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Above-belowground linkages of functionally dissimilar plant communities and soil properties in a grassland experiment

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Abstract. Changes in plant community composition can have long-lasting consequences for ecosystem functioning. However, how the duration of plant growth of functionally distinct grassland plant communities influences abiotic and biotic soil properties and thus ecosystem functions is poorly known. In a field experiment, we established identical experimental subplots in two successive years comprising of fast- or slow-growing grass and forb community mixtures with different forb:grass ratios. After one and two years of plant growth, we measured above- and belowground biomass, soil abiotic characteristics (pH, organic matter, soil nutrients), soil microbial properties (respiration, biomass, community composition), and nematode abundance. Fast- and slow-growing plant communities did not differ in above- and belowground biomass. However, fast- and slow-growing plant communities created distinct soil bacterial communities, whereas soil fungal communities differed most in 100% forb communities compared to other forb:grass ratio mixtures. Moreover, soil nitrate availability was higher after two years of plant growth, whereas the opposite was true for soil ammonium concentrations. Furthermore, total nematodes and especially bacterial-feeding nematodes were more abundant after two years of plant growth. Our results show that plant community composition is a driving factor in soil microbial community assembly and that the duration of plant growth plays a crucial role in the establishment of plant community and functional group composition effects on abiotic and biotic soil ecosystem functioning under natural field conditions.

Key words: nematodes; plant growth rate; soil bacteria; soil fungi; soil microbial respiration; soil nutrients.

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Introduction

Variations in climate and land use are leading to worldwide changes in plant communities, resulting in profound impacts on ecosystem functions and services (Sala et al. 2000, Wardle et al. 2011). In the past decades, an increasing number of studies have experimentally investigated the temporal relationship between plant diversity and above- and belowground properties (Tilman et al. 2001, Zak et al. 2003, Roscher et al. 2004, Cardinale et al. 2011). However, analyses of temporal changes in plant diversity have suggested that at local scales there are no consistent changes in species richness, but rather changes in the composition of plant

communities (Vellend et al. 2013). So far, only few plant diversity experiments have considered how differences in the composition of plant communities with the same diversity level affect above- and belowground properties. Such compositional changes can have profound and long-lasting consequences for belowground ecosystem functioning such as nutrient cycling (Wardle et al. 2011), soil moisture, and soil microbial properties (Meyer et al. 2016).

Plant communities greatly differ in how they influence the soil biochemical cycles by altering soil nutrient concentrations both via their carbon (C) and nitrogen (N) input through litter and rhizodeposition (Rosenkranz et al. 2012, Abbas et al. 2013) and through constant nutrient uptake (Scherer-Lorenzen et al. 2003, Fornara and Tilman 2008, Hobbie 2015). This, in turn, has a major effect on soil microbial communities mineralizing organic matter and enhancing the release of nutrients by mineral weathering (Van der Krift and Berendse 2001, Lange et al. 2014). Such plant-induced changes in substrate availability also affect other parts of the soil community, comprising, for example, bacteria, fungi, and nematodes which all act as mutualists, decomposers, herbivores, or pathogens (Wardle et al. 2003, De Deyn et al. 2004, Wardle 2006). In recent years, plant functional traits have become a very powerful tool to identify the mechanisms by which plants influence above- and belowground ecosystem properties (Diaz et al. 2004, De Deyn et al. 2008, Steinauer et al. 2017). Here, especially, the plant economics spectrum is a typical functional trait and can range from fast to slow based on resource acquisition and processing of a plant species (Reich 2014). Plant species with fast traits have a rapid resource uptake and fast turnover of biomass (e.g., high-quality litter and short-lived plants; Lavorel and Garnier 2002, Reich 2014), promoting bacterial-dominated soil microbial communities and, thereby, faster overall nutrient cycling. In contrast, slow-growing plant species exhibit opposite traits (e.g., low-quality litter and long-lived plants) and are thought to promote a fungal-dominated soil food web (Wardle et al. 2004), leading to slower nutrient turnover. In addition, plant species can be assigned to specific functional groups (such as grasses and forbs) differing in their functional trait compositions related to plant growth and resource acquisition. Those can drive differences in above- and belowground community functioning (Finke and Snyder 2008, Cadotte et al. 2009), which can alter both abiotic and biotic soil conditions (Ehrenfeld 2010, Lange et al. 2014, Heinen et al. 2018). Grasses, for example, often stimulate beneficial bacterial communities and bacteria-feeding nematodes in the rhizosphere (Latz et al. 2015). Further, both forb and grass species are associated with distinctly different plant-feeding nematode communities (De Deyn et al. 2004).

Plant–soil interactions are temporally dynamic (Bardgett et al. 2005, Bray et al. 2012). Plant residues and rhizodeposits accumulate over time, which in the longer-term creates plant community-specific soil organic matter pools. These specific food sources, in turn, shape the soil microbial community (De Deyn and Van Der Putten 2005, Schofield et al. 2018) and drive its governing ecosystem processes. Previous studies found rapid plant effects on the structure of soil microbial communities (Maul and Drinkwater 2010, Kulmatiski and Beard 2011, Knelman et al. 2018). However, other studies reported that it may take several years before changes in plant communities impact on belowground properties such as soil microbial respiration and diversity (DeBruyn et al. 2011, Steinauer et al. 2016, Strecker et al. 2016). Similarly, changes of nematode community composition due to alterations in plant communities have been shown to underly time lags (Korthals et al. 2001, Cortois et al. 2017, Wubs et al. 2019).

In this study, we test whether the duration of plant growth (one and two years) of functionally distinct grassland communities (fast- vs. slow-growing and their forb:grass ratio, see below) of naturally co-existing plant species affects soil properties and functions. In a split-plot field experiment, we established identical experimental subplots in two successive years that comprised of fast- or slow-growing grass and forb communities and that differed in forb:grass ratios (0:100; 25:75; 75:25, or 100:0 percent forb:grass). Due to their opposing traits in resource acquisition and processing, we expected (1) fast- vs. slow-growing plant communities to affect nutrient cycling, and shape distinct soil microbial and nematode communities. Furthermore, we predicted fast-growing plant communities will

have strong effects on soil processes regardless of how long those communities conditioned the soil, while slow-growing plant communities will have more pronounced effects after two years. Lastly, due to higher root biomass and greater C input via rhizodeposition by grasses versus forbs (Pausch and Kuzyakov 2018), we hypothesized (2) higher nutrient availability, increased soil microbial biomass and respiration and higher nematode abundances in plant communities dominated by grasses compared to forbs and that these effects would become more pronounced over time.

Material and Methods

Experimental setup

The experimental site was established in spring 2015 in a restored grassland site (abandoned from agricultural use in 1996), De Mossel (Natuurmonumenten, Ede, the Netherlands, 52°04′ N, 5°45′ E). The area around De Mossel is characterized by a mean daily temperature of 16.7°C in summer months and 1.7°C in winter months and monthly precipitation ranges from 48 to 76 mm (based on open source data from long-term climate models; www.climate-data.org). The soil of the field site is described as a holtpodzol and soil texture is characterized as sandy loam (94% sand, 4% silt, 2% clay, ~5% organic matter, 5.2 pH, 2.5 mg/kg N, 4.0 mg/kg P, 16.5 mg/kg K; Jeffery et al. 2017).

In total, 100 experimental plots (1.66 × 2.50 m) were installed and each plot was divided into two subplots (each 0.83 × 2.50 m) resulting in 200 subplots (De Long et al. 2019). Experimental plots were randomly arranged in four blocks. To study the effects of the duration of plant growth of distinct plant communities, the topsoil (about 4 cm depth) including the previously existing plant communities of 100 subplots was removed in May 2015 (later referred to as two-year subplots) whereupon experimental plant communities were sown immediately. In May 2016 (later referred to as one-year subplots), the topsoil including the previously existing plant communities was removed of the remaining 100 subplots and experimental plant communities were then sown. In total, 24 predominantly perennial plant communities based on their economic spectrum (fast- versus slow-growing species) and differences in forb:grass ratio were chosen. Fast- or slow-growing grasses or forbs were selected from a pool of 24 grassland species that all co-occur locally at this site. Plants were assigned to fast- versus slow-growing species based on known growth rates (Fitter and Peat 1994, Fry et al. 2014) or after consultation with botanists (van Ruijven and Poorter, *personal communication*). Three fast- (Fast 1, Fast 2, Fast 3) and three slow- (Slow 1, Slow 2, Slow 3) growing plant communities were used in the experiment, each mixture consisted of 3 or 6 plant species (three grasses and/or three forbs; Table 1). Specifically, sown plant communities differed in forb:grass ratios as per seeding such as (1) three fast- or slow-growing forb species (100%); (2) three fast- or slow-growing grass species (100%); or (3) three fast- or slow-growing forbs and three fast- or slow-growing grasses (25% grass and 75% forb or 75% forb and 25% grass). Sowing density amounted to 12,000 seeds per subplot, representing each plant species in equal amounts of seeds. Seeds were obtained from specialized suppliers that provide seeds collected from wild plants (Cruydt-Hoeck, Nijeberkoop, the Netherlands, and MediGran, Hoorn, the Netherlands) in 2015. Additionally, after topsoil removal, in each block, two experimental subplots were kept bare one from May 2015 onwards and one from May 2016. These plots served as a control to permit the comparison of ecosystem processes in the absence of vegetation. In 2015 and 2016, during the growth season (May through September) all sown subplots and bare control subplots were regularly weeded to maintain the sown plant community composition. In total, this resulted in 2 temporal treatments (two- and one year; represented by the level of subplots) and 25 plant community treatment combinations (2 community growth rates (fast, slow) × 4 forb:grass ratios (0:100; 25:75; 75:25 or 100:0 percent forb:grass, represented by the level of plots) × 3 species combinations + 1 bare control), which were replicated across four blocks (200 subplots). The efficiency of the establishment of experimental plant communities was reported previously in De Long et al. (2019). Here, it was shown that the six different plant communities (Fast 1, Fast 2, Fast 3, Slow 1, Slow 2, Slow 3) significantly differed in their composition whereas fast-growing plant communities and slow-growing plant

Table 1. Species composition of plant communities consisting of fast- (F1, F2, and F3) or slow- (S1, S2, and S3) growing plants sown on subplots in 2015 and 2016.

Plant community	Plant species
Fast-growing plant species	
Grass	
F1	*Dactylis glomerata* L.; *Holcus lanatus* L.; *Alopecurus pratensis* L.
F2	*Agrostis capillaris* L.; *Lolium perenne* L.; *Holcus lanatus* L.
F3	*Phleum pratense* L.; *Dactylis glomerata* L.; *Lolium perenne* L.
Forb	
F1	*Plantago lanceolata* L.; *Crepis capillaris* ((L.) Wallr.); *Taraxacum officinale* (F.H. Wigg)
F2	*Rumex acetosella* L.; *Crepis capillaris* ((L.) Wallr.); *Achillea millefolium* L.
F3	*Achillea millefolium* L.; *Epilobium hirsutum* L.; *Taraxacum officinale* (F.H. Wigg)
Slow-growing plant species	
Grass	
S1	*Arrhenatherum elatius* ((L.) P.Beauv. ex J.Presl & C.Presl); *Briza media* L.; *Festuca ovina* L.
S2	*Briza media* L.; *Trisetum flavescens* (L.) P.Beauv.; *Anthoxanthum odoratum* L.
S3	*Anthoxanthum odoratum* L.; *Deschampsia flexuosa* (L.) Trin.; *Trisetum flavescens* (L.) P.Beauv.
Forb	
S1	*Tripleurospermum maritimum* ((L.) W.D.J.Koch); *Clinopodium vulgare* L.; *Geranium molle* L.
S2	*Clinopodium vulgare* L.; *Gnaphalium sylvaticum* L.; *Myosotis arvensis* ((L.) Hill)
S3	*Tripleurospermum maritimum* ((L.) W.D.J.Koch); *Myosotis arvensis* ((L.) Hill); *Galium mollugo* L.

Note: There were three different fast and slow plant communities and each community consisted of forb:grass ratios of 0:100, 25:75, 75:25, 100:0 and hence consisted of three (only forbs or grasses) or six plant species.

communities were clustering separately. Furthermore, it was reported that the actual percentage cover of both forbs and grasses corresponded well to the treatments.

Data collection

Above- and belowground biomass.—In the beginning of June 2017, aboveground biomass production was assessed within two randomly selected squares (25 × 25 cm, minimum 10 cm distant to the edge) in each subplot by cutting plants just above the soil level. Aboveground biomass of all samples was determined by weighing after drying at 70°C for 72 h and later converted to g per m^2. After the aboveground biomass was clipped in a subplot, one soil core (diameter 3 cm, 10 cm deep) was taken from the center of the square. The fresh soil weight was determined, and the roots were then washed over a 0.425-mm sieve. Root biomass was then determined after drying at 40°C for 72 h and later converted to gram per gram fresh weight of soil.

Soil sampling.—Soil sampling was carried out in two sampling campaigns. First, soil samples were collected in March 2017. Here, 20 soil samples (metal corer: diameter 1 cm, 10 cm deep) were taken randomly per subplot, pooled, and homogenized. Approximately 2 g of fresh soil was immediately frozen at −80°C for DNA-based determination of the soil microbial community composition. In mid-May 2017, an additional 20 soil samples (metal corer: diameter 3 cm, 10 cm deep) were taken randomly per subplot. The soil samples were pooled, homogenized, and divided into several subsamples. About 50 g of fresh soil for nematode extraction was kept in a fridge (4°C). Approximately 20 g was sieved (2 mm mesh size) to remove stones, roots, and invertebrates >2 mm and then stored at −20°C for measurements of soil microbial properties (soil microbial respiration and biomass); the remaining soil was dried at 40°C and used to measure soil abiotic characteristics.

Soil abiotic characteristics.—The soil was dried at 40°C until the soil weight was stable and sieved (mesh size: 1.4 mm) to remove roots and stones. Three grams of dried soil was mixed with 30 mL of 0.01 M $CaCl_2$ and shaken for 2 h on a mechanical shaker with linear movement at 250 rpm. Samples were then centrifuged for 5 min at 1693 *g*. Then, 15 mL of the supernatant was filtered through a Whatman Puradisc Aqua 30 syringe

filter with a cellulose acetate membrane and 130 μL HNO_3 was added to 12.87 mL of the filtrate. Soil extractable elements (Fe, K, Mg, P, S, Zn) were analyzed using an inductively coupled plasma-optical emission spectrometer (ICP-OES, Thermo Scientific iCAP 6500 Duo Instrument with axial and radial view and CID detector microwave digestion system). The remaining filtrate (2.13 mL) was used to measure soil pH and to measure nitrite (NO_2–N) + nitrate (NO_3–N) and ammonium (NH_4–N) on a QuAAtro Autoanalyzer (Seal analytical).

Soil organic matter.—Soil organic matter content was estimated by the loss-on-ignition (LOI) method (Heiri et al. 2001). Approximately 5 g of soil was oven-dried at 105°C for 16 h and weighed. The sample was then burned at 550°C for 5 h and weighed again. Soil organic matter was calculated as the percentage weight loss between the oven-dried and burned samples.

Soil microbial respiration and biomass.—Approximately 5 g soil (fresh weight) was weighed into 50-mL centrifuge tubes to determine soil microbial respiration and soil microbial biomass. The lid of each tube was sealed gas-tight using an O-ring and a rubber septum in the middle. For basal respiration measurements, the tubes were capped and flushed with CO_2-free air to remove any CO_2 from the headspace. After 24 h of incubation at 20°C, 12 mL of headspace was sampled using a gas-tight syringe. Microbial biomass was determined after addition of D-glucose-monohydrate using the substrate-induced respiration method (SIR) (Anderson and Domsch 1978). Next, 2 mL of 75 mM D-glucose solution was added to each soil sample and placed on a horizontal shaker for 1 h. Tubes were capped, flushed with CO_2-free air, and incubated for 4 h at 20°C. Again, 12 mL of headspace was sampled. Measurements of the CO_2 concentrations were carried out on a Trace CG Ultra Gas Chromatograph (Thermo Fisher Scientific, Milan, Italy). Gravimetric soil water content was determined by drying the soil samples overnight at 60°C to constant weight and calculating the difference in weight between fresh and dried soil.

Soil microbial community composition.—DNA was extracted from 0.75 g of soil using the PowerSoil DNA Isolation Kit (Mo Bio Laboratories, Carlsbad, California, USA) following the manufacturer's protocol. The DNA quantity was measured using a Nanodrop spectrophotometer (Thermo Scientific, Hudson, New Hampshire, USA). Approximately 100 ng of DNA was used for a PCR. We used the primers ITS4ngs and ITS3mix targeting the ITS2 region of fungal genes (Tedersoo et al. 2015) and the primers 515FB and 806RB (Caporaso et al. 2012, Apprill et al. 2015, Parada et al. 2016) targeting the V4 region of the 16Sr RNA gene in bacteria. Presence of PCR product was checked using agarose gel electrophoresis. The PCR products were purified using Agencourt AMPure XP magnetic beads (Beckman Coulter, Brea, California, USA). Adapters and barcodes were added to samples using Nextera XT DNA library preparation kit set A (Illumina, San Diego, California, USA). The final PCR product was purified again with AMPure beads, checked using agarose gel electrophoresis, and quantified using a Nanodrop spectrophotometer before equimolar pooling. We pooled all fungal samples (200) in one Miseq PE250 run and divided the bacterial samples in two separate runs (100 samples each; 1st run: block 1 and 2, 2nd run: block 3 and 4). Libraries were sequenced at McGill University and Genome Quebec Innovation Center, Canada. Extraction negatives were also sequenced. A mock community, containing 10 fungal species, was included to investigate the accuracy of the bioinformatics analysis.

Bacterial sequences and fungal sequences were analyzed using the PIPITS pipeline and the hydra pipeline, respectively (Gweon et al. 2015, De Hollander 2017). In short, fungal sequences were paired using VSEARCH and quality was filtered using standard parameters. The ITS2 region was extracted using ITSx (Bengtsson-Palme et al. 2013). Short reads were removed, and sequences were clustered based on a 97% similarity threshold using VSEARCH and chimeric sequences were removed by comparing with UNITE uchime database. The representative sequences were identified using the RDP classifier against the UNITE database (Kõljalg et al. 2005). For bacterial sequences, VSEARCH was used to pair sequences and cluster them or classification; SINA classification was used with the SILVA database.

Nematode extraction.—Decantation (Cobb 1918) and centrifugal flotation methods (Van Bezooijen 2006) were used to extract nematodes from 50 g

fresh soil collected from block 1 and 2 (100 subplots). Briefly, soil samples were weighed, suspended in 3 L water, and stirred until a homogenous suspension was obtained. The suspension was settled for 15 s and the supernatant decanted into a plastic bowl. This procedure was repeated three times and the obtained suspension was passed through one 75-µm and three 45-µm sieves and collected into a 50-mL centrifuge tube. The nematode suspension was centrifuged for 5 min at 1693 *g* and the supernatant was carefully poured off. Sugar solution (484 g/L) was added, fully mixed with the remaining sediment in the tube and centrifuged again for 5 min at 188 *g*. The supernatant was poured over a 30-µm sieve. Nematodes on the sieve were carefully rinsed with water and collected in a beaker for microscopic analysis. All nematodes were counted and classified by microscopy to one of five feeding types (root feeders, fungivores, omnivores, bacterivores, or predators) according to Yeates et al. (1993). However, predatory nematodes were excluded from further analysis because of low abundance.

Data analysis

We used linear mixed-effects models to test the effects of duration of plant growth (one- and two-year subplots), community growth rates (fast vs. slow), and forb:grass ratios on above- and root biomass, soil abiotic characteristics (pH, Fe, K, Mg, P, S, Zn, NO_2–N + NO_3–N, NH_4–N), soil organic matter, soil microbial properties, and feeding types of nematodes. Thereby, plot and plant community identity (Fast 1, Fast 2, Fast 3, Slow 1, Slow 2, Slow 3) were included as random factors. Above- and belowground biomass, chemical abiotic characteristics, and soil microbial properties (respiration and biomass) data were ln-transformed to meet assumptions of ANOVA. Linear mixed-effects models were performed using lme4 package (Bates et al. 2015), whereas p-values and degrees of freedom were estimated with type III Kenward-Roger approximation using lmerTest (Kuznetsova et al. 2017). We further used comparisons of means for treatment-specific effects (Tukey's HSD test; $\alpha < 0.05$). Tukey's tests were performed using and multcomp package (Hothorn et al. 2016). Bare plots were excluded from analysis, but their values are displayed in graphs.

To test the effects of duration of plant growth, community growth rates, and forb:grass ratios on bacterial and fungal community compositions, we first filtered out taxa that were present in less than ten samples and had an abundance of less than 0.01%. ITS sequences derived from other organisms than fungi were further removed, and for 16S rRNA data mitochondria and chloroplast sequences were removed. For both bacteria and fungi, samples with less than 1000 reads of more than 80,000 reads remaining were removed from the dataset and read numbers were further normalized using total sum scaling (TSS). Mock communities consisting of 10 fungal species were used to inspect the filtering done for fungi. After filtering, we detected 13 fungal OTUs which show that we might be slightly overestimating the diversity. Afterward, we ran permutational multivariate analysis of variance (PERMANOVA, based on Bray–Curtis dissimilarities, 999 permutations) on both bacterial and fungal OTUs using the adonis function in the vegan package (Oksanen et al. 2016). However, here we only used one- and two-year subplots to test for the differences in plant community effect on soil microbial community composition. Since the basic assumption of PERMANOVA is a balanced design and homogenization of samples, bare subplots were excluded due to lower number of samples than the one- and two-year subplots. For visualization, we applied a non-metric multidimensional (NMDS) analysis of the dissimilarities (based on Bray–Curtis dissimilarities) in microbial community composition using ggplot2 package (Wickham 2016). All statistics were performed within the R statistical environment (version 3.5.1; R Core Team 2018).

Results

Above- and belowground biomass

Overall, shoot biomass was 68% higher in two-year than one-year subplots (Fig. 1A, B; Appendix S1: Table S1; $F_{1,88} = 135.40$, $P < 0.001$) but did not differ between fast- and slow-growing plant communities (Fig. 1A; Appendix S1: Table S1; $F_{1,4} = 0.13$, $P = 0.733$). Further, shoot biomass was lower in forb-dominated than in grass-dominated plant communities (Fig. 1B; Appendix S1: Table S1; $F_{3,84} = 18.38$, $P < 0.001$).

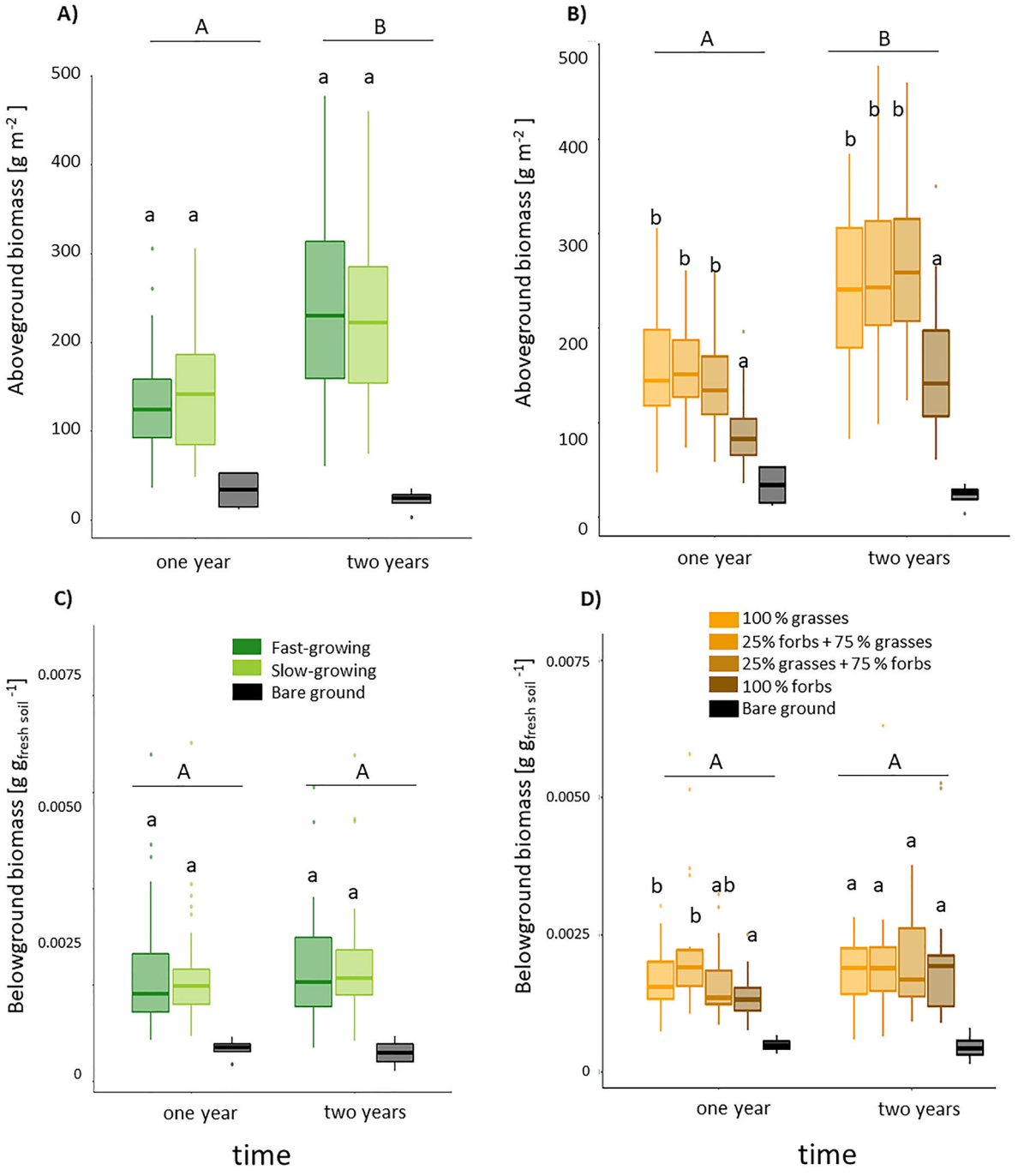

Fig. 1. The effects of (A) time (one- and two-year subplots) and fast- versus slow-growing plant communities on aboveground biomass, (B) time and forb:grass ratio (0:100; 25:75; 75:25 or 100:0 percent forb:grass) on aboveground biomass, (C) time and fast- versus slow-growing communities on belowground biomass, and (D) time and forb:grass ratio on belowground biomass with a total sample size of 200. Black boxes represent the effects on bare soil. The boxes represent 95% confidence intervals, the horizontal line in each box shows the median, and the whiskers show the spread in the data. The circles denote outliers. Bars with different letters vary significantly (Tukey's HSD test, $\alpha < 0.05$).

In contrast to shoot biomass, root biomass did not depend on the duration of plant growth (Fig. 1C, D; Appendix S1: Table S1; $F_{1,88} = 0.98$, $P = 0.325$) and fast- and slow-growing plant communities (Fig. 1C; Appendix S1: Table S1; $F_{1,4} = 0.40$, $P = 0.846$). However, root biomass decreased with increasing forb dominance after one year (Fig. 1D), whereas root biomass was similar across all plant community mixtures after 2 yr of plant growth (Appendix S1: Table S1; time × forb:grass ratio $F_{3,88} = 3.71$, $P = 0.014$).

Soil abiotic characteristics and organic matter

Soil nitrate (NO_3–N) concentrations were 55% higher in two-year than one-year subplots (Fig. 2A,B; Appendix S1: Table S2; $F_{1,88} = 47.45$, $P < 0.001$). Further, NO_3–N concentrations decreased with increasing forb dominance over time, mainly in two-year subplots (Fig. 2B; Appendix S1: Table S2; $F_{3,84} = 2.73$, $P = 0.049$; time × forb:grass ratio: $F_{3,88} = 6.15$, $P = 0.001$). Ammonium concentrations (NH_4–N) were 160% higher in one-year than in two-year subplots (Fig. 2C, D; Appendix S1: Table S2; $F_{1,88} = 136.93$, $P < 0.001$), irrespective of plant community composition. Other soil extractable elements (K, P, S, Fe, Zn, and Mg) as well as pH and soil organic matter showed no differences between the duration of plant growth or any of the plant community treatments (Appendix S1: Table S2).

Soil microbial respiration and biomass

The respiration of soil microorganisms was 9% higher in two-year than one-year subplots (Appendix S1: Fig. S1a, b, Table S3; $F_{1,88} = 9.54$, $P = 0.003$) irrespective of any plant community treatment. However, soil microbial biomass was not significantly affected by any of the treatments (Appendix S1: Table S3).

Soil microbial community composition

The composition of both bacterial and fungal communities significantly differed between the one- and two-year subplots (Fig. 3A, C; Appendix S1: Table S4; bacteria $F_{1,156} = 4.79$, $P = 0.001$; fungi $F_{1,172} = 6.48$, $P = 0.001$). Soil bacterial communities further differed between fast- and slow-growing plant communities (Fig. 3B; Appendix S1: Table S4; $F_{1,156} = 1.54$, $P = 0.007$), whereas fungal communities were most distinct in soils in which 100% forbs had been grown compared to the other plant communities (Fig. 3D; Appendix S1: Table S4; $F_{1,172} = 2.24$, $P = 0.001$).

Nematode community composition

Total nematode abundance per gram soil was 55% higher in two-year subplots than in one-year subplots (Appendix S1: Fig. S1c, d, Table S5; $F_{1,40} = 14.36$, $P < 0.001$), but did not differ between the different plant communities (Appendix S1: Table S5). Bacterial-feeding nematodes were significantly more abundant in two-year subplots than in one-year subplots (Appendix S1: Fig. S1e, f, Table S5; $F_{1,40} = 13.42$, $P = 0.001$). Plant-feeding nematodes were marginal significantly more abundant in two-year subplots than in one-year subplots (Appendix S1: Fig. S1g, h, Table S5; $F_{1,40} = 3.08$, $P = 0.087$). Further, the abundance of plant-feeding nematodes in plant communities with 0:100% forb:grass was higher in two-year than in one-year subplots (Appendix S1: Fig. S1h, Table S5; $F_{3,40} = 3.83$, $P = 0.017$). Abundances of fungal-feeding and omnivorous nematodes were not significantly affected by any of the treatments (Appendix S1: Table S5).

Discussion

Plant community effects on soil microbial communities

In this field experiment, we tested whether the duration of plant growth of functionally distinct grassland plant communities influences abiotic and biotic soil properties including chemistry, microbial communities, and nematodes. We found that fast- and slow-growing plant communities formed different soil bacterial communities, whereas soil fungal communities shaped by plant communities belonging to the 100% forb treatment were most different from plant communities containing grass species. In the same experiment, Heinen et al. (2020) reported that soil pathogenic fungi decreased with higher forb cover. However, arbuscular mycorrhizal fungi and saprotrophs were not affected by the forb:grass ratio of the experiment. These results are in line with our hypotheses that changes in plant community structure, plant functional group composition, and plant growth rate can profoundly affect soil microbial community structure and functions via differences in quantity and quality of resource inputs into the soil, such

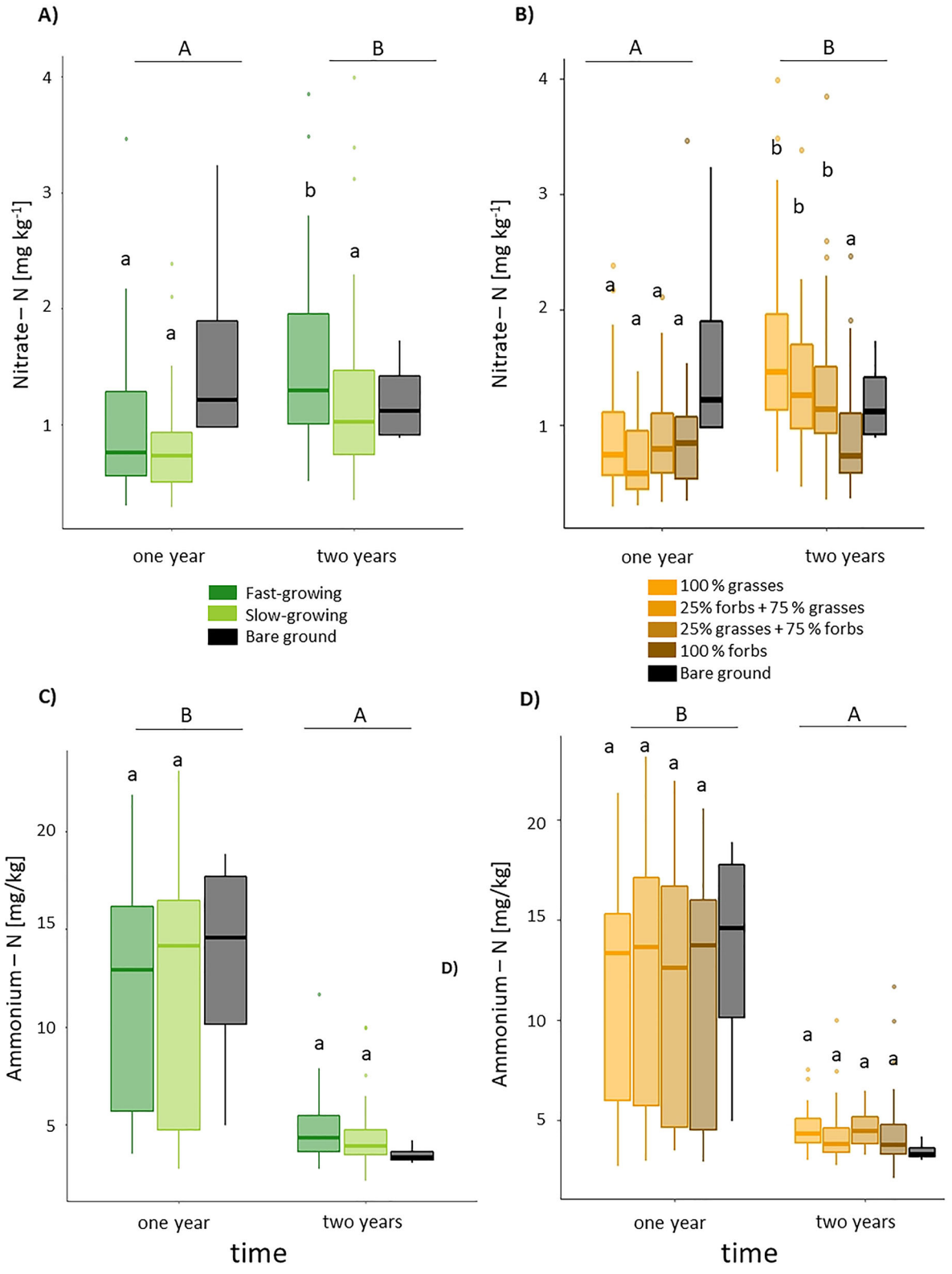

Fig. 2. The effects of (A) time (one- and two-year subplots) and fast- versus slow-growing communities on soil

nitrate-N concentrations, (B) time and forb:grass ratio (0:100; 25:75; 75:25; or 100:0 percent forb:grass and bare ground) on soil nitrate-N concentrations, (C) time and fast- versus slow-growing communities on soil ammonium-N concentrations, (D) time and forb:grass ratio on soil ammonium-N concentrations with a total sample size of 200. Black boxes represent the effects on bare soil. The boxes represent 95% confidence intervals, the horizontal line in each box shows the median, and the whiskers show the spread in the data. The circles denote outliers. Bars with different letters vary significantly (Tukey's HSD test, $\alpha < 0.05$).

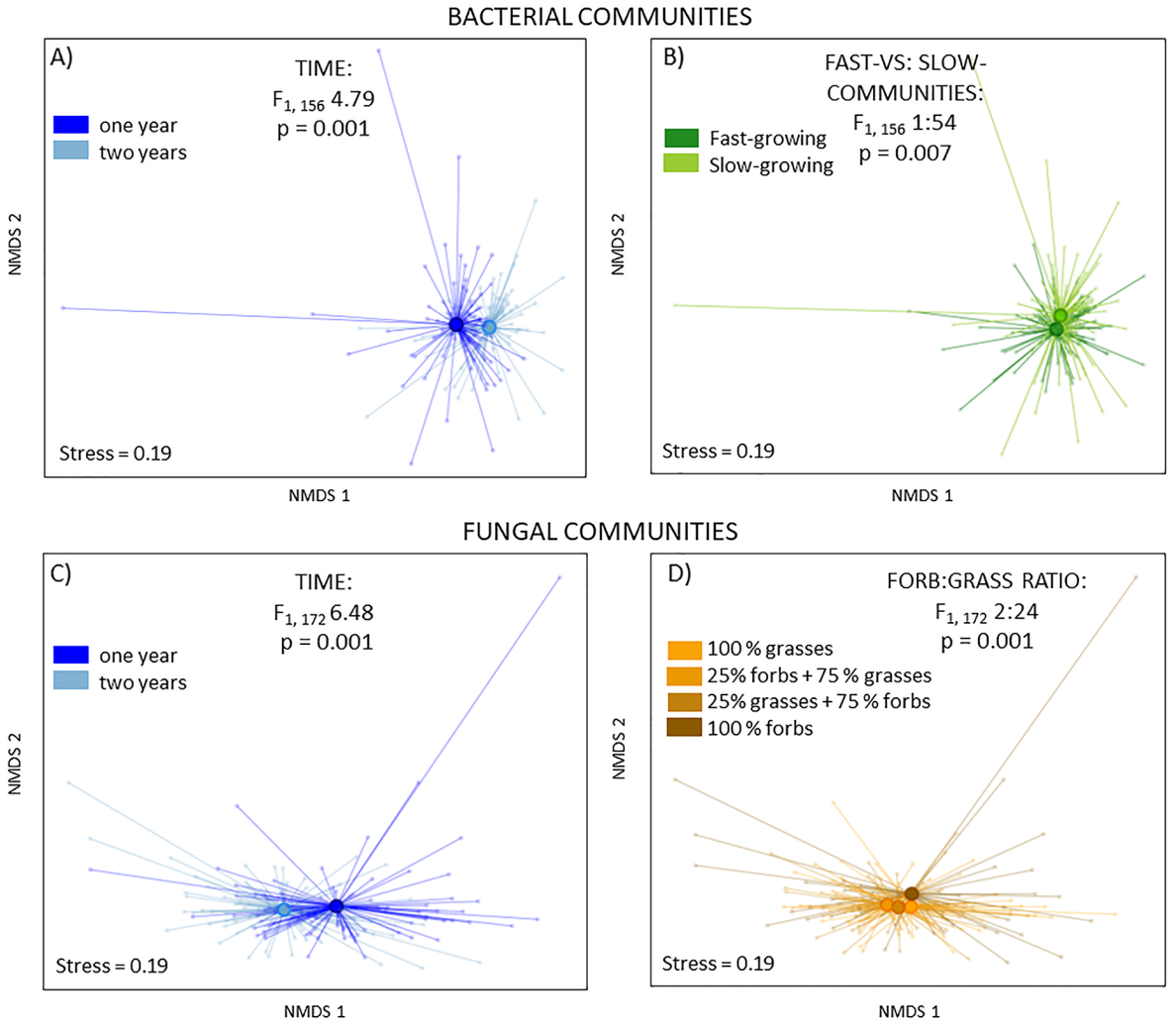

Fig. 3. Non-metric multidimensional scaling (NMDS) plots based on Bray-Curtis dissimilarity. Smaller dots represent individual plots, and larger dots represent averaged centroids. Significance and F-statistic based on permutational ANOVA testing the effect of time and plant community composition on Bray-Curtis dissimilarity matrix. Stress values are given for each NMDS. The effects of (A) time (one and two years) on soil bacterial community composition, (B) fast- versus slow-growing communities on soil bacterial community composition, (C) time (one and two years) on soil fungal community composition, (D) forb:grass ratio (0:100; 25:75; 75:25, or 100:0 percent forb:grass) on soil fungal community composition. Each (bacterial and fungal community composition) had a total sample size of 200.

as root exudates and litter (Zak et al. 2003, Philippot et al. 2013, Bardgett and Van Der Putten 2014, Faucon et al. 2017). Furthermore, plant community effects on soil properties might also depend on plant species that make up the community or on the presence of species that belong to other functional groups such as legumes (Leff et al. 2018), which calls for more specifically designed field studies to understand plant identity effects within plant communities on soil properties.

Plant community effects on soil chemistry and nematodes

Interestingly, there were no major differences between fast- and slow-growing plant communities, notably in terms of plant productivity, nutrient cycling, and nematode abundance, which contradicted our expectations based on previous studies (Reich 2014, Diaz et al. 2016). It is possible that the plant species selected for this experiment were not distant enough within the economic spectrum of fast- or slow-growing species to show clear differences in plant productivity. However, we observed differences between forb and grass communities irrespective of the fast–slow continuum. Communities that were sown with forb seeds only, produced least shoot biomass both after one and two years of plant growth, whereas root biomass of these communities was lowest after one year of plant growth. This is consistent with results from another field experiment reporting a decrease of above- and belowground productivity of plant communities with increasing dominance of forb species because forbs produce less biomass per sampling unit than grasses (Bessler et al. 2009).

Field vs. greenhouse experiments

We speculate that a lack of differences between slow- and fast-growing plant communities in terms of belowground process could be related to the complex interactions between abiotic and biotic factors that are much more important in field studies than in controlled greenhouse experiments, which potentially influence the links between plants and belowground processes and communities. Many studies have shown contradicting results of greenhouse and field experiments when studying aboveground–belowground interactions (Kulmatiski and Kardol 2008, Heinze et al. 2016). Furthermore, interactions in the field are likely to be influenced by small-scale differences in microclimate, soil moisture, and soil texture, and by direct interactions between the co-occurring plant species such as competition for nutrients, water, and light (De Deyn and Van Der Putten 2005). The high number of interactions, the complexity of the grassland system, and our poor understanding of the mechanisms via which plants influence soil properties in mixed plant communities in the field, all hamper our ability to link shoot and belowground processes and interactions in the field. Hence, our results highlight the need for a better understanding of these mechanisms in realistic settings.

Effects of plant growth duration on soil chemistry

Overall, we found that the duration of plant growth had strong effects on plant productivity and soil nutrient availability (such as NO_3–N and NH_4–N), as well as on structural (community composition and abundances) and functional (basal respiration) alterations of soil microorganisms and nematodes compared to plant's growth forms. Aboveground productivity was higher after two years of plant growth due to the extended period of undisturbed plant growth confirming previous long-term field experiments (Tilman et al. 2001). The composition of the plant community and the duration of plant growth can strongly influence the availability of nutrients in ecosystems, because species differ in primary productivity, nitrogen-use efficiency, composition of rhizodeposits, and litter quality (Hooper and Vitousek 1998, Van der Krift and Berendse 2001). Generally, nitrogen availability in soil depends largely on the mineralization—the microbial-mediated conversion of organic nitrogen to inorganic forms like NO_3–N and NH_4–N. After two years, NO_3–N concentrations were lower in soils of plant communities belonging to the 100% forb treatment than in soils of grass communities. Noteworthy, soil fungal communities especially pathogenic fungi were also lower in 100% forb-dominated plant communities. This might be explained by an increased NO_3–N uptake by forbs in the second year of plant growth leading to a reduction in the density of soil fungal pathogens (Snoeijers et al. 2000). In line with previous field experiments,

we observed strong temporal differences on soil NO_3–N and NH_4–N availability (Oelmann et al. 2011, Mueller et al. 2013). NO_3–N concentrations were higher after two years of plant growth and NH_4–N concentrations were higher after one year of plant growth. To fully evaluate the underlying effects and mechanisms of plant community nutrient uptake and release, longer-term observations are of inevitable importance (Oelmann et al. 2011, Mueller et al. 2013).

Effects of plant growth duration on soil microbial communities

After two years of plant growth, shoot biomass was higher than after one year of growth. This, in turn, might have caused greater carbon allocation via rhizodeposition into the soil in two-year-old plant communities (Pausch and Kuzyakov 2018). This, in turn, increased basal soil respiration suggesting increased net activity of soil microorganisms. Furthermore, soil bacterial and fungal community structures were not only affected by plant community composition but also by the duration of plant growth. This finding corresponds to several previous studies showing that temporal changes in soil microbial community composition depend on plant growth duration (Habekost et al. 2008, Lange et al. 2015, Strecker et al. 2016), and this can further lead to functional changes such as basal respiration associated to soil microorganisms. However, compared to those studies which found temporal effects after 4 yr of plant growth, we could detect changes in soil microbial community composition already after 2 yr of plant growth. Hence, our study emphasizes the importance of temporal buildup of plant-derived resources in the soil as a key determinant of soil microbial structure and function.

Effects of plant growth duration on nematodes

Besides soil microorganisms, nematodes are another important component of the soil community and they can have substantial effects on soil ecosystem processes (Yeates 2003, De Deyn et al. 2004). Overall, we found that the total abundance of nematodes and specifically the abundance of bacterial-feeding nematodes increased with the duration of plant growth. Further, we observed that plant-feeding nematodes increased over time in grass-dominated plant communities, showing that plant community-driven differences in nematode communities increase over time (Scherber et al. 2010, Sohlenius et al. 2011). These effects are likely to be driven by differences in resource quality (Wardle et al. 2004, De Long et al. 2016, Veen et al. 2017). Grasses usually provide higher quality root resources (Lavorel and Garnier 2002) and therefore may be more attractive to plant-feeding nematodes (De Deyn et al. 2004). This may explain why plant-feeding nematodes accumulated in grass-dominated plant communities over time in our experiment.

In summary, the results of the present study indicate that temporal dynamics play a crucial role in the establishment of plant community and plant functional group composition effects on abiotic and biotic soil properties (Bardgett et al. 2005, Thakur et al. 2015). Functionally dissimilar plant communities (fast- vs. slow-growing and grasses vs. forbs) form distinct soil microbial communities under field conditions whereas the composition of these communities also depends on the duration of plant growth. Extended plant growth for more growth seasons may result in more substantial effects of the plant communities on soil nutrient cycling and soil organisms due to the slow accumulation of plant-derived resources in the soil (Eisenhauer et al. 2012, Kuzyakov and Xu 2013). These time lags in changes in plant community composition on belowground processes and functions should be considered in future studies. Studying the effects and mechanisms of plant community composition and functional groups on abiotic and biotic soil properties under field conditions remains challenging but is essential for a comprehensive picture of the aboveground–belowground linkages between plant communities, soil communities, and soil functions.

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Data Availability Statement

The sequencing data are stored in European Nucleotide Archive and can be found under accession number PRJEB31856.

Supporting Information

Additional Supporting Information may be found online at: http://onlinelibrary.wiley.com/doi/10.1002/ecs2.3246/full