# Soil-borne disease suppressiveness after short and long term application of fermented, composted or fresh organic amendment treatments in arable soils

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## ARTICLE INFO

## ABSTRACT

Soil-borne diseases can cause significant crop losses and should be tackled sustainably in agroecosystems. Increasing the capacity of soils to suppress the effects of soil-borne diseases (soil suppressiveness) is an important tool in sustainable crop production. Soil suppressiveness can be improved by adding organic amendments to the soil for multiple years, but the effects can vary greatly depending on the processing method of the organic amendment (composted, fermented, or fresh material) and the time since application. To test these impacts we conducted two bioassays using the *Lepidium savitum* (cress) – *Pythium ultimum* model system. We tested the disease suppression capacity of sandy arable soil from a field experiment where fresh plant material, compost, or Bokashi (fermented amendment), all originating from the same plant material had been applied for two consecutive years across 10 field sites subject to conventional farming. In addition, the effect of short term application on soil suppressiveness was tested right after applying the same organic amendments to control arable sandy soil from 2 sites from the field experiment. Field sites strongly differed in cress growth independent of the organic amendment treatments. Absence of field effects in the sterilized soil and their soil chemical characteristics suggested differences in inherent soil pathogen load between the 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 microorganisms in response to Bokashi, which contains more easily decomposable compounds as compared to the other soil amendments, together with Bokashi microorganisms that survive the fermentation and are activated in the aerobic soil condition. 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.

## 1. 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) and might be prohibited in the near future. 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).

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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)" (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., 2006).

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 (>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. It is important to investigate which organic amendment is most effective in increasing in soil suppressiveness because of the urgent demand for developing sustainable soil suppressiveness and the current variability of results of differing 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 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 compared to the fresh material due to the fermentation process the organic material has been through which makes the plant cells weaker that in the fresh material (Luo et al., 2022; Shin et al., 2017). Additionally, Bokashi is a fairly new processing method for organic materials with a high potential in increasing soil suppressiveness due to the high decomposability. Comparing the different processing methods on their effect on soil suppressiveness to potentially unveil a new and sustainable way to improve soil suppressiveness is very valuable. These different organic amendment treatments (fresh, compost, or Bokashi) were not directly compared to each other in former studies or in a real-world field situation. It is therefore interesting to compare these different organic amendments made with the same starting 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 time dependent process (Bonanomi et al., 2010). Compost for example consists of more recalcitrant carbon and when applied to soil might decompose over a longer time frame and therefore have a better effect in the longer term

after multiple applications. Bokashi and fresh amendments might decompose faster when applied to soil and are therefore expected to have a more short term effect. Clocchiatti et al. (2021) also noted this time-scale effect showing that application of different organic materials to soil 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 through increasing the Soil Organic Matter (SOM) content, can improve the soil microbiome over a longer time frame (Bonanomi et al., 2020, 2018; Pérez-Piqueres et al., 2006) and therefore the general disease suppression capacity of the soil over several years (Bonanomi et al., 2018; Schlatter et al., 2017). The need for testing the effects of organic amendments on the soil microbiome and soil suppressiveness over a longer time frame is necessary since also these relationships are highly dependent on multiple factors (Bonanomi et al., 2020; Knapp et al., 2010) which asks for a fair comparison between processing methods. Nevertheless, very few studies investigated both the short and longer term effects of different organic amendments in a conventional farming situation using organic amendments produced from the same starting material but with different qualities due to diverging microbial activity during their preparation process. Therefore, it is relevant to investigate the disease suppression capacity in a conventional 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 expected Bokashi to increase soil suppressiveness in the short term since this method results in 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 multi-site field experiment that received the different organic amendments for multiple years. The soil was used in complementing bioassay experiments.

## 2. 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 applied knowledge and the different organic amendments were added shortly before the bioassay.

### 2.1. Multi-site field experiment

The multi-site field experiment entailed 10 conventional arable field locations with sandy anthrosols (Soil Survey Staff, 2010) across two

provinces (detailed information presented in the appendix Fig. A1). This experimental design allowed us to gain conclusions over a wider region of arable sandy fields instead of only being able to conclude on the effect in one specific field. Although the fields are from individual farms in The Netherlands, the management history was similar with the main crop being maize altering with wheat or potato and once a year tillage at differing depths. The detailed management history of the fields is presented in appendix Fig. A1, C. The experiment was set up in September 2019. At every site, ten-by-ten meter plots (100 m²) 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 each site. Randomization was done for each locations independently to create a randomized block design where the treatments were present in a different order in every site. 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 (low diversity verge), and iv) fresh cuttings from road verges with high plant diversity (high diversity verge).

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 low diversity 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.-a) (lactic-acid bacteria) and eMB starter (Multikraft, n.d.-b) (bacteria that break down cellulose) to the fresh material. Pulverized calcareous shells were added together with the bacteria, a standard procedure in the Bokashi processing protocol (Bij de Oorsprong, 2021) in order to prevent a too acidic end-product. After eight weeks the plastic sheet was opened to use the Bokashi underneath. 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. There were two separate fresh organic amendment treatments since the road verges in the municipality of Sint Anthonis are managed in two different ways. A large part is managed conventionally (low diversity verge cuttings) and a smaller part is managed sustainably to improve flower and insect biodiversity by sowing a diverse plant mixture in the road verge (high diversity verge cuttings). The high diversity verge cuttings are likely to have a higher recalcitrant C content due to the sowing of woody herbs with lignin like structures. It is therefore necessary to treat them as a different treatment since it can result in different outcomes in the soil suppressiveness. The chemical composition of these organic amendment treatments are presented in Table 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. All amendment treatments received the same amount of mineral fertilizer, irrespective of their own NPK concentrations. This approach allows for a practical interpretation of the results that is closest to conventional farming practice. 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).

### 2.2. Soil collection

After two consecutive years of amending the soil with different 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 three samples in the inner two-by-two meters of each plot, resulting in approximately 2 kg of fresh soil 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 h, and then weighed again.

Several soil chemical characteristics were determined per soil sample. 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 2 h 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

**Table 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 | g Lignin/kg dw | Moisture (%) | Organic matter (%) | pH – H₂O |
|---|---|---|---|---|---|---|---|
| Compost | 12.1 ± 0.25 | 177.6 ± 14.0 | 14.6 ± 1.0 | 0.41 ± 0.01 | 62.29 ± 0.70 | 37.71 ± 0.70 | 8.1 ± 0.04 |
| Bokashi | 32.1 ± 0.56 | 267.4 ± 55.2 | 8.4 ± 1.8 | 0.30 ± 0.04 | 68.70 ± 2.34 | 31.30 ± 2.34 | 6.8 ± 0.04 |
| High diversity verge | 29.5 ± 1.87 | 410.0 ± 7.0 | 14.1 ± 1.0 | 0.20 ± 0.01 | 68.47 ± 0.35 | 31.53 ± 0.35 | 7.0 ± 0.09 |
| Low diversity verge | 29.5 ± 1.73 | 436.4 ± 7.6 | 15.3 ± 0.9 | 0.19 ± 0.01 | 82.17 ± 5.76 | 17.83 ± 5.76 | 7.5 ± 0.02 |

sites, Phospholipid fatty acid (PLFA) extraction and analysis took place in samples of every control soil of the 10 field locations. To unravel general disease suppressiveness, data on the total community will be most indicative as opposed to specific microbes. Thus, PLFA giving the size of microbial groups should be a relevant indicator for this study. 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.

### 2.3. 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 10 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 (see also Section 2.1). Half of each soil sample was autoclaved at 121 °C for 20 min to kill soil microorganisms and this soil was then considered sterilized (Trevors, 1996). The advantage of our short bioassay approach is that the cress will mainly use nutrients from the seed and that nutrients released during the sterilization process (Razavi Darbar, 2007; Wolf et al., 1989) has limited influence. Autoclavation is therefore found to be effective for our study since the main expected change in the soil is the release of nutrients which will not affect our results. 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. 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 conventional arable field situation. This resulted in an average amount of soil of 283 g per pot. Due to limited space in the incubator and to prevent further risks in contaminations of different (sterilized) soils, the experiment was split into two batches based on site number and with that, ensuring that an equal amount of treatments was present in both batches. 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. Soils used in the second batch were stored in a fridge at 4 °C for two weeks before the second bioassay which is assumed not to influence the soil biota. Soils were taken out two days before usage in the bioassay to get to room temperature. 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 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. 1). Soil from control plots from 2 field sites from the field experiment was used, site nr 5 and 8. These 2 sites were selected based on low natural pathogen pressure, as shown in the first bioassay. This choice ensured enough cress growth to be able to detect treatment effects on soil suppressiveness since sites with high natural pathogen pressure will result in no cress growth which will not help us in understanding the soil suppressiveness effect of the amendments. To test the impact of the organic amendments on disease suppressiveness we used the same four amendments (compost, Bokashi, high diversity, and low diversity organic material) as were used in our field experiment. The chemical composition of these amendments are presented in Table 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
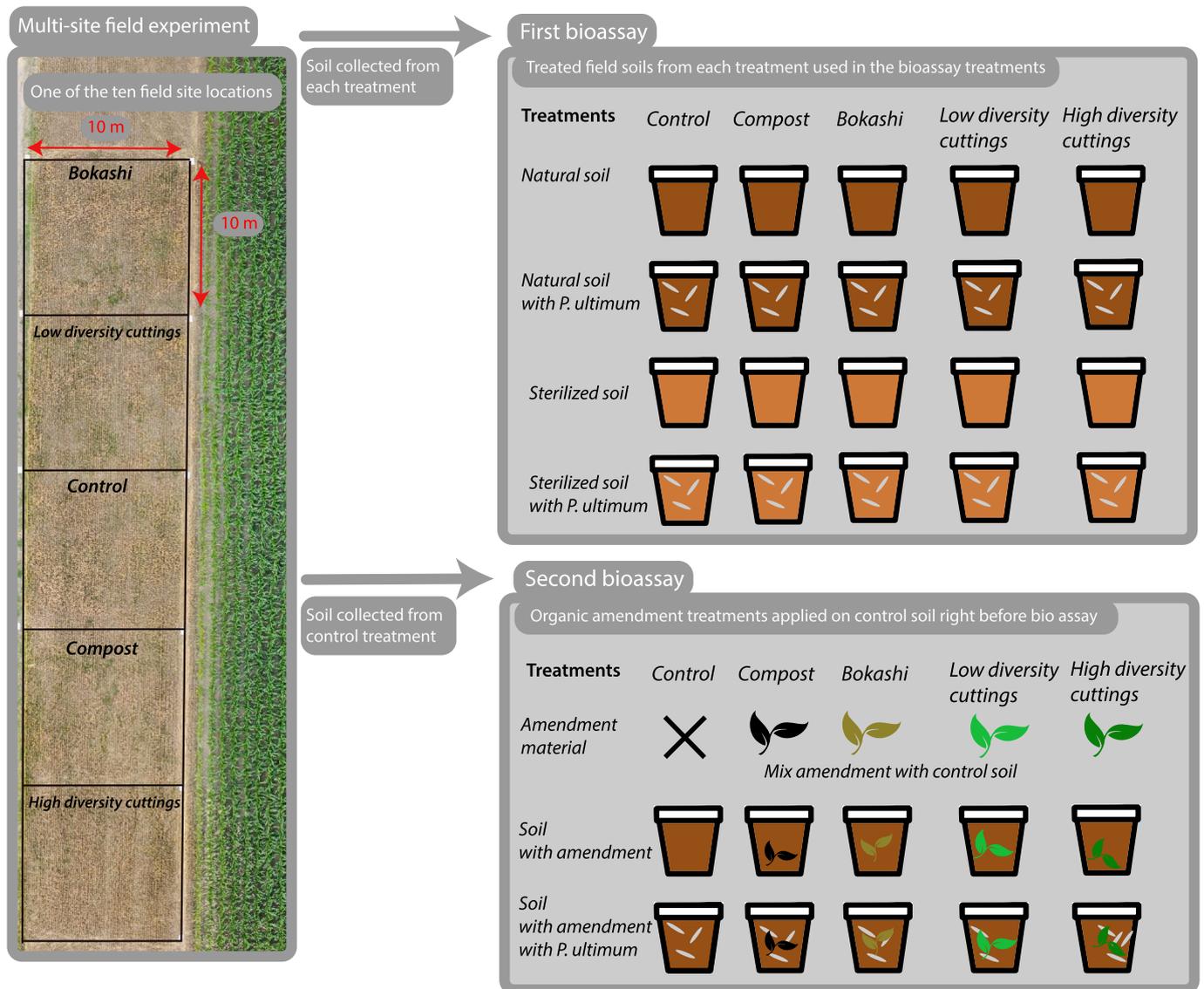
**Fig. 1.** Overview of the experimental design showing the treatments of the multi-site field experiment (left), the first bioassay (above) and second bioassay (below).

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.

### 2.4. 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.

## 3. Results

### 3.1. 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. 2A, Wald Chi$^2$ = 230.45, $p < 0.0001$), suggesting that the different field locations varied in their inherent soil-borne pathogen load. We assigned the sites a parameter of either a high perceived inherent pathogen load (site nr 1, 2, 3, 4, 6, and 10) or low perceived inherent pathogen load (site nr 5, 7, 8, and 9) based on the amount of cress weight grown in non-sterilized soil without *Pythium* addition (Fig. 2A). The effect of *Pythium* addition on cress weight significantly interacted with the perceived inherent pathogen load in not sterilized soil (Fig. 2B), but not in the sterilized soil (Fig. 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 1, 2, 3, 4, 6 and 10 was caused by a biotic rather than an abiotic factor. This was further

supported by the similar soil type (anthrosols: Hartemink and Sonne-veld, 2013; Rijkswaterstaat, 2014) and the absence of significant soil chemical differences (in SOM, pH, plant available N and total amount of N) or management history and crop rotation (Appendix, Fig. A1, C) between the locations with suspected high and low pathogen load (Table 2). Additional information on the general soil microbiome in the control plots of the 10 locations via PLFA analysis (Appendix, Fig. A2) showed that the amount of microbial biomass (both in fungi and bacteria) and the microbial composition in the locations was not clearly different between locations with suspected high (location nr. 1, 2, 3, 4, 6 and 10) versus low (location nr. 5, 7, 8 and 9) pathogen load. The results in sterilized vs unsterilized soil (Fig. 2) and the extra information on soil chemical (Table 2) and biological (Appendix, Fig. A2) data support our idea that the differences in cress growth between field sites are likely to be driven by variation in natural disease presence. Consequently, further analyses made 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 3). In the bioassay, *Pythium* suppressed cress weight only in the field sites with low inherent
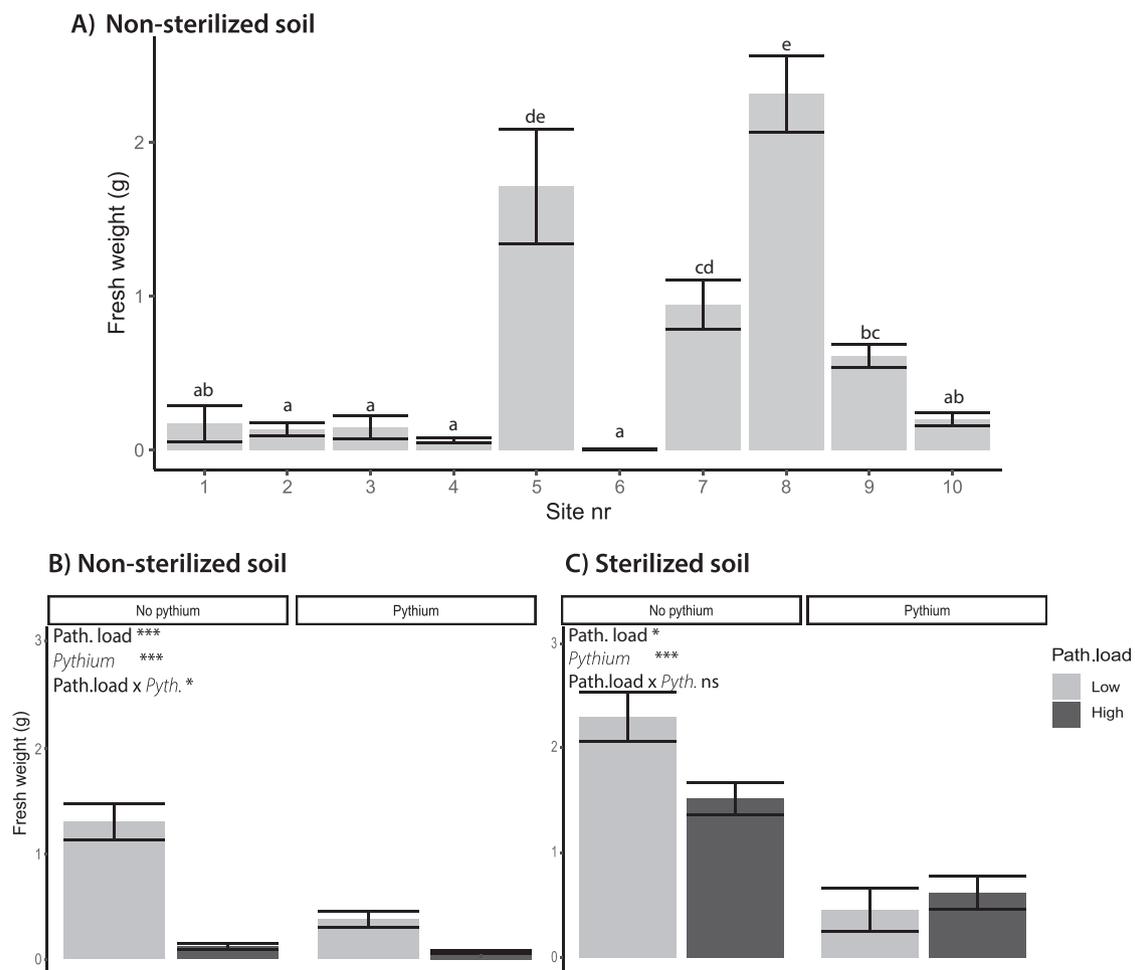


**Fig. 2.** A) Mean cress shoot fresh weight in grams across non-sterilized field soils and across all amendments from 10 experimental field sites without *Pythium* addition. Error bars represent the mean ± standard error (n = 5). Different letters indicate significant differences at p ≤ 0.05 with ANOVA 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. *p < 0.05, **p < 0.01, ***p < 0.001, (*) p > 0.05 & <0.1 ns = non-significant

**Table 2**
Soil 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. *$p < 0.05$, **$p < 0.01$, ***$p < 0.001$, ns = non-significant.

| Location | Organic matter (%) | Moisture percentage (%) | pH - $H_2O$ | 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 ± 0.01 | 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 |

**Table 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. *$p < 0.05$, **$p < 0.01$, ***$p < 0.001$, 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 × Pythium | 6.12 (4) | 0.19 | ns |
| Amendment × Path.load | 12.80 (4) | **0.01** | * |
| Pythium × Path.load | 8.43 (1) | **0.004** | ** |
| Amendment × Pythium × Path.load | 2.71 (4) | 0.61 | ns |

pathogen load. Also, the effect of amendment addition on cress weight depended significantly on the field soil's inherent pathogen load (Table 3).

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 Fig. 2B and C. The sites with a high inherent level of pathogen load showed overall low cress weight (Fig. 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. 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.

### 3.2. 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 5 and 8), 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 high diversity verge cuttings (Fig. 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 high diversity verge cuttings

(Fig. 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, high diversity verge, and low diversity verge but not in the Bokashi treatment, suggesting high disease suppression for Bokashi (Fig. 4A and B).

## 4. Discussion

To investigate whether different processing methods (compost, Bokashi, or fresh road verge cuttings) affected soil suppressiveness of organic amendments after short term or multi-year application we conducted two bioassays using the plant-pathogen *Lepidium sativum* (cress) – *Pythium ultimum* model system (Bongiorno et al., 2019; Mayerhofer et al., 2021; Tamm et al., 2010; Thuerig et al., 2009). We found a large difference in cress weight in the non-sterilized soil of the ten field sites that were used for the first bioassay (Fig. 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). However, we could not find justification for differences in disease suppressiveness due to differing management or crop rotation between the sites (Appendix, Fig. A1, C). We concluded that the difference between locations in how *Pythium* affected cress weight is likely not a chemical (Table 2) but a biological effect supported with evidence from the sterilized treatments (Fig. 2B and C). We also concluded that it is probably 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 deviation in fungal and microbial biomass and had a similar composition across microbial groups between suspected low and high inherent pathogen load (Appendix, Fig. A2), while they did differ in their disease suppressiveness, we assume that differences in inherent pathogen load between fields best explained our results. Therefore, we do have, albeit indirect, evidence that the differences between field sites were biological in origin and probably not related to soil chemical differences. The effect of the organic amendments on soil-borne disease suppression showed a trend ($p < 0.1$) towards significance in fields with a low inherent pathogen load (Table 3 and Fig. 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 but these studies did find similar results where the effect of an organic amendment did not "cure" soil from a plant pathogen (Blok et al., 2000). It would be interesting to investigate this further in future research using natural soils with known pathogen presence and concentrations.
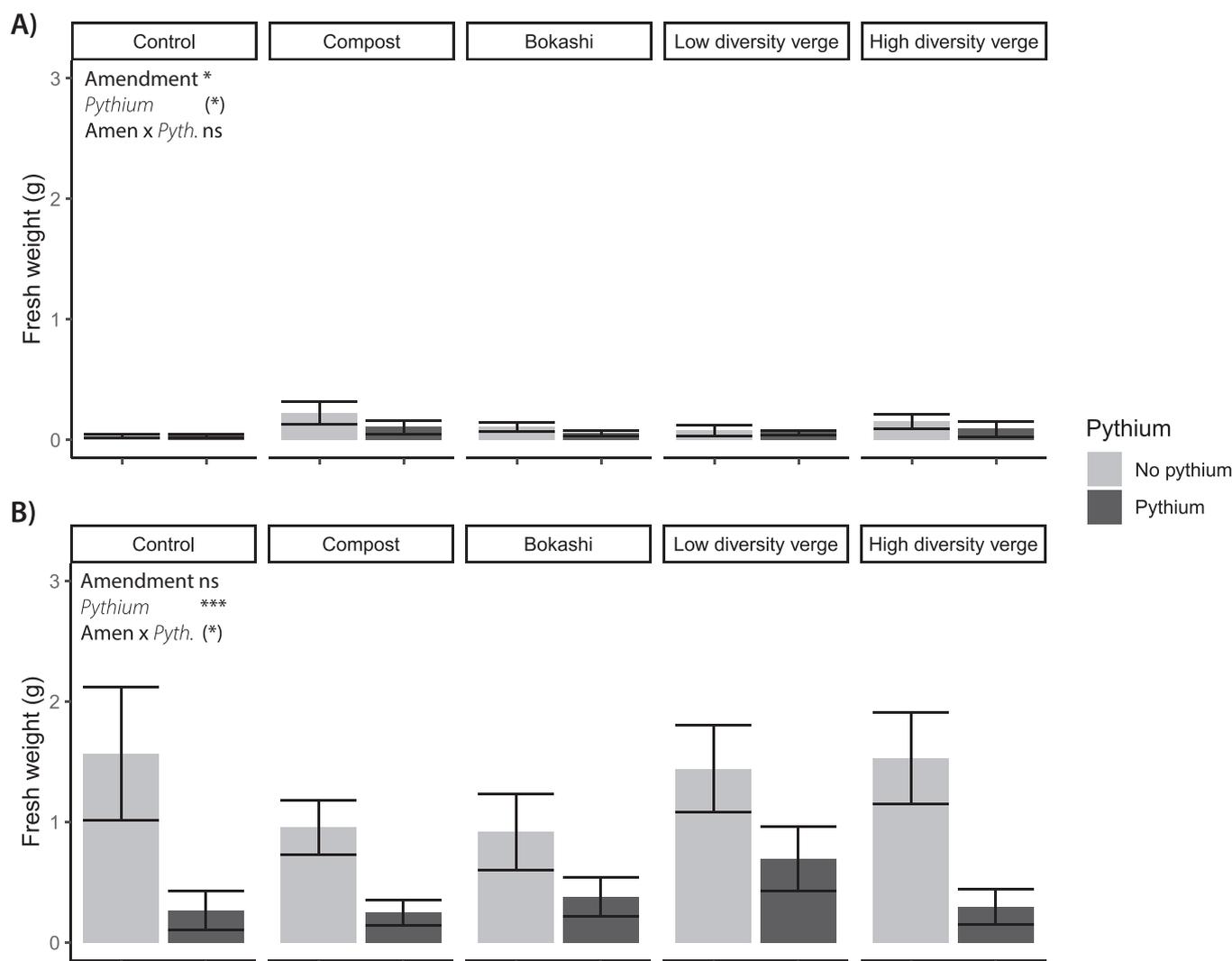
**Fig. 3.** A) Mean cress shoot fresh weight in grams of sites with high perceived pathogen load (nr 1, 2, 3, 4, 6 and 10) effected by the different amendment treatments and *Pythium* addition. The error bars represent the mean ± standard error (n = 6). The effect of the amendment, *Pythium* addition 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 5, 7, 8 and 9) 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. *p < 0.05, **p < 0.01, ***p < 0.001, (*) p > 0.05 & <0.1 ns = non-significant

In our study, 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 by increasing the activity 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 microbial activity and hence 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 (Figs. 3B and 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 starting 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 carbon sources at this stage of composting is low (Mondini et al., 2003) which does not activate or increase the soil microbiome in the short and long term as well as other amendments would (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., 2010, 2007). In our research, the fresh organic amendment treatments did not increase soil suppressiveness in the short or long term (Figs. 3B and 4), nor did they promote or suppress 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 starting 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

**A)**



**B)**
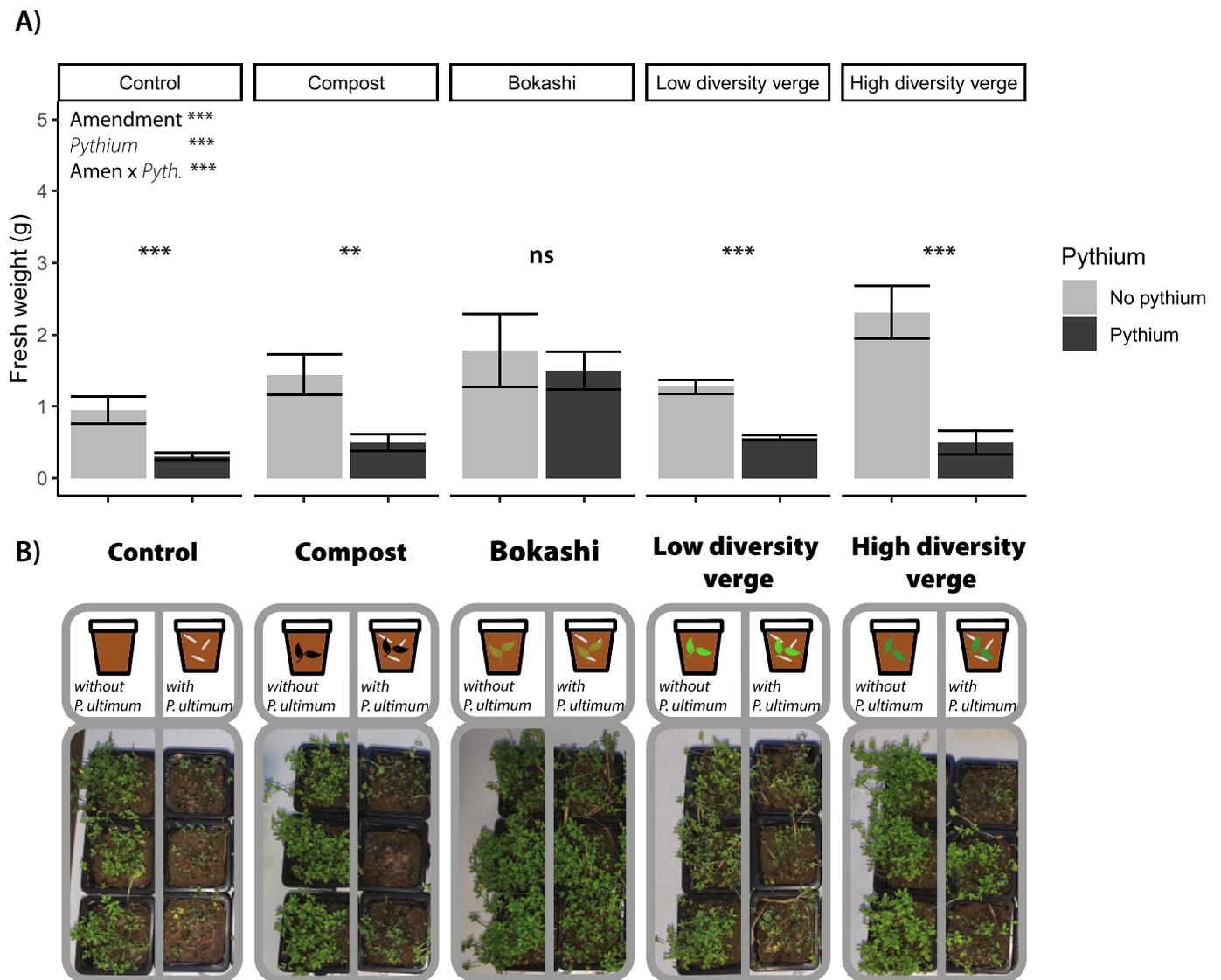


**Fig. 4.** A) Mean cress shoot fresh weight in grams effected by the different amendment treatments and *Pythium* addition on non-sterilised soil from 2 field locations with low pathogen load (site nr 5 and 8). The error bars represent the mean ± standard error (n = 6). The effect of the amendment, *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. B) Visualization of three random replicates are shown of every treatment both with and without inoculation of *Pythium*. *p < 0.05, **p < 0.01, ***p < 0.001, ns = non-significant.

(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). It is unlikely that this effect is the result of an increase in N availability in the soil after swift decomposition of the Bokashi material since during the short time period of cress growth in the bioassay (one week) the cress uses all the necessary nutrients from the seed rather than from the soil solution. 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 when comparing to sterilized Bokashi. Microbial activity was boosted for a week after adding Bokashi (either normal or sterilized) when compared to the control soil without organic amendment addition. This result is in line with our research suggesting that the soil suppressiveness effect of the Bokashi treatment is likely to be linked to a short term boost in microbial activity of the soil inhabiting microbes and which would be 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. 3B) echoing results by Shin et al. (2017) who reported that adding Bokashi enhanced microbial activity only in the first week(s) after addition. The temporal dynamics of the buffering

capacity of Bokashi as soil amendment to combat invasion of soil-borne pathogens and its underlying mechanisms warrants further study.

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. The complexity of the soil system interacting with multiple factors both biological, chemical and physical makes stating general rules regarding soil suppressiveness very difficult. Especially when combining this with realistic time and climate variations. 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 real-world situations but in depth sequencing of microbial communities both in the soil and amendment might increase the mechanistic knowledge surrounding this topic further. It would be interesting, for example, to sequence the microbial community of both compost, Bokashi (made from the same starting material) and fresh amendment and to assess the effect of these communities on the soil microbiome overt time since this would help the mechanistic understanding of these amendments on soil suppressiveness greatly since these complex relationships are considered a black box in this study. It is, however, important to establish a more standardized protocol for Bokashi since the procedures in practice deviate widely which makes future studies on this new organic amendment processing method difficult to compare. Next to that, we recommend that future studies use the same starting material when comparing compost and Bokashi to prevent confounding effects of different materials.

## CRediT authorship contribution statement

**Maartje van der Sloot:** Data curation, Formal analysis, Project administration, Resources, Software, Validation, Visualization, Writing – original draft, Writing – review & editing. **Solomon Maerowitz-Mcmahan:** Data curation, Formal analysis, Methodology, Writing – review & editing. **Joeke Postma:** Conceptualization, Formal analysis, Writing – review & editing, Methodology. **Juul Limpens:** Conceptualization, Formal analysis, Funding acquisition, Writing – review & editing, Supervision. **Gerlinde B. De Deyn:** Conceptualization, Formal analysis, Methodology, Supervision, Writing – review & editing.

## Declaration of competing interest

The authors declare the following financial interests/personal relationships which may be considered as potential competing interests: Maartje van der Sloot reports financial support was provided by Gelderland Province. Maartje van der Sloot reports financial support was provided by Province of North Brabant.

## Data availability

Data will be made available on request.

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## Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.apsoil.2023.105268.

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