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The hidden potential of saprotrophic fungi in arable soil: Patterns of short-term stimulation by organic amendments

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ABSTRACT

Saprotrophic fungi are abundant in soils of (semi-)natural ecosystems, where they play a major role in ecosystem functioning. On the contrary, saprotrophic fungal biomass is remarkably low in intensively managed soils and this can have a negative impact on soil functioning. Nevertheless, arable soils harbour a diverse pool of fungi, which can be stimulated by organic amendments. Management targeted towards increasing soil organic matter often coincides with an increase of fungal biomass, but it can take years before effects are seen. However, a rapid stimulation of fungal biomass at the start of the growing season could immediately benefit crop production, by improving nutrient availability, soil structure and suppression of soil-borne diseases. The objective of this study is to realize a rapid increase of saprotrophic fungal biomass with organic amendments. In controlled pot experiments, dried and milled organic materials of different quality were added to an arable sandy soil. Ergosterol-based fungal biomass and ITS2-based fungal community structure were measured over a period of two months. Wood sawdust of deciduous tree species and paper pulp resulted in a high and lasting increase of fungal biomass, as opposed to transient effects given by cover crops and other non-woody plant materials. Little or no stimulation of fungi was seen for coniferous wood sawdust and agro-industrial by-products. Nitrogen immobilization induced by sawdust and paper pulp was compensated by supplementing mineral nitrogen, which enhanced the stimulation of saprotrophic fungi. The composition of the stimulated fungi was influenced by the quality of organic amendments. In particular, deciduous wood sawdust and paper pulp favoured saprotrophic ascomycete fungi (mainly Sordariomycetes), with no increment in potential plant-pathogenic fungi. Overall, our results point at a good perspective to use woody materials as sustainable soil improver via stimulation of saprotrophic fungi.

1. Introduction

Saprotrophic fungi represent an important component of soil microbial life in many terrestrial ecosystems. In particular, in (semi-) natural ecosystems with high inputs of plant litter, abundance and activity of saprotrophic fungi is typically high (Fierer et al., 2009). The decomposing activity of saprotrophic fungi contributes to important ecosystem functions in soils, such as carbon and nitrogen cycling (Fontaine et al., 2011; van der Wal et al., 2013). In addition, the hyphal networks they form are involved in soil aggregate formation, which is important for water retention and resistance against soil erosion (Beare et al., 1997). Furthermore, saprotrophic fungi have a strong influence on other soil inhabitants, for instance via competitive or mutualistic interactions with bacteria and as food for fungivorous soil biota (Ballhausen and de Boer, 2016; Kramer et al., 2016; Morriën et al., 2017; Deveau et al., 2018).

In contrast to many soils in natural ecosystems, saprotrophic fungal biomass is remarkably low in arable soils, in particular in intensively managed arable land (Djajakirana et al., 1996; van der Wal et al., 2006a; de Vries and Bardgett, 2012). This is ascribed to the application of chemical pesticides, mineral fertilizers and intensive tillage (Duah-Yentumi and Johnson, 1986; Beare et al., 1997; Frey et al., 1999; Bittman et al., 2005; Scotti et al., 2015). When arable soils are taken out of production, fungal biomass and activity increase and are followed by

Abbreviations: ITS2, ribosomal internal transcribed spacer region 2; CRBD, completely randomized block design; ANOVA, analysis of variance; PERMANOVA, permutational analysis of variance; OM, organic matter

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shifts in the soil food web structure, connectedness and soil nutrient cycling (Morriën et al., 2017).

The small size of active biomass of saprotrophic fungi in arable soils implies that these soils are bacteria-dominated and this can have negative consequence for agricultural sustainability. Increase of saprotrophic fungal biomass in arable soil has been reported to coincide with lower losses of mineral nutrients (de Vries et al., 2011), increased carbon sequestration (Six et al., 2006) and better water retention (Beare et al., 1997; Helfrich et al., 2015; Liao et al., 2018). Moreover, saprotrophic fungi can contribute to the suppression of root-infecting fungal pathogens (van Beneden et al., 2010; Xiong et al., 2017; Siegel-Hertz et al., 2018). This is ascribed to competitive interactions between pathogenic and saprotrophic fungi (Fravel et al., 2003; Kepler et al., 2017) or to fungus-suppressing bacteria that are stimulated by the presence of saprotrophic fungi soils (Leeman et al., 1996; van Beneden et al., 2010; de Boer et al., 2015).

The benefits of having high activity and biomass of saprotrophic fungi in arable soils for enhancing sustainability in agriculture are increasingly recognized (de Vries et al., 2006; Six et al., 2006; van der Wal et al., 2013; Frąc et al., 2018). Since growth of soil microbes is mostly limited by biodegradable carbon supply, one of the options is to add materials that can be particularly well degraded by fungi (Lucas et al., 2014; Arcand et al., 2016). Saprotrophic fungi are known as efficient degraders of polymers like hemicellulose, cellulose and lignin that are present in recalcitrant fractions of plant residues. A combination of hyphal growth and production of polymer-attacking extracellular enzymes enables them to enter and decompose solid, lignocellulosic materials (van der Wal et al., 2013). Indeed, addition of lignocellulose-rich organic materials appears to be a key stimulant, as incorporation of straw and wood residues in arable or ex-arable soils were found to be associated with heightened saprotrophic fungal biomass (van der Wal et al., 2007; Moll et al., 2015; Reardon and Wuest, 2016).

Yet, a consistent increase of fungal biomass in arable soils can take several years, if not decades (van der Wal et al., 2006b; García-Orenes et al., 2013; Arcand et al., 2016; Chen et al., 2016). In addition, other management activities like tillage and use of fungicides can interfere with the stimulation of fungi by organic amendments (Williams and Hedlund, 2013; Willekens et al., 2014). Therefore, it is important to examine if there are possibilities to have a rapid increase of saprotrophic fungi at the start of the growing season. This is the period where soils are sensitive to leaching of nutrients, as more fertilizer is added than the amount taken up by seedlings. In addition, many crops suffer from pathogenic fungi at the seedling stage, such as pathogens causing damping-off diseases (Lamichhane et al., 2017). Despite the low fungal biomass, intensively managed arable soils harbour a diverse seed bank of fungal saprotrophs (de Graaff et al., 2019), which efficiently respond to new inputs of organic substrates (van der Wal et al., 2006b; Heijboer et al., 2016). In addition, in intensively managed arable soils saprotrophic fungi become active during the growth season in the root surroundings of mature crop plants (Hannula et al., 2010, 2012). However, there is little information on how a rapid and prolonged fungal biomass increase can be realized within the early growing season.

The objective of this study was to indicate organic amendments that may be used to realize a rapid and lasting stimulation of saprotrophic fungi in fungal-poor arable soils at the start of the growing season of cash crops. To this end, organic materials were selected that are commonly used in both conventional and organic farming, such as composts, agro-industrial residues, cover crop residues (Goss et al., 2013; Scotti et al., 2015) and materials rich in (ligno)cellulose, namely paper pulp and beech wood sawdust. As the latter materials resulted in prolonged fungal biomass stimulation, the study was extended with a comparison of the fungus-stimulating effect of wood sawdust obtained from other tree species. The amendment of high C:N ratio materials can have negative consequences on crop yield, as it causes immobilization of mineral nitrogen by saprotrophic microbes (Gad et al., 2015). Yield depression following the addition of (ligno)cellulose-rich materials needs to be mitigated by a supplement of mineral nitrogen (Mohanty et al., 2013; Gad et al., 2015). At the same time, nitrogen fertilization can have negative impact on fungal development (Treseder, 2008; Zhang et al., 2018). In order to test whether elevated nitrogen could interfere with fungal biomass stimulation by high-C organic amendments, paper pulp and wood sawdust were added to the soil both alone and in combination with supplemental mineral nitrogen. This study was carried out in a series of controlled pot experiments, over a period of two months, during which the response of fungal biomass and community structure were examined.

2. Materials and methods

The fungus-stimulating effect of incorporating organic materials in arable soils was examined in three controlled pot experiments. In the first experiment (Organic Amendments, OA), fourteen organic materials were mixed with an arable sandy soil. Materials included woody and non-woody plant material, paper pulp and other organic by-products. Based on the results of the OA experiment, soil amendment with sawdust was further studied in a second experiment (Wood Amendments, WA). Here wood sawdusts of five tree species were added to the same sandy soil. Finally, the effect of wood sawdust incorporation was studied in four arable soil types (Soil Types, ST).

2.1. Soil collection and soil characteristics

Arable soil samples were collected from four sites and used for the pot experiments. The main site for this study was the experimental farm PPO-Vredepeel of Wageningen University & Research (N 51.32.19, E 5.51.05, Vredepeel, the Netherlands). Vredepeel soil has a sandy texture and has been classified as Hortic Podzol (Table 1; FAO, 2014). Soil was collected from a plot to which conventional management is applied (Quist et al., 2016). Three soil samples were collected from this location, namely on 1st August 2016 (Batch 1), 17th October 2016 (Batch 2) and 5th June 2017 (Batch 3) for use in experiment OA, WA and ST, respectively (Table 1). Batch 1 was collected in between rows of triticale, Batch 2 in between rows of the cover crop oilseed radish and Batch 3 from fallow soil on which maize had been grown during the previous summer. In June 2017 soil was collected from three additional locations, for use in the ST experiment (Table 2). The selected sites were arable fields nearby Panningen (N 51.32.89, E 5.97.94), Lisse (N 52.25.52, E 4.54.77) and Nagele (N 52.38.42, E 5, 43, 29). Panningen soil is a Plaggic Anthrosol with a sandy loam texture, Nagele soil is a

Table 1
Overview of the arable soils used in this study, including soil proprieties and field management.

Sampling site and batch	Experiment	Texture	Sand-silt-clay (%)	pH	OM (%)	Management	Crop
Vredepeel, batch 1	OA	Sand	–	6.1	–	Conventional	Triticale
Vredepeel, batch 2	WA	Sand	–	6.2	–	Conventional	Oilseed radish
Vredepeel, batch 3	ST	Sand	92-6-2	5.7	6.3	Conventional	Fallow
Panningen	ST	Sandy loam	74-25-1	5.3	7.7	Conventional	Sugarbeet
Lisse	ST	Sand	95-4-1	7.5	2.3	Conventional	Hyacinth
Nagele	ST	Silty loam	36-57-1	6.7	5.6	Organic	Wheat

Table 2
Organic materials used for experiment OA, WA and ST, with the quantification of carbon and nitrogen content (%, mean (SD), $n = 3$) C:N ratio and a brief description of the source of each material are indicated.

Material	Experiment	C %	N %	C:N	Description
Paper pulp	OA, WA	42,3 ± 0,3	0,15 ± 0,01	282	Acid-washed cellulose, (SCA Hygiene Products Suameer BV, NL)
Beech sawdust	OA, WA, ST	45,4 ± 0,9	0,14 ± 0,02	331	*Fagus sylvatica*, commercial sawdust for smoke ovens (Sänger Rollenlager GmbH & Co, Waldsolsms, D)
Vetch	OA	20,9 ± 2,8	2,44 ± 0,21	9	Cover crop *Vicia sativa*, collected in March 2016
Radish	OA	32,6 ± 1,2	2,95 ± 0,06	11	Cover crop *Raphanus sativus*, collected in March 2016
Canadian pondweed	OA	34,4 ± 1,2	3,14 ± 0,81	11	Acquatic plant, *Elodea* spp.
Black oat	OA	6,4 ± 1,9	0,28 ± 0,08	23	Cover crop *Avena strigosa*, collected in March 2016
Hay	OA	41,0 ± 0,2	1,28 ± 0,08	32	Collected from a local meadow
Cocoashells	OA	39,9 ± 1,1	2,45 ± 0,03	16	Husks of cacao beans: gardening mulch (Pokon Naturado, Venendaal, NL)
Soy seed meal	OA	41,6 ± 0,6	7,70 ± 0,45	5	Residue from protein extraction and fermentation of soy beans (Ecostyle, Oostervolde, NL)
Brassica seed meal	OA	47,2 ± 0,1	5,57 ± 0,14	8	*Brassica* spp. seed meal (P.H. Petersen, Grundhof, D)
Manure/wood composted mix	OA	33,7 ± 1,9	2,24 ± 0,17	15	Obtained from an organic farmer
Bone meal	OA	21,1 ± 0,3	5,30 ± 0,07	4	Milled residue of pig bones (Ecostyle, NL)
Biophosphate	OA	32,3 ± 9,2	2,05 ± 0,54	16	Fermented pig manure (Eurofins Agro, Wageningen, NL)
Beer waste	OA	40,7 ± 0,9	3,55 ± 0,56	11	Residue from hop fermentation (Ecostyle, NL)
Willow	WA	47,5 ± 1,0	0,33 ± 0,19	144	*Salix alba,* collected in December 2017
Hazel	WA	46,5 ± 0,4	0,30 ± 0,03	157	*Corylus avellana,* collected in December 2017
Poplar	WA	47,3 ± 0,6	0,67 ± 0,04	71	*Populus alba,* collected in December 2017
Douglas fir	WA	49,0 ± 0,2	0,18 ± 0,02	277	*Pseudotsuga menziesii,* collected in December 2017
Green compost	WA	19,4 ± 2,3	0,97 ± 0,10	20	Composted plant prunings (van Iersel Compost, Biezenmortel, NL)
Organic compost	WA	14,0 ± 2,0	1,28 ± 0,16	11	Composted organic municipal waste (Vereniging Afvalbedrijven, 's-Hertogenbosch, NL)

Calcaric Fluvisol with as silty loam texture and Lisse soil is a Calcaric Arenosol with a sandy texture (Table 1; FAO, 2014). All sites are located in the Netherlands, which have a temperate maritime climate. In each site soil was obtained from 0 to 10 cm depth at ca. 15 random spots within a plot of at least 20 m × 20 m. Soil collected from a site was pooled into a composite sample. This was 4-mm sieved, homogenised and stored at 4 °C until use, for a maximum of two months. Soil pH, organic matter content, texture and taxonomy were determined according to standard procedures and are summarized in Table 1. The four soils, used in ST experiment, differed in texture, organic matter content and/or pH. These provide a small, yet representative, selection of arable soils of the Netherlands.

2.2. Experimental design and organic materials

The OA experiment was carried out as a completely randomized block design (CRBD) with three replicates per treatment. Soil amendments tested were paper pulp + N, beech wood sawdust + N, vetch, radish, Canadian pondweed, black oat, cocoa shells, soy seed meal, *Brassica* seed meal, manure/wood compost, bone meal, biophosphate and beer waste (Table 2). Soil without amendments was considered as control treatment. The experiment was sampled two and eight weeks after adding the materials. The selected materials comprise of by-products of farming, industry or forestry and that could gain value as soil improvers. Moreover, these materials cover a broad range in terms of biochemical quality, as shown by the variation in C:N ratios (Table 2). Lignocellulose-rich materials are represented by fresh sawdust of beech, whereas paper pulp consists mostly of cellulose. Non-woody plants are included as well, comprising three common winter cover crops (vetch, radish and black oat), hay and an invasive waterweed. Besides the above mentioned undecomposed materials, several residues were included, such as a composted manure/wood mixture, remainders of manure fermentation, by-products of seed and plant processing, as well as milled animal bones (Table 2).

The WA experiment was arranged in a CRBD with four replications per treatment. The experiment included the following materials: sawdust of different tree species (beech, willow, hazel, poplar and Douglas fir), paper pulp, green compost and organic compost (Table 2). The experiment was sampled two and eight weeks after amendment. Each type of sawdust and paper pulp was added to the soil alone and in combination with mineral N. Both bare soil and soil amended with N only were used as controls. Beech sawdust and paper pulp were included as a repetition of the main result of the OA experiment. Sawdust of four other tree species was obtained from branches of ca. 6 cm diameter. Three of them were fast-growing, deciduous tree species (willow, hazel and poplar), whereas Douglas fir was included as a representative of conifers. In addition to these recalcitrant, fresh materials, two decomposed, recalcitrant materials were tested: a commercial compost derived from plant pruning (green compost) and composted organic municipal waste (organic compost).

In the ST experiment beech sawdust, together with ammonium nitrate, was amended in four contrasting soils, as introduced in Section 2.1. Soils with only added N acted as controls for each soil. In the ST experiment four replicates were used and were organized in a CRBD. Beech sawdust from the same batch was used in all experiments (Table 2). Unlike experiments OA and WA, the ST experiment was sampled at three time points, namely two, four and eight weeks after amendment.

Before addition to the soil, all materials were air-dried at 40 °C for 2 to 14 days, crushed and milled (cutting mill SM 100, Retsch B.V., Haan, Germany) into a fine powder, 2-mm sieved and stored at room temperature until use. Carbon and nitrogen content of each material were determined in triplicate with Thermo flash EA 1112 (Thermo Fisher Scientific, Waltham, Massachusetts, United States).

2.3. Soil preparation, incubation and harvesting

The three experiments were carried out in the same way. Each organic material was added in the form of fine (< 2 mm), dry powder to the soils, at the concentration of 5 g kg^{-1} dry weight soil. Supplemental N was added as ammonium nitrate (170 mg N kg^{-1} soil dry weight) to a part of the treatments,as described in Section 2.2. In this way, the C:N ratio of paper pulp and woody materials was adjusted to < 15:0, which is suitable for preventing plant yield depression (Mohanty et al., 2013). Soil moisture was adjusted to 60% water holding capacity by adding and mixing the amended soils with sterile water. Pots were filled with 230 g moist soil and incubated in a dark climate chamber at 20 °C. Soil moisture was kept constant by adding sterile water twice a week on basis of weight loss. Distinct replicate pots of every treatment were harvested at each time point. The upper 3 cm of soil, which experienced the largest fluctuation in moisture, was discarded from each pot. After that, the remaining soil in a pot was homogenised. For every measurement, one sub-sample per pot was taken, namely for the determination of fungal biomass (ergosterol), fungal community composition

(DNA) and mineral N content. For ergosterol extraction, 1 g of fresh soil was mixed with 4 ml of 10% KOH in methanol and was stored at −20 °C for up to two months before extraction. For DNA extraction, ca. 1,5 g of fresh soil were stored at −20 °C for ca. four months before analysis. About 50 g of the remaining soil was collected in a paper bag and air-dried at 40 °C for 7 days and stored at room temperature for ca. two months before determination of mineral nitrogen and pH.

2.4. Fungal biomass

Alkaline extraction of ergosterol was performed starting from 1 g soil samples, as described by de Ridder-Duine et al. (2006). Briefly, samples were stored in 4 ml methanol 10% KOH, processed by sonication (47 kHz, 15 min), followed by a heat treatment (70 °C for 90 min). Alkaline hydrolysis of esterified ergosterol carried out by the addition of 1 ml water and 2 ml n-hexane, combined with mechanical shaking. The hexane fraction was collected and the solvent was evaporated overnight. The pellet, containing ergosterol, was dissolved in methanol. Finally, ergosterol concentrations were quantified by LC-MSMS (UHPLC 1290 Infinity II, Agilent Technologies and 6460 Triple Quad LC-MS, Santa Clara, California, United States).

2.5. Mineral N and pH

Soil mineral nitrogen (NO_3^-, and exchangeable NH_4^+) and pH were determined using 10 g air-dried soil samples. Soil suspensions were obtained by mixing soil and 25 ml of water and shaking for 2 h at 250 rpm with linear movements. After measuring the pH, 25 ml 2 M KCl was added and the suspensions were shaken for 2 h. The aqueous phase was collected and centrifuged at 10.000 × *g*. NH_4^+ and NO_3^- concentrations in the supernatant were determined with EAL QuAAtro SFA system (Beun-de Ronde B.V., Abcoude, the Netherlands).

2.6. Fungal community structure

For the OA experiment, the fungal community structure was analysed in soils treated with beech sawdust + N, paper pulp + N, vetch, radish, black oat and hay and the un-amended control (OA experiment). For the WA experiment, soils amended with all sawdust types and paper pulp with ammonium nitrate were analysed for fungal community structure, as well as soil amended with beech sawdust and paper pulp without supplemental N.

DNA was extracted from 0.35 g of soil using MoBio Power Soil Kit (MO BIO Laboratories, Carlsbad, California, USA) according to the indications of the manufacturer and eluted in 50 µl PCR-grade water after 15 min incubation at room temperature. The nuclear rDNA internal transcribed spacer 2 (ITS2) region was amplified with fITS9 and ITS4 primer pair (Ihrmark et al., 2012). Barcoded ITS4 primers were used for discriminating each sample. Polymerase chain reactions (PCR) were performed in 25 µl mixtures containing 200 µM each dNTP, 2,5 µl 10 × PCR Buffer with $MgCl_2$, 1 µl $MgCl_2$ 25 mM, 1,25 µl BSA 4 mg ml^{-1}, 0,4 µl of each primer, 0,15 µl FastStart Expand High Fidelity polymerase (Roche Applied Sciences, Indianapolis, Indiana, USA) and 1 µl template DNA (10 ng). The PCR cycling conditions were: denaturation at 95 °C for 5 min, 35 cycles of 95 °C for 45 s, 54 °C for 60 s, and 72 °C for 90 s, followed by a final extension step at 72 °C for 10 min. For each sample, two PCR reactions were performed and the products were pooled before being purified with QIAquick PCR Purification Kit (Qiagen, Hilden, Germany). Quality of PCR-products was checked with Fragment Analyzer (Agilent Technologies, Santa Clara, California, United States). Samples were mixed in equimolar concentrations and sequenced (BGI, Shenzhen, Guangdong, China) on a MiSeq Sequencing System (Illumina, San Diego, California, USA). The resulting sequences can be accessed at the European Nucleotide Archive (accession number PRJEB33534).

2.7. Statistical and bioinformatic analysis

The statistical analysis was carried out in R (version 3.5.1). Two-way ANOVA and a three-way ANOVA were used to compare fungal biomass and soil mineral N across organic amendments, time points (OA, WA and ST), extra N application (WA) and soil type (ST). The assumptions of normality and equality of variances were checked for each ANOVA model. Multiple comparisons were obtained by Tukey's post-hoc test (family-wise error rate 1%). Correlation between NO_3^- and pH was tested for WA and OA experiment (Pearson).

Sequencing data were processed with the automated pipeline PIPITS (Gweon et al., 2015). Briefly, fungal sequences were prepared by joining read pairs and by quality filtering according to standard parameters. The ITS2 subregion was extracted using ITSx (Bengtsson-Palme et al., 2013). Short reads were removed (< 100 bp) and sequences were clustered based on a 97% similarity threshold using VSEARCH. Chimeric sequences were removed by comparing with UNITE uchime database. Taxonomic assignment was performed using the RDP classifier based on the UNITE fungal ITS database (Abarenkov et al., 2010; Kõljalg et al., 2013). Overall, the dataset counted 2.125.972 sequences. OTU abundances were normalized across samples by total sum scaling (TSS). All OTUs other than fungi were filtered out, as well as singletons, resulting in a dataset of 2116 fungal OTUs. Starting from the taxonomy table, fungi were classified into functional groups when possible, based on FUNGuild database v1.0 (Nguyen et al., 2016) and further manually revised based on literature. Functional groups of interest in this study were: plant pathogens, saprotrophs and fungal parasites (Tables S7 and S8). Symbiotrophs and animal pathogens and other groups were classified as “Other”. When needed, fungi were assigned to multiple guilds, in order to account for functional diversity within a taxon (e.g. *Fusarium*: plant pathogen – saprotroph).

Sequencing data were further analysed in R 3.5.1 (phyloseq and vegan). The total fungal community was analysed independently for OA and WA experiment. Permutational multivariate analysis of variance (PERMANOVA) was used to evaluate the effects of type of organic material, time point (OA and WA) and additional mineral N (WA) on the fungal community composition. Bray-Curtis dissimilarities between fungal communities were visualized as a result of principal coordinate analysis (PCoA). Differences in relative abundance across soil treatments and time points were also analysed at phylum, class and family level by generalized linear model (GLM, glm2 and multcomp packages) based on Poisson distribution, followed by post-hoc multiple comparisons (Tukey, family wise error rate 5%). Potential plant pathogenic fungi were recorded in a conservative way, namely including all taxa classified either as “plant pathogen” or as “plant pathogen-saptrotroph” (e.g. *Fusarium*). The relative abundance of each potential plant pathogen in this dataset was summed up in a cumulative relative abundance, which was compared across treatments and time points by two-way ANOVA ($p < 0.05$).

3. Results

3.1. Experiment OA: fungal biomass, mineral N and fungal community structure

Ergosterol concentration in Vredepeel soil changed during the two-months incubation depending on the type of added material (Tab. S1A). Addition of paper pulp and beech sawdust, in combination with ammonium nitrate, resulted in increased ergosterol concentrations as compared to the control (Fig. 1A). High ergosterol was sustained for the whole duration of the experiment and peaked at week 2 and week 8, for paper pulp + N and beech sawdust + N respectively (Fig. 1A, Table S2). Conversely, amendment with non-woody plant materials (vetch, radish, Canadian pondweed, black oat and hay) resulted in a transient stimulation of ergosterol. At week 2, ergosterol had increased to levels comparable to beech sawdust + N, but at week 8 it declined to levels

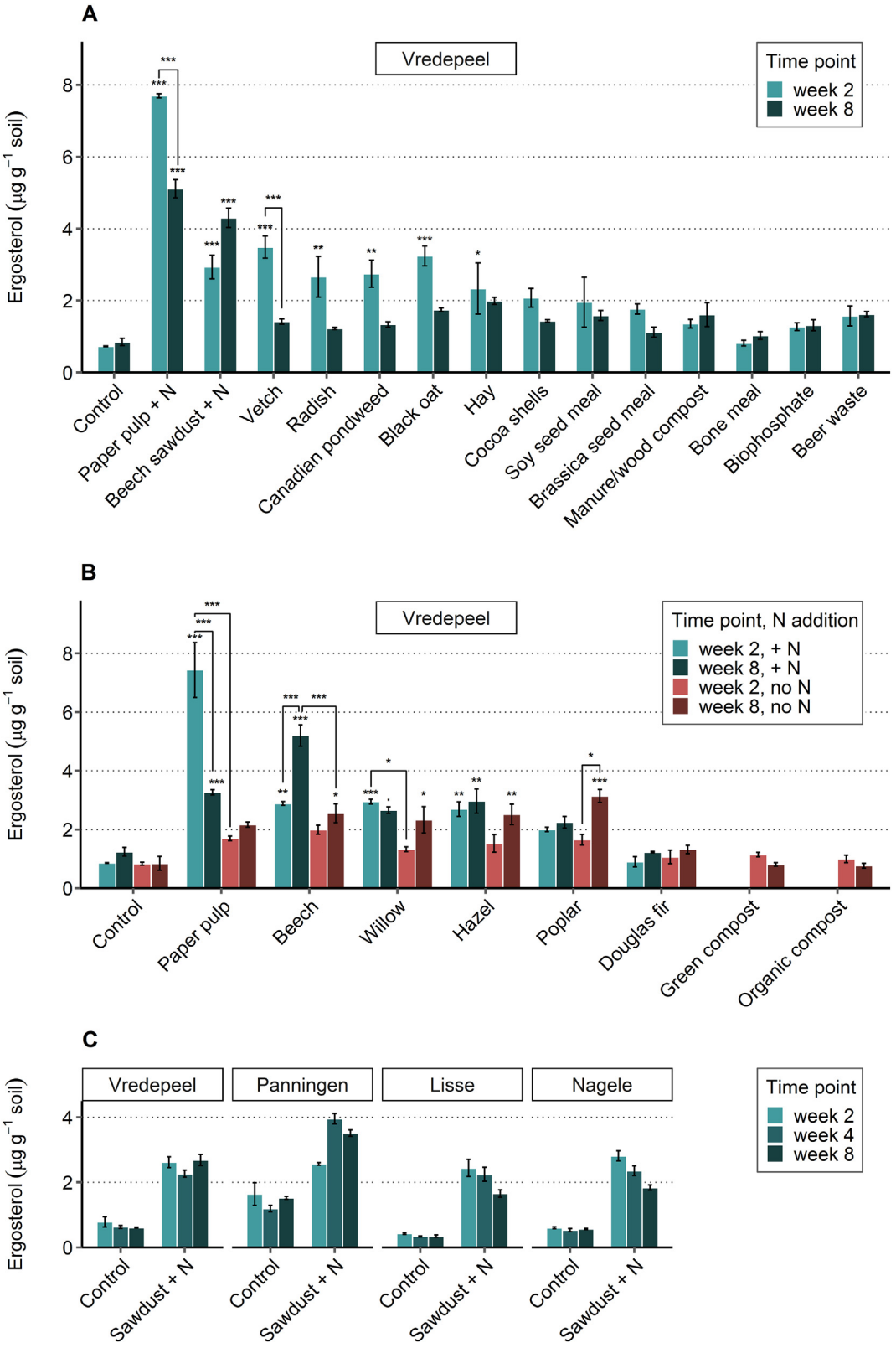

Fig. 1. Effect of organic amendments on fungal biomass (ergosterol) development in arable soil. Ergosterol concentration (mean ± SE, $n = 3$) in Vredepeel agricultural soil amended with fourteen organic materials, sampled after two and eight weeks; Experiment OA (A). Ergosterol concentration (mean ± SE, $n = 4$) in Vredepeel soil amended with nine organic materials, in combination with mineral N and alone, sampled after two and eight weeks; Experiment WA (B). Significant differences between treatments and controls are indicated in A and B for both sampling time points (* on top of error bar). In addition, significant differences within organic amendments between sampling times (A and B) and between with/without nitrogen additions (B) are shown (* on top of connecting line). Experiment ST (C), ergosterol concentration (mean ± SE, $n = 4$) in four soils amended with beech sawdust + N, as compared to N-amended control soils. Ergosterol was measured at three time points for each soil type. The description of each soil characteristics is found in Table 1.

not dissimilar to the control. These dynamics were more pronounced for N-rich plant residues (vetch, radish and Canadian pondweed) than for N-poor plant materials (black oat and hay) (Fig. 1A, Table S2). Finally, cocoa shells, soy seed meal, *Brassica* seed meal, manure/wood compost, bone meal, biophosphate and beer waste had little or no effect on ergosterol concentrations (Fig. 1A).

Out of the fourteen materials, seven increased mineral N concentration in the soil (Fig S1A). Of these, vetch, soy seed meal, *Brassica* seed meal, bone meal and beer waste incremented N at both time points, whereas radish and Canadian pondweed raised soil N after eight weeks. Soil N immobilization occurred after two weeks in soil amended with black oat, hay and cocoa shells, however after eight weeks N levels were again comparable to the control. At the end of the experiment, all mineral N was present as nitrate. A negative correlation was seen between the final soil pH and mineral N concentration in soil, with $R = -0{,}91$ (Fig. S2A).

The native fungal community of Vredepeel soil was dominated by Ascomycota, followed by Basidiomycota and Mortierellomycota. Low-abundant phyla (< 2%) were categorized as "Other" (Fig. 3). Changes in fungal community composition after soil amendment were significantly explained by both the type of added material and the time of incubation, yet most of the variation was ascribed to the type of material (PERMANOVA, $R^2 = 0{,}77$, $p < 0.001$, Table S6A). In particular, pronounced effects on fungal community composition were seen for Paper pulp + N, C-rich plant materials (beech sawdust + N and black oat) and N-rich cover crops (vetch and radish) (Fig. 2A–B). Soil amended with paper pulp + N was dominated by *Chaetomiaceae*, wheareas beech sawdust + N favoured *Chaetomiaceae* as well as other Ascomycota (Sordariomycetes, *Lasiosphaeriaceae* and *Ramophialophora*spp.). N-rich cover crops stimulated Mortierellomycota (Fig. 3C and Table S5A).

The cumulative relative abundance of potential plant pathogens did not increase when organic materials were added to the soil. On the contrary, the relative abundance of the sub-community of pathogens was lower, as compared to the control, in soil amended with paper pulp + N, beech sawdust + N, vetch, black oat and hay (Fig. 3A, ANOVA, $p < 0.05$). Thus, the functional analysis of the fungal community showed that organic materials shifted the indigenous soil fungal community in favour of non-pathogenic, saprotrophic groups, at the expense of potential pathogens. The only exception was radish, where the relative abundance of *Plectosphaerella* spp. was larger than in the control soil and the cumulative relative abundance of potential pathogens was comparable to the control (Fig. 3A).

3.2. Experiment WA: fungal biomass, mineral N and fungal community structure

In experiment WA the development of ergosterol concentration in response to paper pulp + N and beech sawdust + N was similar to that observed in experiment OA (Fig. 1A–B). Moreover, willow and hazel sawdust + N stimulated soil ergosterol concentrations. However, unlike beech sawdust + N, the increase in ergosterol was similar at both time points (Fig. 1B, Table S3). Among deciduous trees, poplar sawdust + N was the only amendment that did not significantly increase ergosterol. For sawdust of all deciduous trees, added without N supplement, ergosterol increase was seen after eight weeks. Conversely, ergosterol concentration was comparable to the control with the addition of paper pulp without N. Sawdust from Douglas fir, the only conifer species examined, had no effect on ergosterol concentrations neither with or without mineral N. Lack of stimulation of fungal biomass was also seen for green compost and organic compost addition (Fig. 1B). Addition of mineral N alone also had no effect on ergosterol.

Addition of solely paper pulp and woody materials caused a depletion of soil mineral N (Fig. S1B). When these materials were added with a dose of 170 mg N kg^{-1}, this was sufficient to maintain high N levels in the soil throughout the eight-week incubation. Only a partial N depletion was observed, which coincided with elevated ergosterol. Douglas fir was an exception to this, since it caused N immobilization without ergosterol increase. Composts had no effect on soil mineral N (Fig. S1B). Overall, soil pH had a negative correlation with mineral N concentration, with $R = -0{,}90$ (Fig. S2B).

In experiment WA, changes in the fungal community composition were mostly explained by the type of added material (PERMANOVA, $R^2 = 0{,}57$, $p < 0.001$, Table S6B). The fungal communities found in control soil, soil amended with paper pulp + N and beech sawdust + N were consistent with those observed in experiment OA (Fig. 3C–D). Similar fungal communities were found in soil treated with sawdust of deciduous tree species + N (Fig. 2C–D), which was especially clear at week 8. Namely, beech, willow and hazel sawdust + N enlarged the relative abundance of Ascomycota (*Chaetomiaceae*, *Lasiosphaeriaceae* and *Ramophialophora*spp.). Relative abundance of Mortierellomycota (*Mortierellaceae*) increased, albeit to a small extent, only with poplar sawdust (Table S5B). Douglas fir + N caused a small, yet significant increase in the relative abundance of Basidiomycota (Tremellomycetes, *Solicoccozyma* spp.). Supplemental mineral N did also affect the fungal community structure (PERMANOVA, $R^2 = 0{,}09$, $p < 0.001$, Table S6B). This was seen from the comparison of paper pulp and beech wood added to the soils with and without N (Fig. 2C–D). Paper pulp alone stimulated not only *Chaetomiaceae*, but also two additional groups, Orbiliomycetes (Orbiliales) and Agaricomycetes (Cantharellales) (Fig. 3D and Table S5B). On the contrary, the addition of beech sawdust alone led to a *Ramophialophora* spp.-dominated fungal community at week 8 (Table S5B).

Similarly to experiment OA, the cumulative relative abundance of the detected potential plant pathogens did not increase, rather was lower than in the control, in soils amended with paper pulp and all sawdust types here examined (Fig. 3B, ANOVA, $p < 0.05$).

3.3. Experiment ST: fungal biomass

When added to four arable soils, beech sawdust + N significantly increased the concentrations of ergosterol, as compared to the same soils amended with ammonium nitrate only ($p < 0.01$, Fig. 1C, Table S4). After two weeks of incubation, ergosterol concentration was similar in all sawdust-amended soils. At later time points, ergosterol concentration further increased (week 4) in sawdust-amended Panningen soil (sandy loam, low pH, high OM), whereas it remained stable in sawdust-amended Vredepeel soil and slightly decreased in Lisse soil (sand, high pH, low OM) and Nagele soil (silty loam, high pH).

4. Discussion

4.1. Effect of organic amendments on saprotrophic fungal biomass

Ergosterol measurements indicated that fungal biomass dynamics in the soil differed strongly for the added materials. In general, three patterns of stimulation of fungi could be recognized (I) No or slight stimulation, (II) Strong initial stimulation followed by a strong decrease, (III) Moderate or strong initial stimulation followed by gradual decrease or further increase.

Pattern (I) was seen for several industrial by-products, composts and coniferous wood sawdust. Industrial by-products and composts probably contain limited amounts of degradable organic compounds as easily available energy sources are depleted during production processes, such as fermentation, extraction, roasting or composting (Goss et al., 2013; Scotti et al., 2015). This can explain the low fungal stimulation observed here. Indeed, field studies have shown that application of compost does not increase fungal biomass, unless compost is added in large amounts (Quintern et al., 2006; Bastida et al., 2008). Fungal biomass stimulation could be expected after a longer time since the application of the by-products here tested in the short-term. On the other end, it is not clear if arable soils harbour slow-growing fungi able

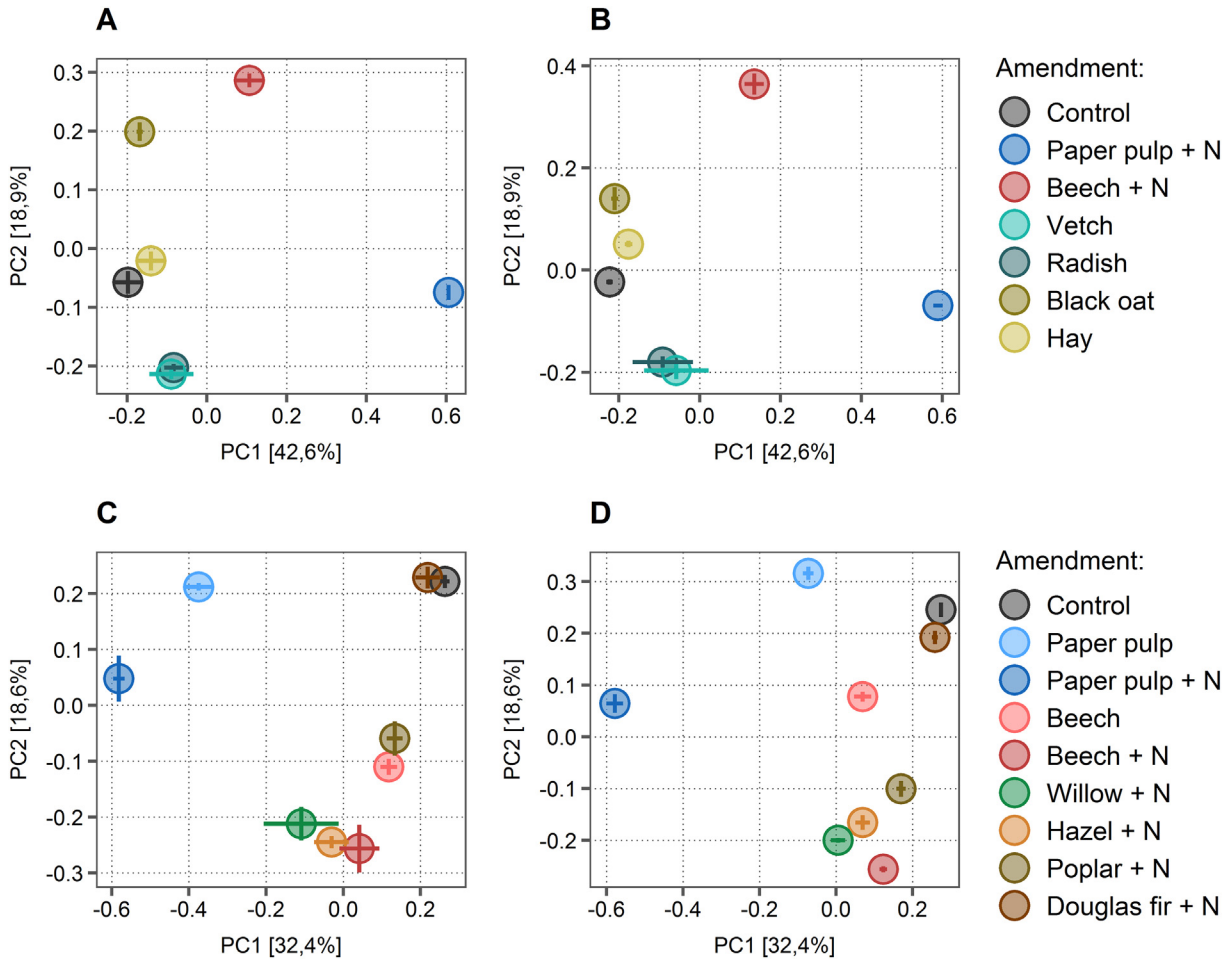

Fig. 2. Effect of organic amendments on the fungal community composition in an arable soil. Ordination (PCoA based on Bray-Curtis dissimilarity matrix) was performed independently for Experiment OA and WA. A and B display the dissimilarity between fungal communities of soil amended with organic materials (Exp. OA) after two and eight weeks of incubation, respectively. Similarly, C. and D. represent the dissimilarity between fungal communities after two and eight weeks of incubation with paper pulp and five sawdust types (Exp. WA). For each amendment and time point, circles are centred on the mean and lines show SE along the first and the second Principal Coordinate.

to further degrade energy-poor residues (Paterson et al., 2011).

Degradation of coniferous wood is a common process in forest ecosystems (van der Wal et al., 2016) and a field experiment showed that conifer wood sawdust caused a long-lasting increase in saprotrophic fungal biomass and soil C stocks, when amended yearly in a wheat monoculture (Wuest and Gollany, 2013; Reardon and Wuest, 2016). Yet, we found a lack of fungal stimulation by Douglas fir sawdust. Hence, it seems that the incubation period of two months used here is too short for Douglas fir sawdust to cause stimulation of fungi. A slower response to conifer wood could be ascribed to a higher concentrations of inhibiting secondary metabolites, resins and terpenes, as well as a distinct structural arrangement and composition of conifer wood polymers (Cornwell et al., 2009).

Pattern (II) was seen for non-woody plant materials. This is in line with studies showing rapid decomposition of dried material of freshly harvested plants and a transient increase in microbial biomass (Lucas et al., 2014). These materials contain a mixture of easily degradable (sugars, proteins) and slowly degradable (cellulose, lignin) compounds (Baumann et al., 2009; Lucas et al., 2014). The labile compounds promote a rapid increase of opportunistic microbes, including so-called sugar fungi. Indeed, for the non-woody plant materials we found a stimulation of the fungi belonging to phylum Mortierellomycota, for which many sugar fungi have been described (see Section 4.3). Yet, within the non-woody plant materials we detected differences in fungal stimulation patterns that appear to be related to the quality of the material reflected mainly in their C:N ratios. For instance, hay had the highest C:N of the non-woody plant materials and gave a lower but more consistent stimulation of fungal biomass. Our understanding of fungal stimulation could be further supported by a detailed chemical characterization of the initial composition of organic amendments (Bonanomi et al., 2018).

Pattern (III) was seen for paper pulp and deciduous woody materials. Common to these materials is that they are recalcitrant and rich in cellulose. This cellulose is crystalline, arranged in microfibrils and fibrils (Rubin, 2008). Many ascomycete and basidiomycete fungi possess a complex of hydrolytic and oxidative enzymes needed to open and degrade it (Wilson, 2011). In intact wood, the presence of lignin limits the accessibility of cellulose for fungi, but this can be partly relieved by milling (van der Wal et al., 2007; Koranda et al., 2014). During paper pulp production from wood, lignin and hemicellulose are removed (Eriksson, 1990) and as a result, paper pulp is mainly composed of cellulose fibres. This can explain the rapid increase in fungal biomass, which was observed in this study, as well as in earlier studies with paper pulp and pure cellulose (Beyer et al., 1997; van der Wal et al., 2006a). Interestingly, the fungal-stimulation effect of (beech) sawdust was consistently observed in the four arable soils tested here. This suggests that the ability to degrade woody materials is common among fungal communities of arable soils, even if they have not received woody residues since a long time. Paterson et al. (2011) showed that prolonged residue exclusion from a soil can limit the ability of resident

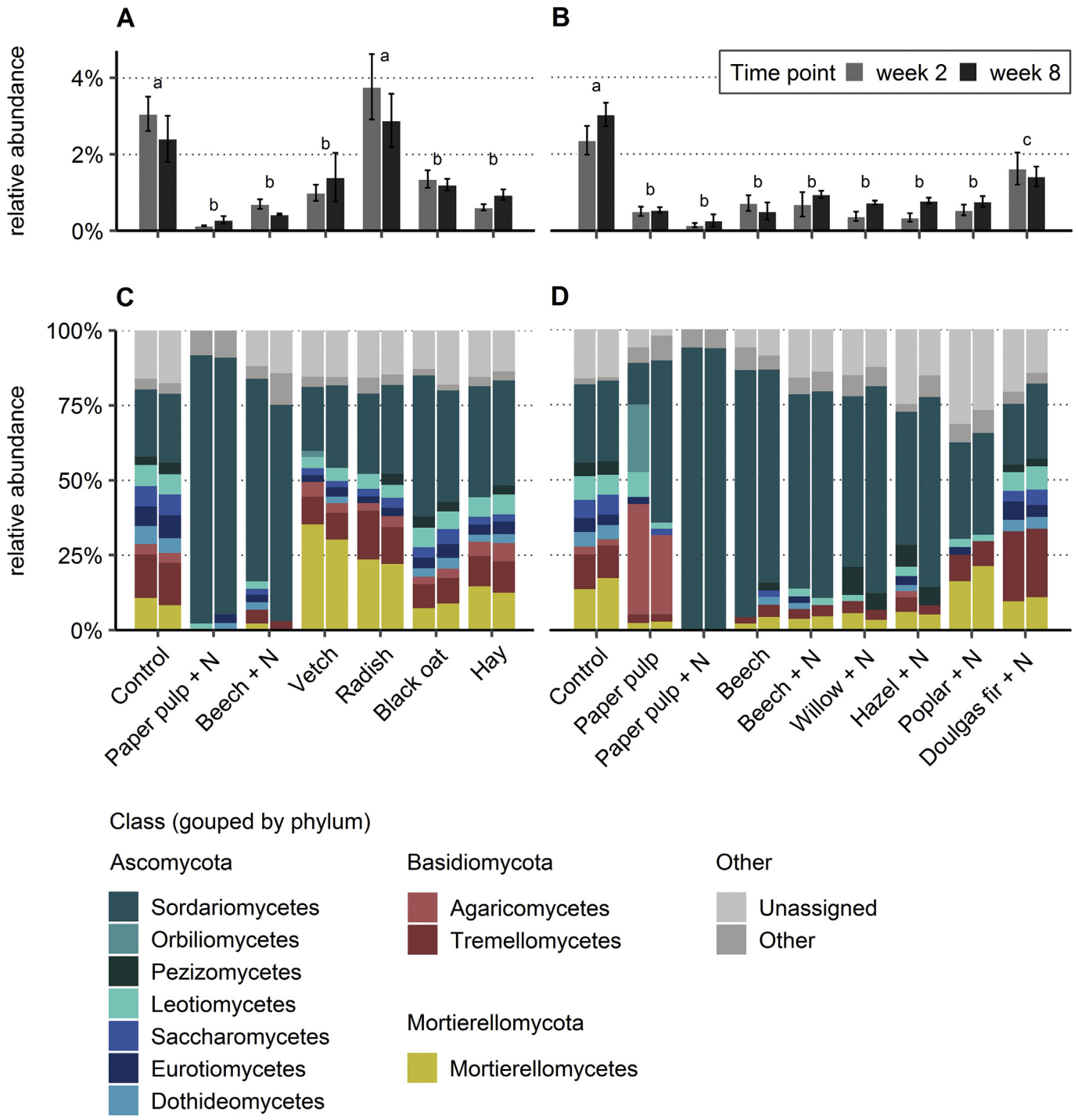

Fig. 3. Effect of organic amendments on potential fungal pathogens and on fugal classes in an arable soil. Relative abundance (mean ± SE) of potential pathogens detected in soil amended with organic materials in Experiment OA (A) and WA (B) after two and eight weeks of incubation. Differences are displayed at $p < 0.05$. C and D show the composition of the total fungal community for Experiment OA and WA, respectively: relative abundances of fungal classes are represented for each soil treatment after two and eight weeks of incubation.

microbes to degrade insoluble fractions of plant material. However, in that study residue exclusion lasted for fifty years, thus it was more extreme than in intensively managed soils, were periods of bare-fallow alternate with cropping. We show that fungi of such arable soils retain the ability to utilize, at least in part, lignocellulose-rich materials.

4.2. Effect of nitrogen on saprotrophic fungal biomass

The initial decomposition of C-rich, fresh materials results in immobilization of mineral N because of an increase in N demands by the microbial decomposers (Mohanty et al., 2013; Gad et al., 2015). Indeed, we detected a depletion of soil mineral nitrogen with addition of wood sawdust, paper pulp and N-poor plant litter. In the field situation this could lead to N deficiency for crops (Mohanty et al., 2013; Heijboer et al., 2016). Thus, stimulation of fungi with fresh, C-rich material should be combined with extra N fertilization to compensate for N immobilization (Mohanty et al., 2013; Toenshoff et al., 2014). At the same time, elevated or prolonged nitrogen fertilization can have a

negative impact on fungal biomass (Zhang et al., 2018) and especially affects lignin-degraders (Entwistle et al., 2018). Reduction in fungal biomass was observed in N-fertilized soils for a range of ecosystems (Treseder, 2008). In our study, mineral N supply did not reduce the biomass of decomposer fungi. On the contrary, mineral nitrogen stimulated fungal biomass in soil amended with paper pulp and wood sawdusts, suggesting that fungal growth was N-limited in amended soils. A similar observation has been made for birch sawdust in an ex-arable soil (van der Wal et al., 2007). In that study, the N-induced increase of fungal biomass in birch sawdust was accompanied with an increased activity of cellulose-degrading enzymes but not of lignin-degrading enzymes. As a legacy to soil management, arable soils likely harbour fungal species that are adapted to using mineral forms of N. Although we did not measure enzyme activities, the predominant stimulation of ascomycetes and not of basidiomycetes by deciduous wood sawdusts and paper pulp is in line with mainly cellulolytic activities (Section 4.3).

Mineral nitrogen supply did not result in fungal biomass stimulation in soils amended with coniferous wood sawdust. This further confirms that other factors constrain the stimulation of fungi. Another exception to the positive effect of mineral nitrogen on fungal biomass stimulation is represented by poplar wood sawdust. Stimulation of fungi was higher when poplar wood was added without N. Of the studied tree species, poplar wood has the highest quality, as suggested by the relatively low C:N ratio. This was also seen for a poplar forest soil that was converted to arable land and amended with poplar post-harvest residues with and without mineral N (Toenshoff et al., 2014).These differences in fungal biomass stimulation and N dynamics after amendment with sawdust types is not fully explained by wood C:N ratio only.

In this study, measurements of mineral N pools indicated that at least 30% (paper pulp) to 50% (wood sawdust) of the added N remained available for plant nutrition. Partial immobilization of the pool of mineral nitrogen by C-rich materials can be utilized as a means for temporarily capturing fertilizer nitrogen that otherwise would be lost from the soil by leaching (Reichel et al., 2018). Our results suggest that fungi may play a major role in N capture, since the maximum nitrogen depletion corresponds with peaks in ergosterol levels, at two and eight weeks after amendment with paper pulp and beech wood, respectively. Increased retention of mineral N in arable soil with increased fungal biomass has been reported earlier(de Vries et al., 2011).

4.3. Effect of organic amendments and mineral N on fungal community composition

The observed rapid stimulation of fungal biomass in soil amended with cover crop fragments, paper pulp and wood sawdust is attributed to an increase in fungi belonging to the phyla Ascomycota and Mortierellomycota. Indeed, such phyla comprehend generally fast-growing fungal species, that are common in arable soils and dominate organic residues in the early stages of decomposition (Poll et al., 2010; van der Wal et al., 2013; Banerjee et al., 2016; Koechli et al., 2019). Interestingly, stimulation of the phylum Mortierellomycota could be an indication of fungi growing on nitrogen-rich organic materials, as this was only seen for the N-rich non-woody plant materials and for poplar wood sawdust. On the other hand, the addition of C-rich materials (black oat, paper pulp and sawdust of deciduous tree spp.) changed the fungal community in favour of ascomycete fungi, especially Sordariomycetes. These comprise a broad diversity of saprotrophic fungi, that inhabit dung, plant litter and wood, where they participate in cellulose degradation (Koechli et al., 2019). In particular, paper pulp combined with mineral nitrogen strongly stimulated *Chaetomiaceae* spp., which are well-known for their ability to degrade cellulose (Banerjee et al., 2016; Koechli et al., 2019). Deciduous wood sawdust, and especially beech, willow and hazel sawdust, stimulated *Chaetomiaceae* and *Lasiosphaeriaceae*. The latter family has been indicated by Hartmann et al. (2015) as responsive to organic soil management. Conversely, coniferous wood caused a shift in favour of basidiomycete yeasts, even though this was not reflected in an increase of fungal biomass. Basidiomycetal yeasts are often found in agricultural soils (Hannula et al., 2012) and are stress tolerators (Treseder and Lennon, 2015), which is in line with the potential presence of antimicrobial compounds and low degradability of conifer wood, discussed above (Section 4.1). This area of research would benefit from a detailed description of the initial C chemistry of the organic materials. This, together with profiling of feeding preferences of fungi and bacteria, is essential to accurately predict the effect of organic amendments on native microbial communities of arable soils (Bonanomi et al., 2018).

Rapidly responding fungal species can include plant-pathogenic fungi, that are commonly found as surviving propagules in resident fungal communities in arable soils (van Agtmaal et al., 2017). Pathogenic fungi are specialized in exploiting living and recently dead plant tissues. For instance, immature compost or fresh plant litter has been shown to increase the incidence of soil borne diseases (Bonanomi et al., 2010). Indeed, in this study we observed an amplification of *Plectosphaerella* spp. in soil amended with radish material. *Plectosphaerella* spp. are known for causing diseases in horticultural plants (Raimondo and Carlucci, 2018). However, other materials did not amplify sequences of potential pathogenic fungi. Conversely, the amendment with other cover crops, and especially sawdust and paper pulp, shifted the fungal community in favour of saprotrophic fungi, with a reduction of potential pathogens. Hence, for the materials here screened for fungal community composition, we did not find indications of an increased risk of pathogen proliferation.

5. Conclusions and application perspectives

This study shows that paper pulp and wood sawdust of deciduous tree species rapidly and consistently increase fungal biomass in arable fungal-poor soils, over a time-frame of two months. In particular, ascomycete saprotrophic fungi were stimulated, whereas potential pathogenic fungi did not increase. Woody materials and paper pulp required additional nitrogen to compensate for nitrogen immobilization. We showed that elevated nitrogen did not inhibit, but rather enhanced fungal biomass stimulation by fresh, high C:N materials. Hence, combining of sawdusts and paper pulp with artificial or organic nitrogen fertilizers could be done in practice. The rapid increase in saprotrophic fungal biomass of arable soils, sustained for a period of two months, can benefit the early growth of crops, for instance by increasing the suppression of soil-borne diseases and by improving the efficiency of nitrogen fertilizers. However, future research should examine these benefits, before paper pulp and woody materials can be safely and effectively implemented as soil management options. In particular, the current study was conducted under controlled growth chamber conditions, without the presence of plants and for a limited span of time. Under field conditions, other factors could interfere with fungal stimulation, such as agricultural management practises and variable weather conditions. Thus, fungal biomass stimulation by sawdusts and paper pulp and its suggested benefits need to be evaluated in field trials, including a longer time frame. Future studies should also include the effect of such amendments on other soil inhabitants. Utilizing wood and paper wastes for sustainable agriculture would be a convenient way of disposing these materials. In particular, sawdust is an abundant waste of the wood industry, as well as of forest and urban landscape management (Harkin, 1969; Heinimö and Junginger, 2009). In recent years, an increasing amount of biomass is used for energy and heat production (Heinimö and Junginger, 2009). However, evidence is growing that large-scale use of wood for energy is a threat for the environment (Schulze et al., 2012; Griscom et al., 2017). We propose that conversion of excess wood biomass into fungal biomass for improving arable soils represents a better perspective for utilization.

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Declaration of interests

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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