are simultaneously harming the food security goal by decreasing arable soil quality (IPCC, 2019; Matson et al., 1997) and decreasing plant and insect diversity in nearby semi-natural habitats (Kleijn and van Langevelde, 2006; Li et al., 2020). Cost-minimizing practices, such as flail mowing without removal of the cuttings, combined with excess nitrogen (N) inputs through air and water are causing plant species diversity in semi-natural habitats to decrease (Cole et al., 2020). However, both of these systems can benefit from an interrelated solution. By removing the cuttings from semi-natural habitats and applying them as organic amendment on arable soils, eutrophication in the semi-natural habitat can be offset while an increase in soil organic matter (SOM) in arable fields can be achieved. An increase in SOM content in agricultural soil is often associated with multiple beneficial ecosystem functions such as increased water retention capacity, improved N retention, improved disease suppressiveness and even increased crop yield (Bonanomi et al., 2007; Diacono and Montemurro, 2010; Emerson, 1995; Franzluebbers, 2002; Lal, 2006; Reeves, 1997; Sullivan et al., 2019; Thiele-Bruhn et al., 2012). Cuttings from road verges represent an easily accessible and local source of organic material from a semi-natural habitat that could benefit from removal of plant material.

The desired increase in SOM content and its associated benefits is highly dependent on the chemical composition of the organic material used (Mohanty et al., 2013; Termorshuizen et al., 2006b). The chemical composition is furthermore affected by the processing methods that are widely used prior to applying organic materials, such as composting or fermenting via the recently popular Bokashi method (Bernal et al., 1998; Olle, 2021; Zmora-Nahum et al., 2005) which in turn may alter the effect on SOM build-up and the additional benefits (Freibauer et al., 2004; Gong et al., 2021; Mohanty et al., 2013; Quiroz and Céspedes, 2019). This research is conducted to explore the effect of road verge cuttings as organic amendment on several benefits (increase in SOM content, water holding capacity, N retention, crop yield and soil disease suppressiveness) and risks (increase in N leaching, weed cover and/or heavy metal and anthropogenic litter contamination) and to evaluate what differences occur when differing processing methods are applied which will increase our knowledge base regarding the application of road verge cuttings and the different processing methods. This thesis, therefore, combined results from highly controlled experimental studies with those from a real-world field study with **the aim to understand the mechanisms, benefits and risks of road verge cuttings as organic amendment in agriculture.**

The multi-site field experiment (**chapter 2**) showed a significant increase in SOM over three years as a result of application of composted and fresh road verge cuttings in the upper 10 cm. Crop yield was found to remain stable in the organic amendment treatment with half of the conventional mineral fertilizer rate compared to the control with the full conventional mineral fertilizer rate. Several potential risks, such as increases in N leaching, weed pressure and heavy metal contamination, were found to be negligible in the field experiment. The only risk that still stands is the presence of anthropogenic litter, primarily beer and soft drink cans and bottles in the organic amendment treatments which would end up in the field.

To increase the knowledge of the different effects from organic amendments quality and quantity, an open air mesocosm experiment was performed where both a range in carbon to nitrogen (C:N) ratio and quantity of organic amendments was applied (**chapter 3**). It proved the importance of the C:N ratio of the used amendment over the quantity of application, especially on crop yield and soil N retention effects. N leaching was found to be influenced solely by the presence (no N leaching) or absence (significant N leaching) of a crop.

The short- and long-term effects on soil disease suppressiveness of applying different types of road verge cuttings as organic amendment was investigated using two bioassays (**chapter 4**). I found little evidence of improved soil disease suppressiveness caused by the application of cuttings since none of the soils that had been exposed to the amendment treatments for two years showed increased soil disease suppressiveness. However, direct application of Bokashi resulted in reduced negative effects of application of the plant pathogen *Pythium ultimum* to the bioassay plant cress.

In this current chapter, these individual studies are interrelated and discussed which is valid since the different studies used the same road verge cuttings source material and/or used the same sandy arable soil. The studies are also put into a wider scientific context and main conclusions and advise on policy changes are considered.

Changes in SOM build-up over time

An important reason to use organic amendments in arable fields is to improve soil quality by increasing the SOM content (Hijbeek et al., 2019). Also the need to increase global SOC levels, which is about 50% of the SOM fraction in Dutch soils (Reijneveld et al., 2021), with a growth rate of 0.4% per year formulated in the 4 per mille target is an important reason when arguing to apply organic amendments (Minasny et al., 2017). Since especially sandy arable soils are prone to lose SOM when they are intensively cultivated for many years (Hijbeek et al., 2017b; Yost and Hartemink, 2019), it could be particularly beneficial to target these soils when applying organic amendments as was done in this thesis. When introducing a new type of organic material as organic amendment obtained via a different processing method compared to conventional organic material such as compost, it is necessary to test if the desired SOM increase is achieved and if different processing methods of the same raw materials generate the same or different results. The hypothesized build-up of SOM content in arable sandy soil as a result of applying road verge cuttings as organic amendment was indeed observed, albeit it not to the same extent with all types of amendment even though they were based on the same raw materials (**chapter 2**). Yearly amending the soil with compost or fresh road verge cuttings created a significant increase in SOM content of 0.81, 0.54 and 0.42 % for compost, fresh species poor, and fresh species rich cuttings respectively. These levels of % SOM change over three years are much higher than the needed 0.4% rate increase that was postulated by the 4 per mille initiative to fight climate change. The field experiment started with an average SOM level of 3.9 % which entails an absolute SOM level of 3.947 % after three years to reach the yearly 0.4 % rate increase. The absolute increases in compost and fresh amendment succeeded this rate increase thus contributing to the Paris Climate Agreement (Minasny et al., 2017).

The field study suggests that application of organic material results in a linear increase of SOM content. In the long-term, this increase probably levels off so that SOM content reaches a certain upper limit. This limit is caused by the balance between carbon (C) inputs and C losses from the soil upon decomposition by the soil biota. If C inputs into the soil are maintained, the C soil balance is driven by the level of C that can be protected from decomposition. Soil aggregates and mineral surfaces can retain newly added C to a certain capacity in the form of minerally-bound C which is unavailable for soil biota. However, when the soils mineral surface storage capacity is exceeded, the additional C cannot be protected from microbial activity and is decomposed (Carter et al., 2003; Hassink, 1997; Six et al., 2002). This saturation level in soils is (mostly) linked to the clay and silt content since these particles increase the surface area for C binding which prevents decomposition of C and therefore increases the C saturation point (Hassink, 1997; Six et al., 2002). Furthermore, the level of physical soil disturbances such as tillage affects the physical protection/exposure of soil C for decomposition. The soils in our field experiment likely have a small percentage of both clay and silt since they are sandy arable soils (Hassink, 1997; Sleutel et al., 2006). These soils are similar in texture to the sandy arable soil used for the mesocosm experiment which consisted of 3 % clay, 16 % silt and 78 % sand according to classification reports (Eurofins Agro) and 3.16 % SOM (**chapter 3**). For the field experiment of **chapter 2**, I do not have the data of g C in the silt and clay fraction/kg soil and thus cannot quantify the level of C saturation. However, to get an idea of the potential C saturation level, I looked at studies that used a comparable soil type as for example in the study of Porre et al. (2020). Porre et al. (2020) worked in an arable cropping system with soil with a high similarity to the soils used in my thesis in terms of clay, silt, sand and SOM percentage, 2 %, 12 %, 86 % and 3.1 % respectively, and in terms of geographic and climatic origin, and showed that the soil was indeed saturated in the level of C that was bound to mineral surfaces. Therefore, it is possible that the soils in my experiments are already reaching C saturation in the mineral associated SOM. However, it is useful to investigate this further by measuring the exact clay, silt and sand content per field site and measuring the different SOM fractions (Particulate Organic Matter and Mineral Associated Organic Matter) to gain more knowledge on the specific fraction where application of the organic amendments most effectively result in an increase in SOM. It will also be interesting to see if the effect of the different processing methods play a role in which SOM fraction the increase is achieved (i.e. mineral associated and particulate organic matter) and if this increase is sustainable or reaching the C saturation point. These are interesting further research opportunities to investigate the limits on SOM build-up after organic amendment application.

Since soils with a higher percentage of clay have a higher C saturation point, it might be useful to apply the organic amendments on clayey soil instead of sandy soil if one only wants to achieve an increase in SOM. Applying cuttings as organic amendment on both clayey and sandy soil was tested by Spijker et al. (2022) by applying Bokashi from road verge cuttings and fresh cuttings in two quantities (10 and 50 ton/ha) on one field with clay soil and two fields with sandy soil. They found a significant N immobilization effect in the clay location hampering maize yield while this effect was not present in the sandy locations. The capacity of the soils to supply N was found to be very low in the clay soil partly due to a much lower SOM content (1.8 %) than the sandy soil in that study (4.0 and 3.8 %). The SOM content in the clay soil was also very low compared to our soils (3.9 %). The low SOM content in combination with no additional fertilization and applying the cuttings right before sowing maize most likely hindered maize growth in the clay soil field site (Spijker et al., 2022). Spijker et al. (2022) looked at direct effects within one cropping season and differences on reaching the C saturation point of the sandy or clayey soil are therefore not investigated yet. Neither my thesis or any study to date could compare the effects of road verge cuttings as organic amendment on SOM between clay or sand. More research investigating the different effects of applying road verge cuttings as organic amendments to both clay and sandy soil for longer periods of time can provide interesting new insights. However, the fact that sandy soils are more susceptible to SOM degradation (Hijbeek et al., 2017a; Johnston et al., 2009; Yost and Hartemink, 2019) makes the choice to focus on sandy soil in this thesis valid.

Since the C saturation point can be expected to be easily reached in sandy soils, it can be argued that adding organic amendments might not be useful to increase soil C in the long-term when the soil is saturated. However, theoretically the soil could still continue to retain soil C in the Particulate Organic Matter which is not protected from decomposition (Stewart et al., 2009). In any case, it would be unwise to stop applying organic inputs at the suspected C saturation level since SOC turnover is faster in soils close to C saturation and refraining from further organic matter additions will result in large initial losses of SOC especially in soils that are subjected to soil disturbances such as tillage (Hassink, 1997; Meyer et al., 2017). Moreover, researchers found that if an arable soil is reaching C saturation level in the upper soil layer (0-5 cm) as a result of organic amendments inputs, the C is translocated to deeper soil layers even with no-tillage management (Nicoloso et al., 2018). The organic amendment used in the last 12 years of the 25 year field experiment from Nicoloso et al. (2018) was very similar to the compost treatment from my thesis. This compost treatment increased the SOC content in the upper 5 cm of soil and achieved an increase below incorporation level at 5-15 cm. It was argued that the saturation point in the upper soil layer is achieved and C was translocated to deeper layer and protected for decomposition. The exact mechanisms for such C translocation in the soil, such as a higher soil porosity to facilitate soluble C transfer or bioturbation caused by fauna or plants roots, are discussed but further research is needed in this respect (Nicoloso et al., 2018). It could, however, explain the result of significant SOM increase by compost in both the incorporation (0-10 cm) and whole cultivation layer (10-40 cm) in my field experiment (**chapter 2**). This would also signal that the C saturation level is achieved in the upper soil layer in the experiment. Increasing SOM content in sandy arable soil by applying organic amendment from composted or fresh road verge cuttings is possible in three years and should be implemented locally to improve arable soil quality.

Organic amendment effect on N cycling: finding a slow release fertilizer without N leaching

Adverse impacts on crop production are a main concern of farmers when application of organic amendments is discussed (Hijbeek et al., 2019). This concern is valid since applying an organic amendment with a high C:N ratio can result in N immobilization which may then leave little N for crop growth (Flavel and Murphy, 2006; Hadas et al., 2004; Kaleeem Abbasi et al., 2015; Mohanty et al., 2013). A low C:N ratio generally results in N mineralization which provides available N during crop growth so that organic amendments basically function as a slow release fertilizer. However, a large amount of freely available N in the soil solution during a fallow period or slow crop growth period increases the risk of N leaching especially after heavy rainfall (Malcolm et al., 2019; Steen Jensen and Ambus, 1999). During periods or rainfall and slow or no crop growth, N immobilization would actually be favourable to limit N leaching. Consequently, when investigating the effects of application of an organic amendment, it is particularly important to examine the balance between net immobilization when the risk of N leaching is high and mineralization of nutrients when the crop is in need of N. It is necessary for the application of road verge cuttings as organic amendment to find the right timing of N mineralization and immobilization to maintain crop yield while improving SOM build-up and preventing N leaching.

The total amount of N applied with both the mineral fertilizer and organic amendment in my field experiment varied from 190-290 kg N/ ha/ year compared to the control of 180 kg N/ ha/ year (**chapter 2**). The N balance calculations showed that this did not result in significant N losses after application of the organic amendment treatments. No significant differences in crop yield were detected, which raises the question of where the extra added N went. The increase in SOM content facilitated by the organic amendment application might explain the discrepancy because an increase in SOM entails an increase in soil N (Van Groenigen et al., 2017) since SOM has a C:N ratio of about 12:1 (Batjes, 1996). However, the soil N retention parameter did not show a significant increase in **chapter 2** compared to the control. This parameter was calculated based on the change in the total amount of N in the entire cultivation soil layer over the three measurements that were done in the final year of the experiment since the whole cultivation layer had not been sampled in the first two years of the study. The increase in SOM in the last year might not have been sufficient to create a significant increase in total amount of N in a single year. However, when comparing the concentration of total N in the soil in September 2022 after three full years of amendment application, I found a significantly higher N concentration in plots that received the organic amendments as opposed to the control, both in the upper soil layer and in the entire cultivation layer (Fig. 5.1). Since crop yield and N loss were not significantly different between the treatments, it is likely that this difference

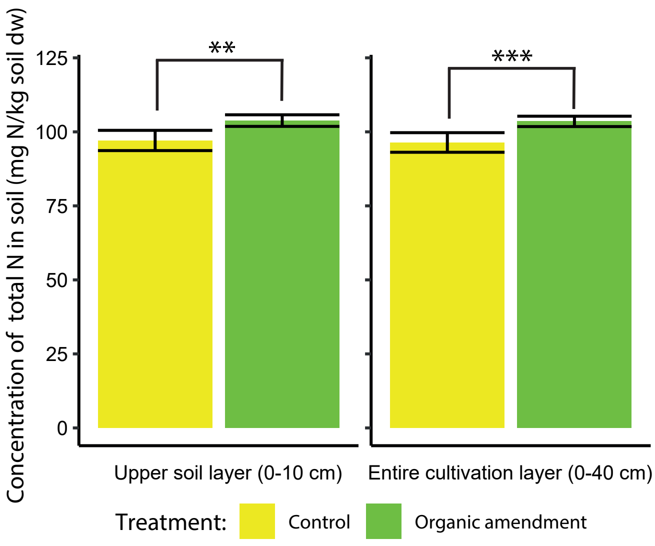


Figure 5.1. Average concentration of total N in soil (as mg N/ kg soil dw) of the control (n=30) and all the organic amendment treatments combined (n=120) in the upper soil layer (left) and entire cultivation layer (right) at the final measuring moment of the field experiment (September 2022). Error bars represent the mean ± standard error. Statistical analysis consisted of a linear mixed effect model for each soil layer separately with the concentration of total N as response variable while treatment was the independent variable. Location nr and crop were added as random factor. Separate models were made for the upper and entire soil layer. Result of the linear mixed effect model is indicated in the graph. Significance is according to (*) $p < 0.1$; * $p < 0.05$; ** $p < 0.01$; *** $p < 0.001$; ns = non-significant.

is explained by the increase in SOM content and associated N content in the SOM (Van Groenigen et al., 2017). Based on our field experiment, it can therefore be concluded that adding N with the organic amendments to sandy soil that contain growing plants does not significantly increase the risk of N leaching as the N is primarily present in organic forms that are not prone to leaching and where most of the mineralized N gets taken up by the growing plants.

More exact measurements on N leaching were done in **chapter 3**. An effect on N leaching as a result of organic amendment application was not visible. The amount of N applied with the organic amendments at 30 ton /ha was 138 kg N/ ha, which was higher than the mineral fertilization rate in the mesocosm experiment (80 kg N/ha). In the experiment in **chapter 3** I incorporated the organic matter in spring and used spring wheat and a shorter cropping cycle (six months) compared to my field experiment, which makes translation to the field experiment of **chapter 2** based on amounts of N added difficult. However, the result on N leaching is similar, namely that neither the organic amendment nor the control treatments showed any increase in N leaching. The only incident of N leaching detected in **chapter 3** was found when no plants were present (control - treatment). Since the field experiment (**chapter 2**) had (cover) crops growing during the whole year, the soil was scarcely fallow. The only fallow period occurred between wheat harvest (end of July) and cover crop sowing (begin of October) which is generally a dry period in The Netherlands. The results align since **chapter 3** proved that leaching only occurs when no plants are present and after intense rainfalls and **chapter 2** shows no sign of leaching probably because the soil was not left bare for extended periods and no heavy rainfall periods occurred during period with bare soil. Growing plants is a well-known method to prevent N leaching (Porre, 2020; Thapa et al., 2018) and underlies Dutch regulations obliging farmers to grow cover crops in certain crop rotations. Despite the good results on N leaching in my thesis, caution still needs to be taken when applying the organic amendment at a different time in the crop cycle since the timing in our field experiment did not result in N leaching but a different timing of application and therefore N mineralization during a fallow or wet period will increase the change of N leaching (Pang and Letey, 2000).

Providing sufficient N to the crop is, next to the effect of a certain C:N ratio, partly dependent on the timing of application of the organic amendment which is also concluded in **chapter 2** and **3**. The timing between application of the amendment and crop sowing differed between these studies, with sowing shortly (**chapter 3**) or longer (**chapter 2**) after amendment incorporation into the soil, which resulted in differences in yield. Applying the organic amendment right before crop sowing without additional fertilization proved to hamper spring wheat growth considerably because of immediate N immobilization (**chapter 3**). In the end of the experiment however, a large amount of mineralized N was present in the soil indicating that some N did mineralize during the experiment and would be available to the next crop. Applying the organic amendment right before sowing the crop was not beneficial despite applying higher absolute levels of N compared to the control treatment (**chapter 3**). In the field experiment, the organic amendments were applied each year in September or October shortly followed by the sowing of winter wheat or a cover crop. The cover crop was incorporated into the soil in April or May and maize was sown right afterwards. This timing probably resulted in N immobilization during the period of least crop growth (winter) and N mineralization in spring when crop N demand is higher. However, in the last year of the field experiment (March 2022, right before mineral fertilizer application) I observed at several locations that the wheat growing in plots that had received organic amendments in the previous years had grown better than wheat in the control plots (Fig. 5.2). This observation is in line with the expectation that some net N mineralization of fresh and old SOM occurred between September and March. These visual differences in crop growth were not present anymore shortly after application of mineral fertilizer and wheat yield did not differ significantly at harvest in July (**chapter 2**).

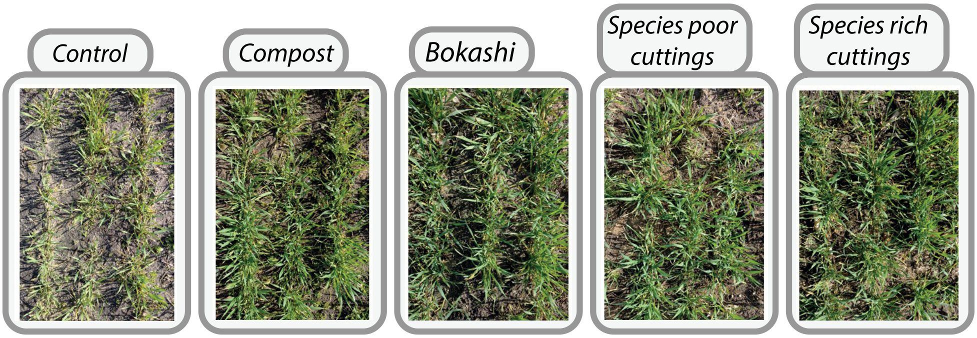


Figure 5.2. Pictures of wheat growing in location 3 of the different treatments from the field experiment right before mineral fertilization in March 2022.

These results indicate that road verge cuttings applied as organic amendments can function as slow release fertilizer without serious risks of N leaching since the timing of application, N immobilization and mineralization matches the demands of this cropping system well. Finding the right timing is a common challenge when changing from direct fertilization from mineral fertilizers towards slow release organic fertilization (Pang and Letey, 2000). This also makes the classification of such organic amendments in policy as fertilizer difficult because it needs to be applied at the right timing without leaving the soil bare to prevent N leaching because N release occurs over a longer time period than from mineral fertilizers. But overall, my results suggest that applying road verge cuttings as organic amendments in September prior to seeding wheat or cover crops followed by maize, in combination with the application of half of the conventional mineral fertilizer rate will result in an increase SOM content, maintain yield of both wheat and maize and does not result in significant risks of N leaching provided a (cover) crop is present.

Soil borne disease suppression and the effect of inherent plant pathogen load on crop growth

To increase the general disease suppression capacity of the soil, organic amendments can be used to improve the soils microbiome in diversity, population size and activity (Bonanomi et al., 2010; Mayerhofer et al., 2021). An improved soil microbiome is more equipped to withstand the invasion of a pathogen but this result is highly dependent on the type and chemical composition of the used amendment (Bonanomi et al., 2007; Termorshuizen et al., 2006b). This notion made it necessary to test for the novel application of road verge cuttings in its different forms as organic amendment (**chapter 4**). The fact that different processing methods (composting and fermenting using the Bokashi method) were tested that were all made from the same start material (which was also added as fresh treatment) makes my study robust and innovative. In the soil disease suppression, only the Bokashi treatment succeeded in increasing the soil suppressiveness shortly after application, and not in longer term. This effect is probably related to the timing of decomposition of the used amendment where only the decomposition of the Bokashi treatment in the short-term aligned to the needs of the soil microbiome and subsequent presence of the pathogen.

Since a long-term increase in SOM can also improve the soil microbiome (Thiele-Bruhn et al., 2012), it is interesting to link the differences in SOM content found in **chapter 2** to the soil suppressiveness bioassays in **chapter 4**. In September 2021, two years into the field experiment and one year after the previous organic amendment application, soil was collected at the field sites to be used for the bioassay experiments. At that timepoint, the control plots had an average of 3.90 ± 0.20 % SOM while the plots receiving compost, Bokashi, species poor and species rich cuttings had statistically significant higher SOM levels at 4.64 ± 0.30, 4.30 ± 0.38, 4.31 ± 0.33 and 4.28 ± 0.32 % respectively. However, I did not find an increase in soil suppressiveness in the bioassay and hence no relation could be made between % SOM and level of disease suppression could be drawn (**chapter 4**). Possibly the inherent pathogen load in the field locations as defined in the study had been interrupting this relation between SOM and disease suppression. However, a linear mixed effect model including both treatment and perceived pathogen load (high or low) as explanatory variable, SOM as response variable and location as random factor did not show an significant interaction (Numerator DF= 4, Denominator DF= 32,F value= 1.02, p= 0.41) indicating that SOM content did not differ between sites with high and low perceived pathogen loads. The difference in SOM content between control and any organic amendment treatment was apparently too small to enhance soil suppressiveness. This result may not be surprising especially because SOM is not the only driver of pathogen suppressiveness (De Corato, 2020; Schlatter et al., 2017; Termorshuizen et al., 2006a). The level of increase in SOM observed in our study might not have increased the soil microbial diversity and activity, the assumed key factor driving pathogen suppression, to create a persistent ecological significant effect. It may still well be that when testing disease suppression in the field shortly after a new application of the organic amendments very similar results to those observed in the controlled bioassay will be seen, which could imply a Bokashi-mediated window of protection for new seedlings during a stage that plants are vulnerable for soil borne pathogen invasion.

In this thesis, the effect of an organic amendment application on general soil suppressiveness was argued to be linked to the peak of decomposition of the applied amendment (**chapter 4**). The assumed mechanism consists of the direct effect of the organic amendment serving as energy source for the soil microbiome to increase the activity and to withstand the invasion of a pathogen. This energy source could potentially also be used by an already present pathogen which aligns with my conclusion on the fact that organic amendments cannot be used to "cure" a soil but merely enhance the defence for subsequent invasion by pathogens (Wim J Blok et al., 2000). Apart from the activation of the soil inherent biota, the organic amendment could also be a source of microorganisms that could help to mediate disease suppression, provided they survived the organic amendment processing and can thrive in soil-like conditions. This was already partly investigated by Shin et al. (2017) using soil respiration analysis and denaturing gradient gel electrophoresis to determine the microbial activity and bacterial composition which found no clear evidence of Bokashi microbes presence in amended soils. However, extensive molecular analysis of the microbial communities in the amendments and in the soil before and shortly and longer after amending the soil will help to pinpoint the main actors in generating Bokashi-mediated disease suppression.

Since the levels of inherent pathogen load in the field significantly influenced cress growth in the bioassay (**chapter 4**) and soil from the multi-site field experiment were used for this bioassay, it is interesting to check if the high perceived pathogen load in certain locations was also reflected in crop growth in the field experiment. To test this, I analysed if fields with high and low inherent pathogen loads had significantly different crop yields using standardized yield data from all the years and all the field sites in a linear mixed effects model. I found that there was a significant interaction between perceived pathogen load and standardized crop yield (Numerator DF= 1, Denominator DF= 255, F value= 23.13, p value< 0.001, Fig. 5.3). Wheat yield did not significantly differ between the locations with high and low perceived pathogen load. Maize yield, however, was significantly lower in locations with high perceived pathogen load.

Typically, maize is a resilient crop but in the field experiment it apparently suffered in certain locations because of a potentially soil borne biological effect (**chapter 4**). An effect in maize but not in wheat could possibly be due to multiple maize cropping seasons prior

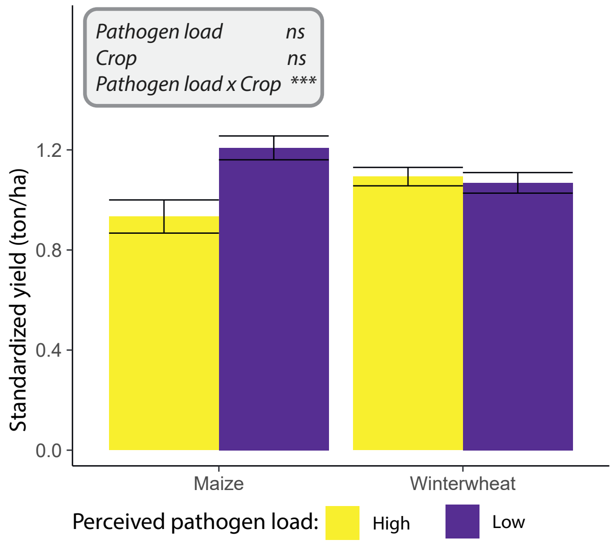


Figure 5.3. Average standardized yield per crop and perceived pathogen load. Yield data of all three years is used and data was standardized by dividing the yield data by the average of that year to adjust for weather influences between years. Error bars represent the mean ± standard error; n= 90 for high perceived pathogen load, n= 60 for low perceived pathogen load. Statistical analysis consisted of a linear mixed effect model with standardized yield as response variable while crop, pathogen load and their interaction were the independent variables. Location nr, year and organic amendment treatment were added as random factor. Result of the linear mixed effect model is indicated in the graph. Significance is according to (*) $p < 0.1$; * $p < 0.05$; ** $p < 0.01$; *** $p < 0.001$; ns = non-significant.

to the experiment and, with that, the build-up of harmful pathogens specific for maize (Van Schooten et al., 2019; Zhao et al., 2021) which are less harmful to wheat. However, the cropping history of the locations did not validate this reasoning because fields 9, 12 and 14 had maize as main crop for multiple years prior to the field experiment and were categorized as low inherent pathogen load. Other location specific observations, i.e. use of fungicides or closeness of certain locations, did also not provide answers to why certain locations suffered from a high perceived pathogen load. If the inherent pathogen load is mainly caused by the natural presence of *Pythium* in the field, the difference in effect between maize and wheat can be explained since *Pythium* is known to hamper maize growth due to root browning (Van Schooten et al., 2019). The effect of *Pythium* on wheat is known to be more pronounced in the germination phase while there is no clear evidence on root browning in later stages (Waller, 1979). The difference in timing of sowing and growing of the crops and peak of activity of *Pythium* in the field underlines this argument even more. The peak of activity of *Pythium* is expected to be in spring (Manici et al., 2014) when the germinating maize is at high risk of getting infected while the winter wheat plants are already past germination phase since they are sown in November. It would therefore make sense that maize plants were suffering more in the fields with high inherent pathogen load than wheat plants. However, I have no conclusive answer on which (combination of) pathogens were present in the field soil since I could not sequence the microbial DNA from the soils and the presence of a *Pythium* contamination as a cause for the differences in crop yield presented in Fig. 5.3 is therefore speculative at best. An additional linear mixed effects model to check if the organic amendment treatments change the effects on crop yield included the standardized crop yield as dependent variable, the inherent pathogen load in interaction with the organic amendment treatments as independent variables and location, year and treatment as random factors. This model showed unfortunately no significant effect of the amendment treatment in either of the crops and pathogen loads (wheat: Numerator DF= 1, Denominator DF= 108, F value= 0.00, p value= 0.99, maize: Numerator DF= 1, Denominator DF= 117, F value= 0.014, p value= 0.91), indicating again that the amendments could not "cure" the soil from the presence of a disease.

The result on the lower maize yield (Fig. 5.3), probably caused by the presence of a pathogen, does suggest that there is a need for an increase in the soils capacity to withstand diseases. This is even of higher priority in the near future with the projected increasing pathogen activity caused by increasing soil temperatures due to climate change (Manici et al., 2014). My thesis, however, also shows that an improved soil suppressiveness caused by organic amendment application is difficult to achieve since only Bokashi achieved an increase in soil suppressiveness, and this effect was short lived. In a real world field situation it will be very difficult to time the decomposition of an applied organic amendment to the period of highest risk of a plant pathogen. It might be more beneficial to apply the organic amendment with the main goal to increase SOM content and achieve the related increase in the soil microbiome's diversity and activity (Thiele-Bruhn et al., 2012). This effect is longer term than my study was able to show, but it will be interesting to follow changes in the soil microbiomes diversity and activity over longer time periods and test soil suppressiveness after short- en long-term application of organic amendments in the field.

The benefits and risks of the different organic amendment treatments

My thesis contains experiments investigating the effect of different organic amendment treatments using the same start material, which is the first study to date that used this approach comparing the effects of compost, Bokashi and fresh organic amendments. Recommendations on which organic amendment treatment and processing method to use are therefore appropriate. The compost and fresh organic amendment treatments were effective in achieving an increase in SOM content while maintaining crop yield with half of the mineral fertilizer rates over three years compared to a control treatment (**chapter 2**). Differences between the compost and two fresh treatments on other measured parameters were rare which verifies the conclusion that merely applying road verge cuttings as organic amendment is effective while the treatment, being compost or a fresh amendment, does not greatly influence the overall effect. Nevertheless, I discuss the slight differences in the coming paragraphs.

The different fresh treatments were included in the study to investigate if the costly processing methods (composting or fermenting via the Bokashi method) are necessary to achieve beneficial outcomes or if fresh cuttings could yield the same results. The only significant effect in favour of a processed treatment compared to fresh amendments was the SOM increase in the entire cultivation layer caused by compost (**chapter 2**) which was explained by the expected high recalcitrant : labile carbon ratio and probable SOM saturation in the upper layer and translocation to deeper layers (Nicoloso et al., 2018). In all the other measured parameters (crop yield, N cycling, water holding capacity, weed pressure and soil suppressiveness), the effect of applying a fresh or composted organic amendment treatment did not change the outcome compared to a control situation.

In the fresh treatments, a division was made between species poor and species rich cuttings since they are expected to differ in their biochemical properties since a higher diversity of flowering herbaceous species is expected to have a higher C:N ratio and/or lignin content than species poor cuttings, creating potentially different effects when this material is applied as organic amendment (**chapter 3**, Flavel and Murphy (2006) and Mohanty et al. (2013)). The difference in both C:N ratio and lignin content between species poor and rich cuttings was not significant in 2019 and 2021. Only a significant difference in 2020 was present in the C:N ratio of 24.8 and 40.9 of species poor and species rich cuttings respectively which was too short to result in significant effects on N cycling and crop growth. Nevertheless, it is positive that the two fresh treatments resulted in beneficial SOM increases without loss of crop yield.

Remarkably, Bokashi did not achieve a significant increase in SOM relative to the control treatment, which was explained by the probable low recalcitrant : labile carbon ratio in the material. Knowing that the auxiliary materials, which are a solution with microbes and clay minerals, needed to make Bokashi can cost between 10- 15 euro's per ton according to Dutch pilot studies (Riechelman and Postma, 2021), it is therefore more expensive than fresh cuttings and might achieve less in terms of SOM build-up.

One could argue that the high decomposability due to the low recalcitrant : labile carbon ratio of Bokashi increases mineral N availability after quick decomposition of the material and with that have a higher fertilization capacity (Olle, 2021). However, I did not find increased crop growth after application of Bokashi compared to the other organic amendment applications (**chapter 2**) which can be explained by the fact that the C:N ratio of the Bokashi (average C:N ratio of 31.0) did not differ from the C:N ratio of the fresh cuttings (average C:N ratio of 25.3 and 31.9 of species poor and species rich cuttings, respectively) and was notably higher than that of compost (average C:N ratio of 15.3). Knowing that the tipping point between N mineralization and immobilization is between a C:N ratio of 10 and 20 as found in **chapter 3** and previous studies (Kaleeem Abbasi et al., 2015; Nicolardot et al., 2001), it is not probable that Bokashi provided more N to the crop than fresh amendments or composts.

The only benefit where Bokashi created a differing and better outcome compared to the other treatments was in the second bioassay experiment demonstrating potential of Bokashi to improve soil suppressiveness in short-term (**chapter 4**). The proposed underlying mechanism causing the short-term effect on soil suppressiveness states that the Bokashi treatment is highly decomposable due to the fermentation process the material has been through which provides a short-term boost of available resources for the soil microbiome's activity which enhances their competition against an added pathogen (Luo et al., 2022; Shin et al., 2017). This mechanism makes sense when considering that the effect on soil suppressiveness is short lived reasoning that this boost in activity disappears when the material is decomposed which was confirmed by the results of the long-term bioassay. The other tested organic amendments did not go through the same fermentation process which possibly made them less effective in achieving an increase in soil suppressiveness (**chapter 4**).

In terms of C conservation and greenhouse gas emissions, Bokashi can also be viewed as a storing method for organic material without losing C as opposed to composting (Olle, 2021). The Bokashi material might, however, decompose more quickly and emit the CO^2 right after application to the soil but the comparison on CO^2 emission after application of compost, Bokashi or fresh material to soil is (to date) not extensively researched yet. I observed such a difference in CO^2 emissions between compost, Bokashi and fresh amendments in a small pilot incubation experiment but the data was not of adequate scientific standard due to the low level of replications (n=3). An extensive life cycle assessment comparing the C footprint of compost, Bokashi and fresh material is needed to justify choices between these treatments when looking for the best outlook on total greenhouse gas emission reduction. However, in terms of SOM build-up effects, which is considered the main reason for applying an organic amendment, using Bokashi as opposed to fresh or composted cuttings is not advised according to this study.

Chemical characteristics of the different organic amendment treatments such as C:N ratio or lignin content are partly explaining the effects I found in SOM build-up (**chapter 2**), N cycling (**chapter 3**), crop growth (**chapter 3**) or soil disease suppressiveness (**chapter 4**). These chemical characteristics are therefore useful to measure but it is difficult to pinpoint a single chemical characteristic that determines the overall quality of an organic amendment treatment. For example, a review article linking the quality of an organic amendment to N cycling showed similar importance of C:N ratio and lignin content but mentioned soluble carbohydrates, cellulose, hemi-cellulose and poly-phenols to be equally important for decomposition of an organic amendment and linked mineralization of N (Mohanty et al., 2013). The same can be found when looking for a chemical parameter determining the link between decomposition and SOM build-up or soil suppressiveness (Bonanomi et al., 2010; Pane et al., 2011). The measured chemical parameter to determine overall quality of an amendment therefore also depends on what a farmer wants to achieve with the organic amendment. Nevertheless, my thesis shows that applying fresh or composted organic amendments is generally improving soil quality when compared to no organic application and that additionally applying half of the mineral fertilizer rates was sufficient to maintain crop yield. Since none of the investigated risks differed between the organic amendment treatments, I conclude that composted or fresh road verge cuttings are a good source of organic amendments on sandy arable soil.

Concluding remarks and advice for farmers and policy changes

The overall conclusion of my thesis must be that fresh and composted road verge cuttings can function well as organic amendment on sandy arable soil by improving SOM content in the long-term while maintaining crop yield with half of the mineral fertilizer application. Since there are two main periods of road verge cutting availability in the Netherlands, being June and September, I would advise Dutch farmers to apply the June cuttings as compost in September to achieve the highest SOM increase possible. Bokashi did not seem the preferred treatment for SOM increase according to my thesis but more detailed research investigating the capability of this treatment to increase soil suppressiveness in the field might show that this treatment is preferred to the other treatments when improving soil suppressiveness is the main goal of the farmer. Mimicking the timing of application (i.e. in fall) and crop sowing of this thesis is important to prevent crop yield losses due to N immobilization when applying the amendments in spring. I would therefore not advise to compost the fresh cuttings in September and applying them in spring but I would apply the fresh cuttings in September since this also achieved a SOM increase while not resulting in more risks such as increases in weed pressure, N leaching or contaminations when compared to another organic amendment treatment or the control.

Outside of improvements in soil quality, this application can result in less fertilization costs for farmers and less disposal costs for municipalities while the road verges might have an improved species diversity helping pollinator populations. The only risk and disadvantage that still stands is contamination with anthropogenic litter. Extra attention and actions can be implemented to decrease this risk, such as collecting the litter (mechanically) prior to or post cutting, implementing deposit schemes for cans and beverage packaging or choosing to use cuttings from nature areas. A major barrier regarding the implementation of this application of road verge cuttings is current policies and laws concerning this organic material. Road verge cuttings are currently classified as a waste stream in policies in The Netherlands and Europe. A European expertise network on Recycling of

Agricultural, Municipal, and Industrial Residues to Agriculture (RAMIRAN) and several Dutch organizations such as 'Programma Circulair Terreinbeheer' and 'Biomassa alliantie' are asking for policy and system change to implement this application and valuing the road verge cuttings as organic resource instead of treating them as a waste stream (Misselbrook et al., 2012; Spijker et al., 2022). With that, new concepts can be introduced based on the perspective of 'harvesting' and 'applying' the road verge cuttings instead of 'mowing' and 'disposing'.

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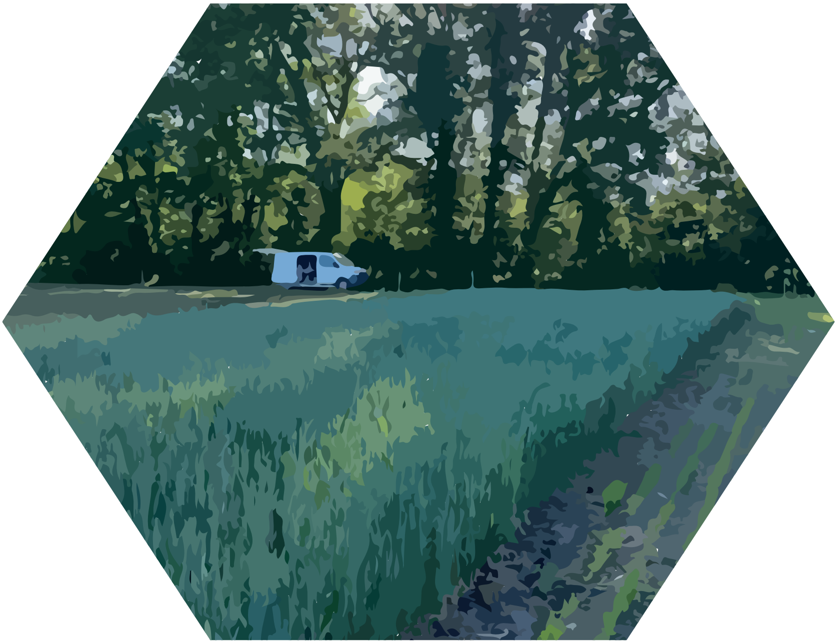
Summary



Over the past decades, an increasing demand for food resulted in agricultural intensification to create the highest crop productivity possible. However, practices used in intensive agriculture, such as deep tillage and heavy reliance on mineral fertilizers and herbicides, are reducing the quality of arable soils. These practices furthermore have severe negative effects on semi-natural habitats bordering these arable fields, such as roadside verges. The main impacts on biodiversity come from large nutrient emissions from agriculture and cost-minimizing practices which result in species-poor vegetation with few flowers, providing poor habitat quality for pollinators and other insects. These two declining systems, both the arable fields and road side verges, can benefit from an interrelated solution: actively removing cuttings from the road verge and applying those plant cuttings as organic amendments on arable fields. This practice can prevent eutrophication and promote restoration of species diversity in the road verge, while the organic amendments can improve the quality of arable soil via an increase in Soil Organic Matter (SOM) content. Increasing SOM content in arable fields can be linked to several beneficial ecosystem functions such as improved water retention, nutrient cycling, disease suppressiveness, and (with that) increased crop yield. However, before road verge cuttings can be advised to function as organic amendment to arable soil, a number of issues need to be resolved.

Firstly, the mechanisms by which road verge cuttings, in fresh or processed form, could best promote soil-based functions in arable fields are not yet investigated sufficiently which are crucial since the type and chemical composition of the used organic amendment are expected to determine the success of achieving the desired SOM content increase and associated beneficial functions. Secondly, the trade-off between the overall benefits (improved water retention, nutrient cycling, disease suppressiveness, and crop yield) and potential risks (increased nutrient leaching, weed cover and application of contaminations) needs to be assessed under realistic conditions in order to be able to advice on the use (or not) of road verge cuttings as organic amendment. This is particularly relevant since using road verge cuttings as amendment is currently perceived by most farmers as undesirable because of the high risk of contaminating the arable fields with weed seeds, anthropogenic litter or heavy metals.

The objective of my thesis was, therefore, to investigate the usage of road verge cuttings as organic amendments in arable soil, and provide more knowledge of the underlying mechanisms, potential benefits and risks of this application. I used road verge cuttings in different forms, namely fresh, composted and fermented via the Bokashi method, in order to find conclusive answers which processing method has the highest potential in improving soil quality and maintaining crop yield. A combination of short-term experiments and a three year multi-site field experiment on farmers' fields using the same organic amendment start materials allowed me to understand both the

mechanistic effects of the organic amendments while also gaining practical knowledge on the potential benefits and risks.

In **chapter 2**, I investigated the potential benefits (possible increases in SOM content, water holding capacity, nitrogen (N) retention and crop yield) and risks (possible increases in N leaching, weed pressure, heavy metal and anthropogenic litter contamination) of the usage of road verge cuttings. A real-world multi-site field experiment was used where processed (composted or fermented as Bokashi) and fresh road verge cuttings (species poor cuttings and species rich cuttings) were applied in a maize-winter wheat crop rotation on fifteen fields at commercial farms on arable sandy soils. The organic amendment plots received half of the conventional rates of mineral fertilizer application, while the control plots on each field site received 100% of the conventional mineral fertilizer application rates. The experiment ran for three years over which a significant increase in SOM content was found in the upper 10 cm of soil after multiple applications of compost and the two fresh cutting treatments. Compost even enhanced SOM significantly in the entire cultivation layer (0-40 cm). Despite a 50% reduction in applied mineral fertilizer in the organic amendment treatments, both the maize and wheat achieved similar yields as in the control treatment. This finding demonstrates that the use of these organic amendments allows for equivalent yields with only half the amount of mineral fertilizer. The application of road verge cuttings did not result in significant increases in N leaching, weed pressure, nor did it surpass the legally defined thresholds for heavy metal content that would prohibit their use. However, we did notice a substantial presence of anthropogenic litter, predominantly beer and soft drink cans and bottles. If this risk can be prevented, by removing the litter pre- or post-cutting, the application of road verge cuttings as organic amendment represents a promising solution for simultaneously promoting soil and road verge quality.

In **chapter 3**, I performed an open-air mesocosm experiment in which I tested the impact of organic amendment quantity and quality on soil N cycling and plant growth. Thereto I used road verge cuttings mixed with slurry or straw to create a range in carbon to nitrogen ratio (C:N ratio) and applied these in different quantities to sandy field soil. This experimental design allowed for a more mechanistic understanding of the combined effect of the C:N ratio and quantity of the used organic amendment. The effect of the range in C:N ratio (10 to 60) and quantity (10 to 30 tons per ha) on crop growth, N leaching and soil N retention was investigated during 6 months. With this experiment, the short-term effect of the organic amendments on N mineralization and N immobilization, the associated crop (spring wheat) growth, N leaching and the N retained in the soil after crop harvest was researched. I found that the balance point between net N immobilization and mineralization is between a C:N ratio of 10 and 20 since the amendments with a C:N ratio of 20 and higher decreased crop biomass due to N deficiency. In contrast, organic amendments with a C:N ratio of 10 promoted crop biomass. Applying larger quantities of amendments reinforced the effect of the C:N ratio and could not alleviate the reduced crop growth caused by amendments with a C:N ratio of 20 or more. N leaching was unaffected by either C:N ratio or quantity of the amendment and occurred only in the control treatment without fertilizer application and plants. These results suggest that growing a crop is probably adequate to reduce the most significant N leaching risks of organic amendment application. This study also indicates that it is important to apply additional fertilizer when growing a crop immediately after amending the soil with fresh organic material, because of the risk of crop yield losses when organic amendments with a high C:N ratio are used.

In **chapter 4**, I described the effects of organic amendment treatments on the soil's capacity to withstand the presence of soil-borne diseases (soil suppressiveness). This effect can be highly dependent on the type and processing method of the used organic amendment and timing since application. The objective of the study was therefore to test the disease suppression capacity of sandy arable soils after application of several road verge cutting treatments. Fresh plant cuttings, or compost or Bokashi (a fermentation method) made from the same cuttings, have been applied for two consecutive years across 10 field sites and soil suppressiveness was determined to gain insight in the long-term effects of these organic amendment treatments. Next to that, the potential disease suppression effect of short-term applications from the same organic amendment treatments was tested using the same sandy arable field soil without any previous road verge cutting additions. Soil suppressiveness was measured with a bioassay using the *Lepidium savitum* (cress) – *Pythium ultimum* model system. The individual field sites greatly influenced cress growth in the bioassay. This effect was likely the result of inherent pathogen load in certain fields as the cress grew well on sterilised soil of all field sites. Evidently, the organic amendment treatments do not have the ability to "cure" a soil from a disease, but also when looking at the sites with suspected low inherent pathogen load, I did not see an effect from the long-term application of the organic amendment treatments. However, significant short-term effects were visible but only in the Bokashi treatment which suppressed the effect of the soil borne pathogen used in the bioassay. I propose that the observed effects are related to the decomposability of the organic amendments which can promote disease suppression by stimulating saprotrophic soil biota which compete with the soil pathogens. It is hypothesized that the peak of decomposition of a material in the soil boosts the natural soil microbiome, both in number and activity, and with that increases general disease suppressiveness by competing with the pathogen. However, only the peak of decomposition of Bokashi apparently lined up with the timing of the short-term bioassay. It is one of the first studies that compared compost, Bokashi, and fresh cuttings from the same start material on soil suppressiveness and illustrates that the potential to increase the soil suppressiveness is there, but needs further testing in a realistic field situation.

This thesis used the same organic amendment material throughout the three chapters, allowing me to interrelate these individual studies and put them in a wider scientific context in **chapter 5**. At first, the effect on SOM build-up shown in **chapter 2** is discussed and the capacity of soils to continue increasing in SOM content is questioned. The carbon (C) saturation point of the soils used in my field experiment are examined and the data necessary to calculate the C saturation points is explained.

The impact of organic amendments on N cycling and the effect on crop growth were investigated in both **chapter 2** and **3**, and showed that both adequate timing of amendment application to the soil and some additional fertilization is needed to prevent crop yield losses. The timing of **chapter 2**, consisting of amendment application in fall and crop planting and additional fertilization of half of the conventional fertilizer rate in spring, is argued to be effective for preventing crop yield losses due to N immobilization. The combined results from **chapter 2** and **3** suggest that N leaching was not increased by the application of the organic amendments as long as there was continuous presence of a (cover) crop. I proved that it is possible that the applied organic amendments can be used as slow release fertilizer without risk on N leaching.

The effect of the organic amendment treatments on soil suppressiveness (**chapter 4**) is discussed and linked to the SOM increase found in **chapter 2** which was not significant due to the limited increase in SOM which did probably not result in an improved soil microbiome. Next to that, I found that the field locations with high perceived pathogen load in **chapter 4** also resulted in significantly lower maize yield in the field experiment. This result proved that indeed the maize was likely suffering from a disease which was not alleviated by the organic amendment, proving again that the application of the road verge cuttings as organic amendment can at best buffer against a new pathogen invasion but does not eliminate pathogens that are already present.

The differences between the organic amendment treatments (compost, Bokashi, species poor or species rich cuttings) were also discussed in **chapter 5**. Bokashi was found to improve disease suppressiveness in the short-term but failed to effectively increase SOM content over the years due to the low recalcitrant: labile carbon ratio. Since no other differences in N cycling, crop yield, water retention, or risk of contaminations were detected, compost and fresh amendments are therefore preferred as soil improver. It is hard to find a single chemical characteristic of the organic amendment treatment that can predict the quality since it depends on the desired effect to generate, being soil suppressiveness, SOM build-up or fertilization capacity.

Finally, concluding remarks and advice for both farmers and policy changes are given in the final paragraphs of **chapter 5**. Classifying road verge cuttings as a valuable resource as opposed to a waste stream, which is done currently, seems the first step towards using this material to close local nutrient cycles and to improve road verge and arable soil quality for a more sustainable future.

Samenvatting

In de afgelopen decennia heeft een toenemende vraag naar voedsel geleid tot intensivering van de landbouw om de hoogst mogelijke gewasproductiviteit te bereiken. Echter, praktijken uit de intensieve landbouw, zoals diep ploegen en grootschalig gebruik van kunstmest en herbiciden, verminderen de kwaliteit van de landbouwbodems. Bovendien hebben deze praktijken ernstige negatieve effecten op de naastgelegen habitats, zoals sloten en wegbermen. Een grote negatieve impact op de biodiversiteit van deze habitats is het resultaat van grote nutriënten emissies uit de landbouw in combinatie met kosten minimaliserend maaibeleid die leiden tot soortenarme vegetatie met weinig bloemen in de wegbermen, wat een slechte habitatkwaliteit biedt voor bestuivers en andere insecten. Deze twee achteruitgaande systemen, zowel de landbouwbodems als de wegbermen, kunnen profiteren van een onderling verbonden oplossing: het actief verwijderen van maaisel uit de berm en het aanbrengen van dit plant materiaal als organische toevoeging op de landbouwbodems als bodemverbeteraar. Deze praktijk kan eutrofiëring voorkomen en de soortendiversiteit in de berm bevorderen, terwijl de organische toevoegingen de kwaliteit van de landbouwbodems kunnen verbeteren door een toename van het organisch stof gehalte in de bodem. Het verhogen van het organisch stof gehalte in landbouwbodems kan worden gekoppeld aan verschillende gunstige ecosysteemfuncties, zoals een verbeterde waterretentie, nutriëntencyclus, ziekteonderdrukking en daarmee een verhoogde gewasopbrengst. Voordat bermmaaisel echter kan worden aanbevolen als organische toevoeging voor landbouwbodems, moeten een aantal vraagstukken worden beantwoord.

Ten eerste zijn de mechanismen waarmee bermmaaisel, in verse of verwerkte vorm, de bodemfuncties in landbouwbodems het best zou kunnen bevorderen nog niet voldoende onderzocht. Dit is heel belangrijk aangezien het type en de chemische samenstelling van de gebruikte organische toevoeging het succes zal bepalen in het bereiken van een verhoogde organisch stof gehalte en de daarmee geassocieerde gunstige functies. Ten tweede moet de afweging tussen de algehele voordelen (verbeterde waterretentie, nutriëntencyclus, ziekteonderdrukking en gewasopbrengst) en potentiële risico's (toegenomen uitspoeling van nutriënten, onkruiddruk en aanwezigheid van andere verontreinigingen) worden beoordeeld onder realistische omstandigheden om advies te kunnen geven over het gebruik (of niet) van bermmaaisel als bodemverbeteraar. Dit is met name relevant omdat het gebruik van bermmaaisel als bodemverbeteraar momenteel door de meeste boeren als onwenselijk wordt beschouwd vanwege het hoge risico op verontreiniging van het akkerland met onkruidzaden, zwerfafval of zware metalen.

Het doel van mijn proefschrift was daarom om het gebruik van bermmaaisel als organische toevoeging en bodemverbeteraar in landbouwbodems te onderzoeken en meer kennis te verschaffen over de onderliggende mechanismen, mogelijke voordelen en risico's van deze toepassing. Ik heb bermmaaisel in verschillende

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vormen gebruikt, namelijk vers, gecomposteerd en gefermenteerd via de Bokashi-methode, om definitieve antwoorden te vinden over welke verwerkingsmethode het grootste potentieel heeft om de bodemkwaliteit te verbeteren en de gewasopbrengst te handhaven of zelfs verhogen. Een combinatie van korte termijn experimenten en een driejarig veldexperiment op meerdere locaties op boerenland met hetzelfde organische startmateriaal stelde me in staat om zowel de mechanische effecten van de organische toevoegingen te begrijpen als praktische kennis op te doen over de mogelijke voordelen en risico's.

In **hoofdstuk 2** onderzocht ik de mogelijke voordelen (mogelijke toenames in het organisch stof gehalte, waterretentiecapaciteit, stikstof (N) vasthouding en gewasopbrengst) en risico's (mogelijke toenames in N-uitspoeling, onkruiddruk, zware metalen en verontreiniging met zwerfafval) van het gebruik van bermmaaisel. Er werd een realistisch veldexperiment gebruikt waarbij verwerkt (gecomposteerd of gefermenteerd als Bokashi) en vers bermmaaisel (soortenarm maaisel en soortenrijk maaisel) werd opgebracht in een teeltrotatie van maïs en wintertarwe op vijftien velden bij commerciële boerderijen op zandige landbouwbodems. De percelen met de bermmaaisel toevoegingen ontvingen de helft van de gebruikelijke hoeveelheid kunstmest, terwijl de controlevelden op elke locatie 100% van de gebruikelijke hoeveelheid kunstmest ontvingen. Het experiment liep drie jaar waarin een significante toename van het organisch stof gehalte werd gevonden in de bovenste 10 cm van de bodem na meerdere toevoegingen van compost en de twee behandelingen met vers maaisel. Compost verhoogde zelfs de organische stof aanzienlijk in de hele teeltlaag (0-40 cm). Ondanks een 50% vermindering van de toegepaste kunstmest in de maaiselbehandelingen, behaalden zowel de maïs als de tarwe vergelijkbare opbrengsten als in de controlegroep. Deze bevinding toont aan dat het gebruik van deze bermmaaisel toevoegingen een gelijke opbrengst mogelijk maakt met slechts de helft van de hoeveelheid kunstmest. De toepassing van bermmaaisel als bodemverbeteraar leidde niet tot significante toenames in N-uitspoeling of onkruiddruk, noch overschreed het de wettelijk vastgestelde drempels voor de gehaltes aan zware metalen die het gebruik zouden verbieden. We merkten echter een aanzienlijke aanwezigheid van zwerfafval op, voornamelijk bier- en frisdrankblikjes en -flessen. Als dit risico kan worden voorkomen door het zwerfafval voor of na het maaien van de berm te verwijderen, vertegenwoordigt de toepassing van bermmaaisel als organische toevoeging en bodemverbeteraar een veelbelovende oplossing om tegelijkertijd de bodem- en bermkwaliteit te bevorderen.

In **hoofdstuk 3** voerde ik een mesocosm-experiment uit in de open lucht waarin ik de impact van de kwantiteit en kwaliteit van de organische toevoegingen op de stikstofcyclus in de bodem en de plantengroei testte. Hiervoor gebruikte ik bermmaaisel gemengd met mest of stro om een variëteit in koolstof-stikstofverhouding (C:N verhouding) te creëren en paste ik deze in verschillende kwantiteiten toe op zandige grond. Deze experimentele opzet zorgde voor een meer mechanistisch begrip van het gecombineerde effect van de C:N verhouding en de kwantiteit van de gebruikte organische toevoeging. Het effect van het bereik in C:N verhouding (10 tot 60) en kwantiteit (10 tot 30 ton per ha) op gewasgroei, N-uitspoeling en N retentie in de bodem werd onderzocht gedurende 6 maanden. Met dit experiment werd het korte-termijneffect van de organische toevoegingen op N-mineralisatie en N-immobilisatie, de bijbehorende gewasgroei (in zomertarwe), N-uitspoeling en het in de bodem behouden N na de oogst van het gewas onderzocht. Ik ontdekte dat het evenwichtspunt tussen netto N-immobilisatie en mineralisatie tussen een C:N verhouding van 10 en 20 ligt, aangezien de toevoegingen met een C:N verhouding van 20 en hoger de gewasbiomassa verminderden door een stikstoftekort. In tegenstelling hiervan bevorderden organische amendementen met een C:N verhouding van 10 juist de gewasbiomassa. Het toepassen van grotere hoeveelheden organische toevoegingen versterkte het effect van de C:N verhouding en kon het verminderde gewas groei effect van toevoegingen met een C:N verhouding van 20 of meer niet verhelpen. De N-uitspoeling werd niet beïnvloed door de C:N verhouding of de kwantiteit van de toevoeging en trad alleen op in de controlegroep zonder bemesting en planten. Deze resultaten suggereren dat het waarschijnlijk voldoende is om een gewas te telen om de grootste risico's van N-uitspoeling bij het toepassen van organische toevoegingen te verminderen. Deze studie geeft ook aan dat het belangrijk is om extra meststoffen toe te passen wanneer er direct na het toevoegen van organisch materiaal een gewas wordt geteeld, vanwege het risico op verlies van gewasopbrengst wanneer organische toevoegingen met een hoge C:N verhouding worden gebruikt.

In **hoofdstuk 4** beschreef ik het effect van bermmaaisel als organische toevoeging op het vermogen van de bodem om de aanwezigheid van bodem ziektes te weerstaan (bodem ziekteonderdrukking). Dit effect kan sterk afhankelijk zijn van het type en de verwerkingsmethode van de gebruikte organische toevoeging en het tijdstip van het aanbrengen. Het doel van de studie was daarom om de capaciteit van zandige landbouwbodems om ziekten te onderdrukken te testen na het toepassen van verschillende behandelingen van bermmaaisel. Gedurende twee opeenvolgende jaren werd er vers, gecomposteerd of gefermenteerd (volgens de Bokashi methode) maaisel opgebracht van hetzelfde bronmateriaal, toegepast op 10 veldlocaties en werd de bodem ziekteonderdrukking bepaald om inzicht te krijgen in de lange termijn effecten van deze toepassing van bermmaaisel. Daarnaast werd het potentiële effect op bodem ziekteonderdrukking op korte termijn van dezelfde maaisel behandelingen getest met behulp van dezelfde zandige landbouwbodems zonder eerdere toevoegingen van bermmaaisel. Bodem ziekteonderdrukking werd gemeten met een biotoets met behulp van het *Lepidium sativum* (tuinkers) - *Pythium ultimum* model systeem. De individuele veldlocaties hadden aanzienlijke invloed op de groei van tuinkers in de biotoets. Dit

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effect was waarschijnlijk het gevolg van de al aanwezige ziektes in bepaalde velden, aangezien de tuinkers goed groeide op gesteriliseerde grond van alle veldlocaties. Kennelijk hebben de bermmaaisel behandelingen niet het vermogen om een bodem te "genezen" van een ziekte, maar zelfs wanneer er wordt gekeken naar de locaties met vermoedelijk een lage concentratie inherent aanwezige ziektes, zag ik geen effect van de lange termijn toepassing van de bermmaaisel behandelingen. Er waren echter significante korte termijn effecten zichtbaar, maar alleen in de Bokashi-behandeling, die het effect van de gebruikte bodemziekte in de biotoets onderdrukte. Ik beargumenteer in dit hoofdstuk dat de waargenomen effecten verband houden met de afbreekbaarheid van de verschillende maaiselbehandelingen, die bodem ziekteonderdrukking kunnen bevorderen door de aanwezige bodemorganismen te stimuleren die concurreren met de bodemziekte. Er wordt verondersteld dat de piek van ontbinding van een materiaal in de bodem de natuurlijke bodemorganismen versterkt, zowel in aantal als in activiteit, en daarmee de algemene bodem ziekteonderdrukking verhoogt door te concurreren met het pathogeen. Echter, alleen de piek van ontbinding van Bokashi kwam blijkbaar overeen met het tijdstip van de korte termijn biotoets. Het is een van de eerste studies die compost, Bokashi en vers organisch materiaal van hetzelfde bronmateriaal vergeleek wat betreft bodem ziekteonderdrukking en het illustreert dat het potentieel om de bodem ziekteonderdrukking te verhogen aanwezig is, maar verdere experimenten in een realistische veldsituatie zijn nodig om meer vat te krijgen op dit potentiële voordeel van het toevoegen van bermmaaisel als bodemverbeteraar.

Deze scriptie maakte gebruik van hetzelfde organische materiaal als bodemverbeteraar in de drie hoofdstukken, wat me in staat stelde om deze individuele studies met elkaar te verbinden en ze in een bredere wetenschappelijke context te plaatsen in **hoofdstuk 5**. Allereerst wordt het effect op de opbouw van het organisch stofgehalte zoals getoond in **hoofdstuk 2** besproken en wordt de capaciteit van bodems om verder te blijven toenemen in dit gehalte in twijfel getrokken. Het koolstof verzadigingspunt van de bodems die zijn gebruikt in mijn veldexperiment wordt onderzocht, en de gegevens die nodig zijn om de koolstof verzadigingspunten te berekenen, worden uitgelegd.

Het effect van organische toevoegingen op de stikstofcyclus en het effect op gewasgroei werden onderzocht in zowel **hoofdstuk 2** als **3**, en toonden aan dat zowel een adequate timing van de toepassing van het maaisel op de bodem als extra bemesting nodig is om verlies van gewasopbrengst te voorkomen. De timing van **hoofdstuk 2**, bestaande uit opbrengen van het materiaal in de herfst, gewasplanting en extra bemesting van de helft van de conventionele bemestingsdosis in het voorjaar, wordt beschouwd als effectief om verlies van gewasopbrengst als gevolg van N-immobilisatie te voorkomen. De gecombineerde resultaten van **hoofdstuk 2** en **3** suggereren dat N-uitspoeling niet werd verhoogd door de toepassing van de organische toevoegingen, zolang er continu een (vang) gewas aanwezig was. Ik heb aangetoond dat het mogelijk is om bermmaaisel als organische toevoeging te gebruiken als langzaam vrijkomende meststof zonder risico op N-uitspoeling.

Het effect van de organische amendementbehandelingen op bodem ziekteonderdrukking (**hoofdstuk 4**) wordt besproken en gekoppeld aan de toename van het organisch stofgehalte zoals gevonden in **hoofdstuk 2**, die niet significant was vanwege de beperkte toename van het organisch stofgehalte, wat waarschijnlijk niet heeft geleid tot een verbetering in het bodemleven. Daarnaast heb ik ontdekt dat de veldlocaties met een hoog waargenomen ziekte belasting in **hoofdstuk 4** ook resulteerden in aanzienlijk lagere maïsopbrengsten in het veldexperiment. Dit resultaat bewees dat de maïs inderdaad waarschijnlijk leed aan een ziekte die niet werd verlicht door de organische toevoeging, wat opnieuw aantoont dat de toepassing van bermmaaisel als organische toevoeging hoogstens kan beschermen tegen een nieuwe ziekte invasie, maar geen ziektes elimineert die al aanwezig zijn.

De verschillen tussen de organische bermmaaisel behandelingen (compost, Bokashi, soortenarm of soortenrijk maaisel) werden ook besproken in **hoofdstuk 5**. Bokashi bleek op korte termijn de bodem ziekteonderdrukking te verbeteren, maar slaagde er niet in om gedurende de jaren effectief het organisch stofgehalte te verhogen vanwege de lage verhouding tussen moeilijk afbreekbare en labiele koolstof. Aangezien er geen andere verschillen werden gedetecteerd in N-cycli, gewasopbrengst, waterretentie of het risico op verontreinigingen, hebben compost en de verse toevoegingen daarom de voorkeur als bodemverbeteraar. Het is moeilijk om een enkele chemische karakteristiek van de maaiselbehandelingen te vinden die de kwaliteit kan voorspellen, aangezien dit afhangt van het gewenste effect, namelijk een verhoging in bodem ziekteonderdrukking of organisch stof opbouw of een grote bemestingscapaciteit.

Ten slotte worden afsluitende opmerkingen en adviezen gegeven voor zowel boeren als beleidsveranderingen in de laatste alinea's van **hoofdstuk 5**. Het classificeren van bermmaaisel als een waardevolle bron van organisch materiaal in plaats van een afvalstroom, zoals momenteel wordt gedaan, lijkt de eerste stap te zijn naar het gebruik van dit materiaal om lokale nutriëntenstromen te sluiten en de kwaliteit van bermen en landbouwbodems te verbeteren voor een duurzamere toekomst.

Acknowledgements

I found myself in deep contemplation, struggling with how to start my acknowledgments section. Curiously, I saved this part for last, and I believe it's because expressing my huge gratitude for the incredible people who have supported and guided me over the past four years feels difficult to put into words. Nevertheless, I am going to give it a try which is what I have been doing throughout most of my PhD trajectory.

Allereerst **David**, wat heb ik ongelooflijk veel van je geleerd op wetenschappelijk vlak. Je kennis en kunde over ecologie is ontzettend indrukwekkend. En ondanks dat je vaak vertelde dat mijn onderzoek wat meer aan de rand van je expertise zat heb je heel veel geholpen en bijgedragen. Door jou heb ik meer inzichten gekregen en meer focus kunnen leggen op wat er nu voor de boeren en het beleid belangrijk is en heeft mijn onderzoek meer toegevoegde praktische waarde gehad. Misschien wist je wel dat ik dit heel erg belangrijk vond want dit is een grote reden dat ik doorzette in moeilijke tijden. Ook ben ik je erg dankbaar voor één zin die je een paar keer herhaalde als we in onze 'Bermmaaisel meetings' met Gerlinde en Juul te enthousiast werden. Jij zei dan de zin: "Maar we hebben maar één Maartje met twee handen." en daarmee zorgde je dat het experiment toch niet nog 4 extra behandelingen erbij kreeg. Bedankt voor alles!

Lieve **Juul**, je bent een hele fijne begeleider geweest in mijn team omdat je net iets vaker dan gemiddeld vroeg hoe het echt met me ging. Vaak was dan het antwoord; "Ja het gaat écht goed hoor" maar mocht het een keer wat drukker zijn of minder goed zijn gegaan dan voelde ik bij jou altijd de openheid om daarover te praten. Ook de hulp in de berekeningen van de stikstof excel sheets en je hulp tijdens mijn allereerste veldwerkdag (die erg chaotisch en regenachtig was uiteraard) was fantastisch. Ik ga jouw vrolijke lach over de afdeling niet snel vergeten en ik wens je alle succes en geluk voor je verdere carrière en leven toe!

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I am very grateful for the wonderful time I had with my **PEN colleagues**. Especially having a PEN trip two weeks after the start of my contract in 2019 made me feel immediately a home in the group. **Jan**, bedankt voor al je harde werken voor mijn metingen maar natuurlijk vooral bedankt voor de ontzettend gezellige tijd tijdens de koffie en lunch pauzes. Ik wens je nog een heel mooi leven met je vrouw, kinderen en alle kleinkinderen. **Hennie**, bedankt voor alle flauwe grapjes waar ik gedurende de tijd van mijn PhD steeds meer flauwe grapjes tegenover ging zetten! Ik nodig je graag een keer uit om te komen luisteren bij de harmonie zodat je kan horen hoe dat nu echt klinkt.

The PEN office layout changed frequently during my stay but luckily I remained at the same desk and I had some really nice office mates. **Maaike, Chen, Miguel, Tony** and **Reinier** thanks for the lovely time and support in our office! I am also very grateful for the support of all the other PhD's in the PEN group. Especially during the PhD/Post doc "How are you doing?"-meetings, PEN pizza parties and the frequent PEN patat pauzes. Thanks to all the PEN PhD's during the last four years: **Wiene, Gabriella, Sina, Tony, Zakaria, Jan-Markus, Mark, Timea, Klara, Iryna, Abbey, Zulin, Maarten, Sasja, Jasper, Enahu, Reinier, Rik, Rùna, Thijs** and **Eline**.

Special thanks to the students that helped my during my research. **Jana, Solomon** and **David**; your results were crucial for the success of my thesis and I wish you the very best in your future careers.

Mijn experimenten waren onmogelijk om uit te voeren zonder de hulp en ondersteunig van al mijn deelnemende boeren. Dus **Paul**, **Manon, Rik, Mark, Wil, Marcel, Geert-Jan, Wim, Pieter, Sander, Albert** en **Willy** ontzettend bedankt! Speciale dank aan **Paul** en de **gemeente dienst** van de vroegere gemeente Sint Anthonis voor het jaarlijks maken van de compost en Bokashi en het helpen met verspreiden van het maaisel.

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Al mijn **studievrienden uit Nijmegen** wil ik graag bedanken. Bij de start van mijn PhD waren jullie mijn familie en ondanks dat we uitgevlogen zijn en iedereen zijn weg vind blijven we een groep waar ik heel graag avonden en weekenden mee spendeer. Speciale dank aan alle helpers bij mijn experimenten, **Hannah, Tom, Gesa, Max, Maud** en **Marieke**, en dank voor de hulp tijdens het thuiswerken in corona tijden aan **Wytske**.

Pap en **mam**, jullie waren een geweldige ondersteunende factor de laatste jaren. Door altijd te zeggen hoe geweldig jullie het vonden wat ik aan het doen was en hoe knap het is zorgde jullie ervoor dat ik inzag hoe bijzonder het is om een promotie onderzoek te doen. Want tja, in mijn omgeving ben ik niet de enige en vergeet ik soms hoe bijzonder het is en dankzij jullie voelde dat wel zo. Dankzij de stabiele basis vanuit thuis heb ik die zelf ook kunnen maken en leven we nu (apart maar op een kleine afstand) heel fijn samen in Boxtel. En **pap**, nog extra bedankt voor alle hulp in het veld. Ik zag je genieten en trots zijn op wat je dochter aan het doen was en daarom stond je (zelfs in de stromende regen) altijd gemotiveerd op mijn veldjes. Het feit dat jullie samen op een vrije zondag even langsfietsen/wandelen bij mijn veldje in Vessem bewijst hoe betrokken jullie waren, bedankt!

Lieve **Robbie**, het laatste bedankje is zoals beloofd voor jou. Muziek is onze verbindende factor. Samen naar de repetitie rijden en concerten samen kunnen beleven is iets wat ik het allerliefst met jou doe. Maar je trof nou eenmaal ruim 2 jaar geleden tijdens de tournee van vakantieorkest Ad Hoc een bioloog en tijdens de wandeling over het klompenpad in Uddel vroeg je naar mijn werk. Je luisterde geïnteresseerd en je bleef altijd geïnteresseerd luisteren tijdens onze relatie. Je zorgt ervoor dat ik niet teveel maaisel/hooi op mijn vork neem en ook mijn frustraties in de laatste periode van mijn PhD beantwoorde jij altijd met bemoedigende woorden en een onmisbare knuffel. Ik ben je daar ongelofelijk dankbaar voor en zal altijd proberen dezelfde steun voor jou te zijn. Ik hou van je en wil nooit meer zonder je!

About the author

Maartje van der Sloot was born on the 5th of June 1995 in ‘s Hertogenbosch in The Netherlands. During her childhood in Boxtel, she frequently walked through nearby nature areas with her parents and brother which might have started her appreciation for plants and nature. Especially her grandmother Trees Pijnenburg showed Maartje the beauty of nature by always having many plants and flowers in her garden which was regularly visited by Maartje.

After graduating from high school, Maartje choose to study Biology at the Radboud University in Nijmegen. At first, she started this study because of the many medical biology courses available in the BSc programme but after the first botany course she discovered her true passion in biology namely plant-soil interactions. Many more courses in ecology and plant physiology followed in both her BSc (graduated in 2016) and MSc (graduated in 2019) in Nijmegen. Maartje particularly enjoyed the field courses to Terschelling and the Swiss Alps and always appreciated courses where she could develop, design and maintain a realistic experiment with plants. Therefore, Maartje made sure to always have an experiment with plants in both her BSc and MSc thesis projects.

The choice to apply for this PhD project was therefore clear because it entailed a large realistic field experiment with many project management tasks which Maartje enjoyed very much. She also had a preference for working in the agricultural field because she feels that these systems are highly managed which means that a new sustainable practice can easily be implemented if the pros and cons are known. Maartje believes that creating a more sustainable farming system is a worthy goal to achieve.

During her PhD project, she worked with many farmers, policy makers and researchers (both in and out academia) which taught her to always consider each other’s perspectives when making choices in policy. She therefore aspires to work in this interface between farmers, policy and (practical) research in the future.

Outside of her career, Maartje makes (classical) music playing oboe and clarinet for most of her live. She enjoys making music in large wind orchestra’s very much which contributes to her (mental) well-being. She believes that this musical outlet during her years as a PhD helped her through difficult (corona) times and advises anyone who aspires to play anything to not be afraid, seek out a teacher, join a music association and start playing.

List of publications

Published paper

van der Sloot, M., Kleijn, D., De Deyn, G.B., Limpens, J., 2022. Carbon to nitrogen ratio and quantity of organic amendment interactively affect crop growth and soil mineral N retention. Crop Environ. 1, 161–167.

Under second round of revision at Applied Soil Ecology

van der Sloot, M., Maerowitz-Mcmahan, S., Postma, J., Limpens, J., De Deyn, G.B. Soil-borne disease suppressiveness after short and long term application of fermented, composted or fresh organic amendment treatments in arable soils. (modified form of *chapter 4* of this thesis)

In preparation

van der Sloot, M., De Deyn, G.B., Limpens, J.,Kleijn, D. The potential of using cuttings from semi-natural habitats as organic amendments in arable fields (*chapter 2* of this thesis).

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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/project proposal (4.5 ECTS)
- Using road verge cuttings as soil improver and green manure for arable farming

Post-graduate courses (6.7 ECTS)
- Laboratory skills course in soil biology and biochemistry; SBL (2019)
- Summer school soil ecology in the circular agroecology; SBL (2022)
- Bayesian data analysis; PE&RC (2022)
- Grasping sustainability; SENSE (2023)

Competence strengthening/skills courses (3.6 ECTS)
- Scientific writing; Wageningen in'to Languages (2020)
- Efficient writing strategies; Wageningen in'to Languages (2022)
- Communication with the media and the general public; WGS (2022)
- Critical thinking and argumentation; WGS (2022)

Scientific integrity/ethics in science activities (0.9 ECTS)
- Integriteit workshop; WUR/PEN (2020)
- Scientific integrity; WGS (2022)

PE&RC Annual meetings, seminars and the PE&RC weekend (1.5 ECTS)
- PE&RC First years weekend (2019)
- PE&RC Last year retreat (2022)

Discussion groups/local seminars or scientific meetings (4.95 ECTS)
- Circulaire terrreinbeheer discussion groups from the Dutch biomassa alliantie (2019-2023)
- Re-design of agroecosystems symposium (2022)
- SBL Carbon symposium (2022)

International symposia, workshops and conferences (3.5 ECTS)

- Netherlands annual ecology meeting; Lunteren (2020)
- Wageningen soil conference; Wageningen (2023)

Societally relevant exposure (5 ECTS)

- Presenting my work yearly for several organizations such as the municipality of Sint Anthonis or the province of Brabant and Gelderland (2019-2023)
- Writing and publishing a Dutch report of my thesis work (2022)

Lecturing/supervision of practicals/tutorials (3.2 ECTS)

- Landscape geography (2019)
- Ecology (2019)
- Supervising an ACT group (2022)

BSc/MSc thesis supervision of students (7.5 ECTS)

- Application of fresh cuttings from road verges can increase fungal :bacterial ratio in Dutch arable soil
- Disease suppressiveness of treated and untreated roadside verge cuttings after long- and shortterm application to the soil
- The effects of amending agricultural soils with road side clippings on excess weed growth

Colophon

The research described in this thesis was carried out at the Plant Ecology and Nature Conservation Group of Wageningen University and was financially supported by the province of Noord-Brabant, the province of Gelderland, the water boards Brabantse Delta, De Dommel and Aa en Maas and the municipalities of Sint Anthonis and Gilze Rijen.

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