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Influence of plant traits, soil microbial properties, and abiotic parameters on nitrogen turnover of grassland ecosystems

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Abstract. Although it is known that multiple interactions among plant functional traits, microbial properties, and abiotic soil parameters influence the nutrient turnover, the relative contribution of each of these groups of variables is poorly understood. We manipulated grassland plant functional composition and soil nitrogen (N) availability in a multisite mesocosm experiment to quantify their relative effects on soil N turnover. Overall, root traits, arbuscular mycorrhizal colonization, denitrification potential, as well as N availability and water availability, best explained the variation in measured ecosystem properties, especially the trade-off between nutrient sequestration and plant biomass production. Their relative contributions varied with soil N availability. In relatively N-poor soils (10–20 $\mu\text{g}\cdot\text{N}\cdot\text{g}^{-1}$ soil), N turnover was mainly controlled by microbial properties and abiotic soil parameters, whereas in the relatively N-rich soils (110–120 $\mu\text{g}\cdot\text{N}\cdot\text{g}^{-1}$ soil), N turnover was mainly controlled by plant traits and microbial properties. This experiment is a strong demonstration of the importance of functional characteristics of both plants and soil microbes, and their interplay with soil N availability, for N turnover in grassland soils.

Key words: ammonia-oxidizing archaea and bacteria; arbuscular mycorrhizal colonization; ecosystem properties; grasslands; leaf traits; nitrite oxidizers; nitrite reducers; nutrient availability; root traits; water availability.

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INTRODUCTION

Changes in land use and land management practices can radically modify the functioning of terrestrial ecosystems (Zeller et al. 2000, Schmitt

et al. 2010, Schulze et al. 2010) by simultaneously altering soil abiotic conditions, but also biotic factors such as plant community composition and the structure and functioning of soil biota (Sala et al. 2000, Laliberte and Tylianakis 2012).

However, most studies to date on management impacts on ecosystem processes have focused on the individual effects of these different variables (Wardle et al. 2004, Van der Putten et al. 2009), and as a result, our understanding of the contributions of each group of biotic or abiotic variables, and of their interactive impacts, is limited (Le Roux et al. 2013). This represents an important gap in knowledge given the need for an improved understanding of the mechanisms by which changes in land use and management intensity impact the ecosystem functioning and the goods and services that it underpins (Lavorel 2013).

In addition to the well-documented direct effects of abiotic soil parameters on nutrient cycling (Qian and Cai 2007), there is increasing evidence that plant functional traits (plant traits) (Denef et al. 2009, Laughlin 2011, Legay et al. 2014) and microbial community structure (Krause et al. 2014) can mediate strong indirect effects on processes of soil nutrient cycling (De Vries et al. 2015, De Vries and Bardgett 2016). For instance, both aboveground and belowground biomass productions have been related to the availabilities of nutrients and water in soil (Hawkins et al. 2003), which are directly related to plant traits (Mokany et al. 2008, Lavorel et al. 2011), and to microbial community composition, such as the proportion of fungi to bacteria in soil (van der Heijden et al. 2008). Most studies that have explored the effects of plant traits or microbial properties on ecosystem properties have considered these aboveground and belowground variables separately. However, the contribution of plant traits and microbial properties to ecosystem properties related to the nutrient turnover can be captured only if both groups of variables are considered simultaneously. For instance, Orwin et al. (2010) showed that the relative abundance of fungi and bacteria and the measures of soil N cycling were related to leaf and root tissue quality across a range of grassland plant species, which reflect the leaf litter quality (Aerts 1999) and the amount and quality of root exudates (Jones et al. 2004), respectively. Furthermore, recent studies have shown relationships between plant traits, microbial properties, and abiotic soil parameters at the landscape scale (Grigulis et al. 2013, Legay et al. 2014, Manning et al. 2015). De Vries et al. (2012) showed across a wide range of English

grasslands that while fungi-dominated belowground communities were associated with plant traits disclosing a conservative strategy (low specific leaf area [SLA] and leaf N content [LNC]), bacteria-dominated communities were associated with exploitative plant traits (high SLA and LNC).

The aforementioned studies reveal the existence of relationships between plant traits, soil microbial properties, and nutrient turnover. However, they were carried out in field conditions, which limit the possibility of experimentally disentangling the relative roles of plant traits, soil microbial properties, and abiotic parameters, as well as their interactions, in driving the nutrient turnover. To address this gap, and identify the relative contributions of each of these three groups of variables to ecosystem properties, we designed a common garden experiment whereby we manipulated grassland plant community composition and soil nutrient availability across three European locations with contrasting climatic conditions and different locally abundant plant species and soils. This allowed us to experimentally quantify the relative influence of plant traits (aboveground and belowground), microbial properties, and abiotic soil parameters on a range of ecosystem properties associated with carbon (C) and N turnover. We hypothesized that (1) in addition to the well-known effects of abiotic soil parameters, microbial properties contribute significantly to the nutrient turnover, especially in nutrient-poor soils, and (2) high N availability (e.g., in a more nutrient-rich site or after the fertilizer application) accelerates the rates of nutrient mineralization and fodder production and increases the contribution of plant traits to the variation in ecosystem properties. We also propose that, in the same way that some plant traits (LNC and SLA) are considered as markers for plant nutrient economics at the levels of individual plants (Freschet et al. 2010) and communities (Perez-Ramos et al. 2012), key plant traits, microbial properties, and abiotic soil parameters can be used as markers of nutrient turnover at the ecosystem level. Based on our previous work (Grigulis et al. 2013, Legay et al. 2014, 2016), we expect root C/N ratio, denitrification enzymatic activities, and soil N to be good markers of soil nutrient turnover across our experimental systems.

MATERIALS AND METHODS

Study sites and plant species

A common garden experimental design was set up at three sites across Europe: the Lautaret Pass (French Alps, 45°02' N, 6°20' E), the Stubai Valley (Austrian Alps, 47°7' N, 11°18' E), and Hazelrigg Field Station, Lancaster University (UK, 54°1' N, 2°46' W), to provide a range of climatic conditions typical of western European upland grasslands. At each site, artificial plant communities of constant species richness were assembled to produce a gradient of community-level trait variation. This experimental design was used to study the effect of varying species evenness and avoids the potential confounding effects of species richness on ecosystem functioning. Plant communities were constructed in mesocosms using four locally abundant grassland species, namely two forbs and two grasses; for both grasses and forbs, one species was selected to represent a more exploitative strategy (higher specific leaf area [SLA], leaf N concentration [LNC], and lower leaf dry matter content [LDMC]) and a more conservative strategy, respectively. The two exploitative species *Dactylis glomerata* (L.) and *Geranium sylvaticum* (L.) were common across the three sites, as was the conservative forb, *Achillea millefolium* (L.). In contrast, the conservative grass species were chosen to be representative of extensively managed grasslands at each site, with *Nardus stricta* (L.), *Festuca paniculata* (L.), and *Anthoxanthum odoratum* (L.) being selected for the Austrian, French, and British sites, respectively. The four species were combined within each site to represent a broad range of community-weighted SLA, LNC, and LDMC.

Experimental design

At the French and the British sites, eight planting schemes each with 36 individual plants, but different relative abundance of the four plant species, were combined with two levels of fertility and replicated four times in a randomized block design (Appendix S1). At the Austrian site, because of high rates of plant mortality, only five planting schemes for a total of 40 mesocosms were used (Appendix S2). Except for one of the planting schemes in Austria, where species were planted in even proportions (4 sp. × 9

individuals), all planting schemes were representative of field proportions of grasses and forbs commonly found in upland grasslands, namely 60–80% for grasses and 20–40% for forbs (Gross et al. 2007). We aimed to produce a continuous gradient from plant communities dominated by conservative species to communities dominated by exploitative species (Appendix S2). The experiment ran for the two growing seasons of 2010 and 2011.

Plants were collected during summer 2009 from grasslands near each experimental site. Mother plants were separated into 3 cm long pieces of rhizome for *A. millefolium*, one rosette with two leaves for *G. sylvaticum*, or one to two tillers for the two grasses, then grown in garden or glasshouse conditions for 4–6 weeks. To limit the transfer of soil microbes with plants, the roots were carefully washed after field collection and then grown in a standardized loam sand mixture in order to homogenize the microbial communities among plant individuals. Following this, the plants were planted in mesocosms (40 cm deep, 45 cm diameter) filled at the bottom with 5 cm of quartz gravel (6–10 mm diameter) and topped with 30 cm of homogenized soil in autumn 2009. The soil used at each site was excavated (0–20 cm) from a nearby grassland with soil of low N availability (Grigulis et al. 2013); soil inorganic N concentrations ranged from 11.7 $\mu\text{g}\cdot\text{N}\cdot\text{g}^{-1}$ dry soil at the Austrian site (Dystric Cambisol soil with 31% sand, 23% clay, and 46% lime), to 14.5 $\mu\text{g}\cdot\text{N}\cdot\text{g}^{-1}$ dry soil at the French site (Brown Earth with 23% sand, 30% clay, and 47% lime), to 23.9 μg inorganic $\text{N}\cdot\text{g}^{-1}$ dry soil at the British site (Brown Earth soil with 48% sand, 11% clay, and 41% lime excavated nearby Hazelrigg Field Station). Half of the mesocosms received 100 $\text{kg}\cdot\text{N}\cdot\text{ha}^{-1}$ (37–42 $\text{mg}\cdot\text{N}\cdot\text{kg}^{-1}$ dry soil) in the form of a urea-based slow-release N:P:K fertilizer (14:13:13, Osmocote), applied immediately after the initiation of growth in spring 2010 and 2011. Weeds were manually removed at the beginning and throughout the two growing seasons. Plants in all mesocosms were cut to 5 cm shoot height in summer 2010, consistent with agricultural practices of each site; the particular date at each site depended on phenology and normal hay harvest date in the different regions (e.g., mid-/late July at Lancaster, late July at Stubai, and early August at Lautaret).

Harvest and plant trait measurements

At peak vegetation biomass, which occurred at the end of June, early July, and end of July 2011 for the British, French, and Austrian sites, respectively, the plants were harvested and four soil cores were taken per mesocosm forming a square around the center of the mesocosm (4.5 cm diameter, 10 cm deep). Soil cores from each mesocosm were pooled and passed through a 5.6-mm sieve, and root mass was collected for biomass estimation. One extra core (205 cm³) at the center of the mesocosm was sampled to measure the soil bulk density and the soil water availability. Prior to the vegetation harvest, plant traits were measured.

Aboveground plant traits (LNC, LCC, LDMC, and height) were measured using standardized protocols (Cornelissen et al. 2003). Depending on the number of individuals, three to eight replicate samples were taken for each species per mesocosm. Because surface area of the dissected leaves of *A. millefolium* could not be measured with satisfactory precision, SLA was not used in this study. During the harvest, aboveground biomass was sorted to the individual species to determine the dry weight of each species within the realized communities. Community-weighted mean traits (CWM; Garnier et al. 2004) and functional divergence (FD; Mason et al. 2003) were calculated following Casanoves et al. (2011). The roots were carefully washed in tepid water to allow the separation of roots by floatation, placed into an ethanol–acetic acid solution (ethanol 10%, acetic acid 5% v:v), and stored at 4°C. Root length and diameter were measured by suspension in 1 cm of demineralized water in a clear acrylic tray and scanned at 300 dpi with an Epson Expression 10000XL flatbed scanner (Long Beach, California, USA). Digital root images were processed using WINRHIZO software (Regent Instruments, Sainte-Foy-Sillery-Cap-Rouge, Canada). Then, the roots were weighed, dried at 70°C, and reweighed to calculate root dry matter content (RDMC) and specific root length (SRL). Finally, dry roots and leaves were ground to a fine powder (5 µm diameter) for the analysis of N and C using an elemental analyzer (FlashEA 1112: Fisher Scientific, Waltham, Massachusetts, USA, for France and Austria; Vario EL III: Elementar Analysensysteme, Hanau, Germany, for England). Because these root trait measurements were obtained from the soil cores with mixed

plant roots, they were considered as community-weighted means.

Abiotic soil parameters

Fresh sieved soil subsamples were weighed and stored at –20°C (for the quantification of marker genes) or at 4°C (for microbial activities and for soil chemical analysis). Soil subsamples were air-dried and ground to measure the total soil C and N, as above. Bulk density and soil porosity were obtained by measuring the dry mass of a fixed volume (205 cm³). Prior to drying, 100 mL of distilled water was added to saturate each soil core, allowing for the calculation of water-filled pore space (WFPS) (Robertson et al. 1999). In situ soil inorganic N sorption was measured using ion-exchange resin bags inserted in the center of each mesocosm (10–15 cm deep at a 45° angle) for 6 weeks prior to the final harvest (Robertson et al. 1999). Resin bags were made using nylon bags (10 × 5 or 5 × 5 cm) containing 5 g of mixed anion–cation-exchange resin (Amberlite IRN150; VWR International S.A.S., Fontenay-sous-Bois, France). Soil nutrients (ammonium [NH₄⁺-N], nitrate [NO₃⁻-N]) were measured from 0.5 M K₂SO₄ soil extracts, analyzed using a FS-IV colorimetric chain (OI-Analytical, College Station, Texas, USA).

Soil microbial properties

Potential rates of nitrification were estimated following Dassonville et al. (2011). Abundance of nitrifiers (ammonia-oxidizing archaea [AOA] and ammonia-oxidizing bacteria [AOB]) as well as nitrite oxidizers (*Nitrospira* sp. [NIP] and *Nitrobacter* sp. [NIB]) was measured based on the gene copy numbers in the soil of corresponding marker genes, which were (1) the ammonium mono-oxygenase gene *amoA* for archaea and bacteria (*amoA*-AOA *amoA*-AOB), (2) the 16S ribosomal RNA gene of NIP, and (3) the nitrite-oxido-reductase gene (*nrxA*) of NIB (Brankatschk et al. 2011).

Potential denitrification activity (DEA) was determined according to Attard et al. (2011), and abundance of denitrifiers was quantified based on the abundance of the nitrite reductase genes (*nirK* and *nirS*).

Fungal and bacterial biomasses were measured using phospholipid fatty acid (PLFA) analysis (Bardgett et al. 1996). Total PLFA was used as a

measure of active microbial biomass, and the fungal-to-bacterial PLFA ratio was calculated by dividing the fungal PLFA marker (18:2 ω 6) by the summed bacterial PLFA markers (i15:0, a15:0, 15:0, i16:0, 16:1 ω 7, a17:0, i17:0, cy17:0, cis18:1 ω 7, and cy19:0) (Bardgett et al. 1996). The intensity of mycorrhizal colonization (Mycor), which gives an estimation of the amount of colonized root cortex in the whole root system, was evaluated according to the method by Trouvelot et al. (1986) using the MYCOCALC program (<http://www.dijon.inra.fr/mychintec/Mycocalc-prg/download.html>).

Ecosystem properties

We selected seven variables as ecosystem properties likely driven jointly by abiotic soil parameters, microbial properties as well as plant functional traits. Total aboveground plant biomass (ABM) and belowground plant biomass (BBM) and plant digestibility were selected because they reflected the total plant biomass production and quality of the ecosystem. Soil organic matter content (SOM), potential leaching of NO₃⁻-N, and microbial biomass N (MBN) were retained to represent the abilities of the ecosystem to retain C (SOM) and N (MBN) or to lose N (leaching of NO₃⁻-N). Finally, in the context of this study, potential N mineralization (PNM) was retained as an ecosystem property rather than a microbial property because it reflects the ability of the ecosystem to mobilize soil organic matter and supply mineral N.

Total ABM and RM were measured as described earlier. Plant digestibility was determined on aboveground plant subsamples using near infrared reflectance spectroscopy analysis (Antaris II FT-NIR analyzer; Thermo Electron Scientific Instruments, Madison, Wisconsin, USA). SOM was measured by loss on ignition. Potential leaching of NO₃⁻-N was measured from the percolate of the central core leached with a given volume of distilled water. PNM rates were estimated using anaerobic incubations of fresh soil subsamples (dark, 7 d, 40°C), during which organic N was mineralized and accumulated as NH₄⁺-N (Wienhold 2007). The difference between NH₄⁺ content before (*t*1) and after the incubation (*t*2) gave PNM = [(NH₄⁺-N)*t*2 - (NH₄⁺-N)*t*1]/soil dry weight/7 d. Finally, soil microbial biomass N was measured using the chloroform fumigation extraction technique (Vance et al. 1987).

Data analyses

The effects of site and fertilization as well as their interactions on plant traits, microbial properties, abiotic soil parameters, and ecosystem properties (EP) were tested using analyses of variance (ANOVAs). We used post hoc Tukey's tests to examine a posteriori differences among the sites and the fertilization treatments. Where necessary, data were transformed to better comply with the criteria of normality and homoscedasticity, or when this was not possible, the nonparametric Kruskal–Wallis test was applied.

To identify the significant sources of variation in EP across the three sites and quantify the respective contributions of plant traits, microbial properties, and abiotic soil parameters to variation in each measured ecosystem property individually, we used linear mixed models with restricted maximum-likelihood (REML) estimates. Prior to REML analysis, the number of explanatory variables was reduced by checking for and removing highly correlated variables. Site was specified as a random effect to remove the variation associated with differences between the sites, while the fixed effects tested were all plant traits, microbial properties, and abiotic soil parameters retained after the variable number reduction. The selection of the most parsimonious model was carried out as described in Diaz et al. (2007). Final combined models reported the variance explained by sites and once this variance was accounted for, the variance explained by plant traits, microbial properties, and abiotic soil parameters.

To quantify the relative contribution of plant traits, microbial properties, and abiotic soil parameters to EP, we applied multitable analyses for each individual site using four data tables with all samples of the site as rows. After an initial step removing highly correlated variables, the relationships between EP and the three other data sets (plant traits as well as microbial properties and abiotic soil parameters) were measured using the R_v coefficient (vectorial correlation coefficient; Robert and Escoufier 1976). The significance of the R_v coefficient was tested by randomly permuting (1000 times) rows within the tables. The statistical significance was determined from the proportion of null values that were greater than the observed R_v coefficients. The relative importance of each explanatory data set (plant traits as well as microbial properties

and abiotic soil parameters) in determining the EP data set was tested as described in Foulquier et al. (2011). Multiple co-inertia analysis (MCOA) was used to provide an ordination of samples along the variables included in the analysis (Chessel and Hanafi 1996) and to summarize in a common structure the information shared by the four data sets.

All statistical analyses were performed with the software R version 3.2.2, using the *ade4* (Dray et al. 2007), *nlme* (Pinheiro et al. 2016), and *vegan* (Oksanen et al. 2008) packages.

RESULTS

Over two growing seasons, plant traits, microbial properties, and abiotic soil parameters, as well as measured ecosystem at each site, cover the range of values typically found in the field (Grigulis et al. 2013, Legay et al. 2014). With the exception of two microbial properties, namely the abundance of archaeal ammonia oxidizers and kinetic parameters of potential nitrification, all variables studied were influenced by site (Tables 1 and 2). Fertilization significantly influenced 21 of 36 variables studied (Tables 1 and 2). Individual plant communities deviated from initial plantings in terms of relative abundance of component species, although plant species richness remained unchanged. In all cases, these deviations were the result of the high relative success of propagation of one of the four plant species (e.g., *Dactylis glomerata* in Austria and *Achillea millefolium* in France). However, the treatments still provided a gradient of plant traits community-weighted means and functional divergences (Appendices S6, S7, and S8), with CWM values of plant traits covering the range of values found in the field (Grigulis et al. 2013, Legay et al. 2014).

Contribution of plant functional traits, microbial properties, and abiotic soil parameters to the variation in individual ecosystem properties

The REML analyses revealed that the overall amount of variation explained by site ranged from 2% (N leaching) to 62% (SOM), and once the site variation was removed, plant traits, microbial properties, and abiotic soil parameters explained between 3% (N leaching) and 42% (SOM) of variation in EP (Table 3). The percentage of variance

explained by site, and then by selected variables, differed among EP: It was low for N leaching (from 2% to 6%), showed strong variations for SOM (from 13% to 62%), and was relatively consistent for belowground biomass (from 15% to 34%) (Table 3). Fertilization affected the percentage of variation explained by site and selected variables, but no consistent pattern was found among EP, regardless of sites and fertilization treatments (Table 3). The proportion of variation explained by each of plant traits, microbial properties, and abiotic soil parameters (once the site variation was removed) also differed among EP. Microbial properties explained between 10% and 75% of the variation mostly for belowground EP (five of six cases) (Table 3). Also, abiotic soil parameters explained the variation for different belowground EP, between 19% and 100% of the six of 10 cases (Table 3). Plant traits explained between 36% and 100% of the variation for different mostly aboveground EP (five of nine cases) (Table 3). Overall, for our 14 models (seven ecosystem properties and two fertilization treatments), we identified five variables that were retained at least in three models. Soil porosity, DEA, and LDMC were retained three times, while root C/N ratio and total soil N were retained four and five times, respectively (Table 3).

To explore the influence of soil N availability on the contribution of each group of selected variables (plant traits, microbial properties, and abiotic soil parameters) to the variation in EP, we averaged the proportion of variation explained by each group for all EP. These averaged proportions were plotted on N fertility axes based on the mean of total dissolved N concentration of all unfertilized and fertilized mesocosms for each site (Fig. 1). Overall, the contribution of each group of selected variables ranged from 18% to 48% and showed nonlinear behavior. This representation showed that the contribution of plant traits increased with soil N availability, whereas the contribution of microbial properties showed an inverse bell-shaped response and abiotic soil parameters a bell-shaped response (Fig. 1).

Contribution of plant functional traits, microbial properties, and abiotic soil parameters to the variation in all ecosystem properties

The site-specific approach using MCOA and multitable analysis was carried out to better

Table 1. Range of values for the plant functional traits, microbial properties, abiotic soil parameters, and ecosystem properties with the effects of sites, fertilization, and their interactions.

Characteristics	Austria	France	England
Plant functional traits			
Vegetative height (cm)	10.7–27.5	14.8–28.2	9.7–20.4
Leaf dry matter content (mg·dry mass·g ⁻¹ fresh mass)	0.30–0.34	0.18–0.31	0.22–0.33
Leaf carbon content (mg·C·g ⁻¹ dry mass)	427–462	409–498	425–448
Leaf nitrogen content (mg·N·g ⁻¹ dry mass)	12.4–21.8	17.6–28.6	13.9–19.9
Root dry matter content (mg·dry mass·g ⁻¹ fresh mass)	0.06–0.36	0.13–0.26	0.15–0.52
Root carbon content (mg·C·g ⁻¹ dry mass)	362.8–457.9	405.9–484.7	311.5–448.1
Root nitrogen content (mg·N·g ⁻¹ dry mass)	5.96–12.09	3.76–12.04	3.96–11.46
Root carbon/nitrogen ratio	36.9–67.9	37.9–117.41	36.9–79.6
Root diameter (mm)	0.17–0.36	0.44–0.74	0.19–0.52
Specific root length (m of root/g ⁻¹ dry mass)	51.4–218.9	39.6–126.01	33.6–225.9
Microbial functional traits			
Abundance of ammonia-oxidizing archaea	855 × 10 ³ –50 × 10 ⁶	47 × 10 ³ –35 × 10 ⁶	470 × 10 ³ –25 × 10 ⁶
Abundance of ammonia-oxidizing bacteria	1.1 × 10 ⁶ –8.7 × 10 ⁶	257 × 10 ³ –28 × 10 ⁶	2.3 × 10 ⁶ –14 × 10 ⁶
Abundance of nitrite oxidizers— <i>Nitrobacter</i>	5.4 × 10 ³ –896 × 10 ⁶	15 × 10 ³ –4.5 × 10 ⁶	1.2 × 10 ⁶ –13 × 10 ⁶
Abundance of nitrite oxidizers— <i>Nitrospira</i>	117 × 10 ⁶ –460 × 10 ⁸	330 × 10 ³ –194 × 10 ⁶	31 × 10 ⁶ –392 × 10 ⁸
Kinetic parameters of potential nitrification— K_m (mg·N-NH ₄ ⁺ ·mL ⁻¹)	0.02–3.51	0.02–9.50	0.01–2.29
Abundance of nitrite reductase gene— <i>nirS</i>	626 × 10 ³ –6.3 × 10 ⁶	25 × 10 ³ –1.1 × 10 ⁶	207 × 10 ³ –9.5 × 10 ⁶
Abundance of nitrite reductase gene— <i>nirK</i>	13.1 × 10 ⁶ –148 × 10 ⁶	70 × 10 ³ –19 × 10 ⁶	7.3 × 10 ⁶ –73 × 10 ⁶
Potential denitrification enzyme activity (mg·N-N ₂ O·g ⁻¹ ·dry soil·h ⁻¹)	0.42–1.25	0.13–0.81	1.41–2.30
Fungal-to-bacterial PLFA ratio	0.10–0.19	0.17–0.45	0.16–0.24
Intensity of mycorrhizal colonization (%)	0.33–21.7	0–25.9	0–22.1
Soil properties			
Total soil porosity (%)	73.8–84.9	61.4–86.0	89.3–93.5
Water-filled pore space (%)	15.0–60.4	6.5–32.0	6.3–9.7
Soil nitrogen (mg·N·g ⁻¹ dry soil)	1.90–4.58	1.28–4.12	2.25–2.83
Soil carbon (mg·C·g ⁻¹ dry soil)	23.2–52.4	11.9–38.4	25.2–32.5
Soil carbon/nitrogen ratio	9.55–21.92	8.83–13.16	10.99–12.77
Soil nitrate (mg·N-NO ₃ ⁻ ·g ⁻¹ dry soil)	0.69–57.61	0.04–59.35	0.03–55.39
Soil ammonium (mg·N-NH ₄ ⁺ ·g ⁻¹ dry soil)	1.40–11.00	0.92–85.58	0.29–96.43
Soil ammonium/nitrate ratio	0.07–8.89	1.34–66.22	0.66–87.40
In situ nitrate absorbed in resin (mg·N-NO ₃ ⁻ ·g ⁻¹ ·resin·d ⁻¹)	0.10–2.85	0.07–24.63	0.00–0.74
Ecosystem properties			
Leached nitrate (mg·N-NO ₃ ⁻ ·g ⁻¹ dry soil)	0.06–11.26	0.02–23.06	0.01–23.76
Soil organic matter (%)	6.9–11.0	3.7–11.1	7.8–9.4
Microbial biomass N (mg·N·g ⁻¹ dry soil)	22.9–79.5	2.3–174.9	0.2–86.3
Potential N mineralization (μg·N-NH ₄ ⁺ ·g ⁻¹ ·dry soil·d ⁻¹)	3.42–12.01	0.35–11.34	3.42–27.70
Plant aboveground biomass (g/m ²)	48.6–521	75.2–2312	214.5–539
Plant belowground biomass (mg/g ⁻¹ dry soil)	0.46–5.49	1.24–4.89	0.25–3.23
Plant digestibility (%)	48.9–72.7	50.0–66.5	52.4–69.9

Note: The abundance of bacterial groups is expressed in the number of gene copies per gram of dry soil.

understand the influence of soil N availability on the contribution of each group of variables to the variation in EP. The ordination of EP provided by MCOA (plant digestibility, aboveground plant biomass (ABM), belowground plant biomass (BBM), microbial biomass N, SOM, PNM, and N leaching) was inconsistent across the

three sites and fertilization treatments (Appendices S3, S4, and S5). However, the contribution of the different properties to the first two MCOA axes showed a switch from soil nutrient sequestration (microbial biomass N and soil organic matter) to plant biomass production when soil N availability increased (e.g., due to the original

Table 2. Effect of sites, fertilization, and their interactions on plant functional traits, microbial properties, abiotic soil parameters, and ecosystem properties.

Characteristics	Site		Fertilization		Site × Ferti.	
	F	P	F	P	F	P
Plant functional traits						
Vegetative height (cm)	77.59	***	179.7	***	13.92	***
Leaf dry matter content (mg·dry mass·g ⁻¹ fresh mass)	226.1	***	43.54	***	6.57	**
Leaf carbon content (mg·C·g ⁻¹ dry mass)	33.49	***	17.08	***	7.83	***
Leaf nitrogen content (mg·N·g ⁻¹ dry mass)	123.9	***	91.44	***	16.51	***
Root dry matter content (mg·dry mass·g ⁻¹ fresh mass)	8.61	***	3.38	.	0.22	ns
Root carbon content (mg·C·g ⁻¹ dry mass)	38.87	***	0.22	ns	0.66	ns
Root nitrogen content (mg·N·g ⁻¹ dry mass)	12.83	***	23.44	***	3.06	*
Root carbon/nitrogen ratio	24.44	***	23.95	***	2.57	.
Root diameter (mm)	444.8	***	1.33	ns	0.06	ns
Specific root length (m of root/g ⁻¹ dry mass)	36.08	***	2.03	ns	2.29	ns
Microbial functional traits						
Abundance of ammonia-oxidizing archaea (no. gene copies/g ⁻¹ dry soil)	2.01	ns	3.22	ns	2.96	.
Abundance of ammonia-oxidizing bacteria (no. gene copies/g ⁻¹ dry soil)	3.78	*	18.53	***	2.83	.
Abundance of nitrite oxidizers— <i>Nitrobacter</i> (no. gene copies/g ⁻¹ dry soil)	40.75	***	0.18	ns	0.19	ns
Abundance of nitrite oxidizers— <i>Nitrospira</i> (no. gene copies/g ⁻¹ dry soil)	138.04	***	0.02	ns	1.79	ns
Kinetic parameters of potential nitrification— K_m (mg·N-NH ₄ ⁺ ·mL ⁻¹)	0.14	ns	4.58	*	1.56	ns
Abundance of nitrite reductase gene— <i>nirS</i> (no. gene copies/g ⁻¹ dry soil)	278.9	***	0.07	ns	0.36	ns
Abundance of nitrite reductase gene— <i>nirK</i> (no. gene copies/g ⁻¹ dry soil)	139.45	***	0.89	ns	4.59	*
Potential denitrification enzyme activity (mg·N-N ₂ O·g ⁻¹ ·dry soil·h ⁻¹)	1,961	***	24.91	***	22.87	***
Fungal-to-bacterial PLFA ratio	275.2	***	19.92	***	4.22	*
Intensity of mycorrhizal colonization (%)	7.46	***	0.07	ns	0.15	ns
Soil properties						
Total soil porosity (%)	521.6	***	0.31	ns	1.08	ns
Water-filled pore space (%)	380.1	***	1.29	ns	4.32	*
Soil nitrogen (mg·N·g ⁻¹ dry soil)	112.3	***	0.02	ns	1.34	ns
Soil carbon (mg·C·g ⁻¹ dry soil)	206.5	***	1.05	ns	1.58	ns
Soil carbon/nitrogen ratio	35.06	***	4.98	*	0.64	ns
Soil nitrate (mg·N-NO ₃ ⁻ ·g ⁻¹ dry soil)	3.17	*	194.8	***	2.92	.
Soil ammonium (mg·N-NH ₄ ⁺ ·g ⁻¹ dry soil)	20.6	***	130	***	30.19	***
Soil ammonium/nitrate ratio	4.91	**	32.18	***	2.92	.
In situ nitrate absorbed in resin (mg·N-NO ₃ ⁻ ·g ⁻¹ ·resin·d ⁻¹)	7.51	***	9.46	**	3.42	*
Ecosystem properties						
Leached nitrate (mg·N-NO ₃ ⁻ ·g ⁻¹ dry soil)	6.74	**	74.18	***	6.75	**
Soil organic matter (%)	216.7	***	0.52	ns	0.49	ns
Microbial biomass N (mg·N·g ⁻¹ dry soil)	13.32	***	12.09	***	17.36	***
Potential N mineralization (μg·N-NH ₄ ⁺ ·g ⁻¹ ·dry soil·d ⁻¹)	104.5	***	9.02	**	1.25	ns
Plant aboveground biomass (g/m ²)	122.1	***	50.99	***	0.37	ns
Plant belowground biomass (mg/g ⁻¹ dry soil)	95.58	***	27.21	***	1.71	ns
Plant digestibility (%)	27.02	***	52.21	***	11.39	***

Note: Values are the results of ANOVAs or Kruskal–Wallis and significance (*** $P < 0.001$, ** $P < 0.01$, * $P < 0.05$, ns, not significant).

soil N availability (sites) and/or fertilization) (Fig. 2). Co-inertia analysis (CoiA) revealed that in unfertilized mesocosms, EP were significantly correlated with abiotic soil parameters, plant traits, and microbial properties across all sites, albeit with different strengths (Fig. 3).

All the three data sets (plant traits, microbial properties, and abiotic soil parameters) were

significantly linked to the EP data set ($R_v > 0.23$, simulated $P < 0.05$), and their relative contribution differed between the sites and according to the gradient of original N availability of unfertilized mesocosms. At the Austrian site, both abiotic soil parameters and microbial properties were better correlated ($R_{v[0.48]} = R_{v[0.42]}$, simulated $P = 0.278$) with EP compared with plant

Table 3. Fixed effects of plant functional trait, microbial properties, and abiotic soil parameters variables retained within the multiple variable REML models for each of the ecosystem properties; also presented is the percentage variation in each ecosystem property explained by the site, the retained fixed effects, the proportion of explanation afforded by the fixed effects due to microbial properties, abiotic soil parameters, and plant functional traits, respectively, and the significance (P) and magnitude of the standardized magnitude of the standardized effect for each of the retained fixed effects.

Ecosystem properties	Treat.	Response variable	% explained by sites	% variation explained after site was removed				P	Effects
				% explained by fixed effects	% of variation explained by microbes	% of variation explained by soil	% of variation explained by plants		
Microbial biomass N	No	Soil porosity	20.11	5.20	0.00	100.00	0.00	0.0013	0.073
		Soil total N						0.0013	1.085
	F	V_{\max} Soil $\text{NH}_4^+/\text{NO}_3^-$	10.06	7.11	74.53	25.49	0.00	0.0031	1.224
Soil organic matter	No	Soil total N	37.50	41.90	12.39	87.61	0.00	<0.001	1.502
		DEA						<0.001	1.626
	F	Soil total N	61.63	13.31	12.97	51.16	36.02	0.0004	1.026
		SRL DEA						0.0008 0.0166	-0.144 1.103
Potential nitrogen mineralization	No	Soil total N	37.39	15.21	22.25	77.78	0.00	0.0030	1.269
		NH_4^+ sorption DEA						0.0017 <0.001	1.041 3.190
	F	Root C Content	30.61	2.99	0.00	0.00	100.00	0.0074	0.062
Leaching of nitrate	No	LDMC	2.29	5.43	0.00	0.00	100.00	0.0036	-2.502
	F	K_m Soil total N	6.25	3.65	10.81	89.31	0.00	0.0096	3.360
Plant digestibility	No	Height	6.56	11.23	9.73	0.00	90.29	<0.001	-0.422
		LDMC						0.0099	-37.658
		F/B ratio						0.0006	-16.352
	F	NH_4^+ sorption Root C/N ratio	29.92	5.00	0.00	62.59	37.43	0.0202	0.249
Plant below-ground biomass	No	Soil porosity	29.05	17.00	0.00	58.90	41.36	0.0001	0.099
		Root C/N ratio RDMC						0.0003 0.0171	82.215 5.857
	F	Soil porosity	34.20	14.90	0.00	100.00	0.00	<0.001	0.113
Plant above-ground biomass	No	Root C/N ratio	35.70	10.41	0.00	0.00	100.00	0.0096	21,660.614
		LDMC						0.0152	-3188.816
	F	Height Root C/N ratio						0.0062 0.0001	26.845 34,473.540

Notes: Treat., treatment; F, fertilized; no, none; C, carbon; N, nitrogen; NO_3^- and NH_4^+ sorption, in situ nitrate and ammonium absorbed in resin; F/B ratio, fungal-to-bacterial PLFA ratio; DEA, potential denitrification enzyme activity; V_{\max} and K_m , kinetic response variables of potential nitrification; LDMC, leaf dry matter content; SRL, specific root length; RDMC, root dry matter content; height, vegetative height.

traits ($R_{v[0.48]} > R_{v[0.32]}$, simulated $P = 0.005$; $R_{v[0.42]} > R_{v[0.32]}$, simulated $P = 0.029$) (Fig. 3a). At the French site, abiotic soil parameters were better related to EP compared with microbial properties ($R_{v[0.59]} > R_{v[0.49]}$, simulated $P = 0.006$) and plant traits ($R_{v[0.49]} > R_{v[0.32]}$, simulated $P = 0.007$) (Fig. 3c). At the British site, which had the highest original soil N availability, plant traits were more strongly correlated with EP compared with abiotic soil parameters ($R_{v[0.33]} > R_{v[0.24]}$,

simulated $P = 0.035$) and microbial properties ($R_{v[0.33]} > R_{v[0.23]}$, simulated $P = 0.017$); abiotic soil parameters and microbial properties had similar levels of correlation with EP ($R_{v[0.24]} = R_{v[0.23]}$, simulated $P = 0.426$) (Fig. 3e). It should be noted that in most cases, plant traits, microbial properties, and abiotic soil parameters were not significantly related to each other. Relationships were only found between abiotic soil parameters and microbial properties data set at the Austrian ($R_v = 0.35$,

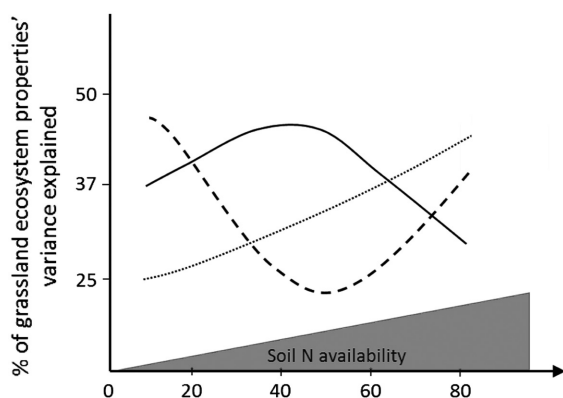


Fig. 1. Schematic diagram representing grassland plant functional traits (dotted line), microbial properties (dashed line), and abiotic soil parameters (solid line) contributions to the variation in a selection of ecosystem properties as a function of the total soil dissolved nitrogen concentration.

simulated $P < 0.008$) and French ($R_v = 0.54$, simulated $P < 0.001$) sites and between the abiotic soil parameters and plant traits data set at the French site ($R_v = 0.17$, simulated $P = 0.04$) (Fig. 3a, c). Across all the sites in unfertilized treatments, the first and second axes of the MCOA explained from 55.2% to 72.2% of the overall covariance between EP and the three other data sets (Appendices S3, S4, and S5). The selected variables at the three sites taken individually were more numerous and did not allow for the identification of generic driving variables in contrast to the REML approach. However, this co-inertia approach showed that the number of factors linked to abiotic soil parameters (such as soil N) and microbial properties (such as DEA or mycorrhization) contributing to the variance of EP decreased with increasing background soil N availability (from Austrian to British sites), whereas the number of factors related to plant traits increased (such as RDMC or SRL) (Appendices S3, S4, and S5).

Fertilization differently affected the relationships between the plant traits, microbial properties, and abiotic soil parameters and the EP across sites. Microbial properties at the Austrian site ($R_v = 0.30$, simulated $P < 0.06$) and abiotic soil parameters at the British site ($R_v = 0.13$, simulated $P < 0.42$) were no longer correlated with EP when compared with unfertilized soils. Furthermore, the relative contribution of the three data sets to the EP data set was also modified by fertilization

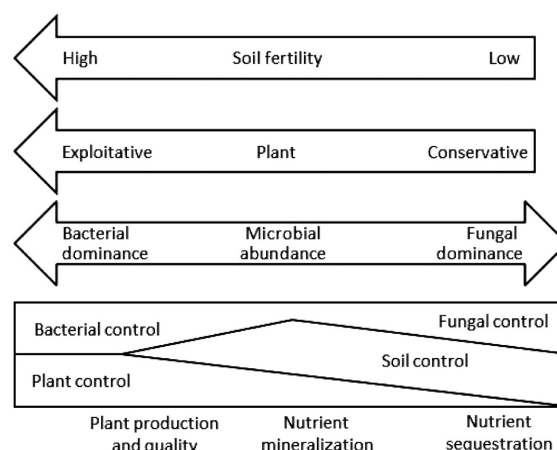


Fig. 2. Schematic overview of simultaneous switches from fungal and soil control to bacterial and plant control underpinning the variation in soil fertility, plant functional strategies, microbial functional composition and activities, and ecosystem properties. Adapted from Grigulis et al. (2013).

and differed between the sites (Fig. 3). At the Austrian site, EP were now related to plant traits together with abiotic soil parameters with similar strengths ($R_{v[0.34]} = R_{v[0.33]}$, simulated $P = 0.214$), but no longer with microbial properties (Fig. 3b). At the French site, EP were always related to all three explicative data sets, now with similar strengths ($R_{v[0.37]} = R_{v[0.35]}$, simulated $P = 0.399$; $R_{v[0.37]} = R_{v[0.33]}$, simulated $P = 0.172$; $R_{v[0.35]} = R_{v[0.33]}$, simulated $P = 0.253$) (Fig. 3d). At the British site, EP were again linked to plant traits and microbial properties, now with similar strengths ($R_{v[0.36]} = R_{v[0.28]}$, simulated $P = 0.079$), but were no longer related to abiotic soil parameters (Fig. 3f). Like in unfertilized mesocosms, few relationships between plant traits, microbial properties, and abiotic soil parameters were also found when fertilizer was added. Abiotic soil parameters and microbial properties were related to each other at the French site ($R_v = 0.22$, simulated $P < 0.008$), as well as plant traits and microbial properties at the British site ($R_v = 0.34$, simulated $P < 0.001$) (Fig. 3d, f). Fertilization did not substantially affect the degree of explanation provided by the first two axes of the MCOA across all sites. The first and second axes explained from 54.6% to 69.5% of the overall covariance with EP (Appendices S3, S4, and S5), and some changes were observed in the types of variables retained

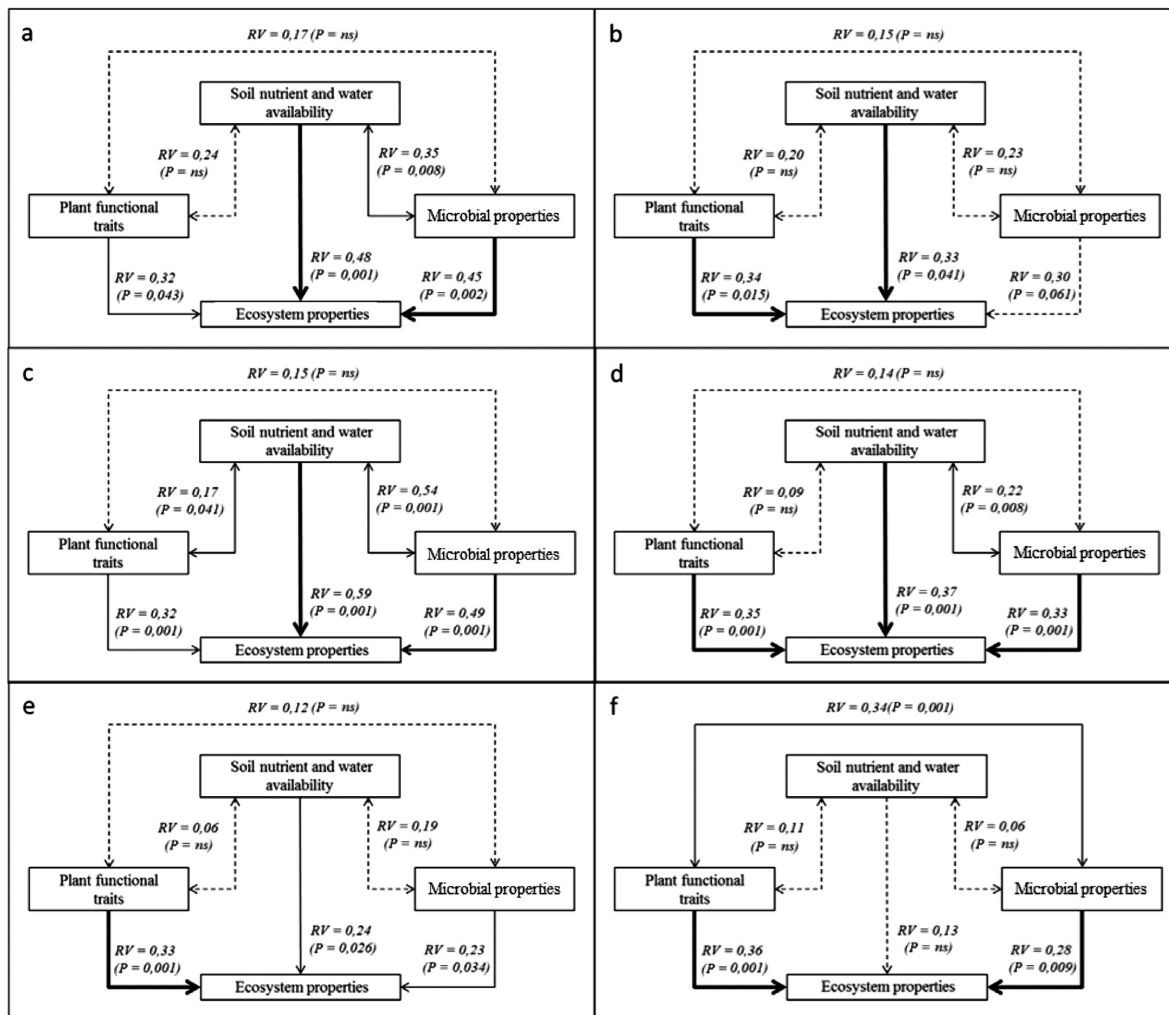


Fig. 3. Synthesis of the relationships between the studied plant functional traits, microbial properties, soil nitrogen and water availabilities, and ecosystem properties in unfertilized (left) or fertilized (right) mesocosms cultivated in Austria (a, b), France (c, d), and England (e, f). Each figure shows R_v coefficients with simulated P values (broken arrows represent nonsignificant correlation). The strength of the relationships between plant traits, abiotic soil parameters, and microbial properties on ecosystem properties is represented by the width of the arrows (plain lines represent a significant correlation, and bold lines represent the relationships explaining most of the variation in ecosystem properties).

within each data set at each site as compared to unfertilized mesocosms. Indeed, the number of factors linked to abiotic soil parameters contributing to variance of EP followed the same pattern and decreased with the background soil N availability of sites (from Austrian to British sites), whereas the number of factors linked to plant traits increased. In contrast to unfertilized mesocosms, the number of factors linked to microbial properties contributing to the variance of EP

increased with the soil N availability at the sites (Appendices S3, S4, and S5).

DISCUSSION

Contribution of plant traits, microbial properties, and abiotic soil parameters to ecosystem properties

Our experimental design aimed to disentangle the relative roles of plant traits, microbial properties, and abiotic soil parameters, as well as their

interactions, in driving ecosystem properties. Our results suggest that there is a switch from soil (microbial properties and abiotic soil parameters) to biotic (microbial properties and plant traits) factors controlling N turnover when soil N availability increases (Fig. 1). Consistent with our first hypothesis, we found that microbial properties contributed most strongly to the variation in a range of ecosystem properties related to nutrient sequestration in nutrient-poor soils, which broadly confirmed the results from previous field studies at the same three sites (Grigulis et al. 2013) (although inconsistencies were found for some ecosystem properties, such as PNM, which was better explained by microbial properties in N-rich soils than in N-poor soils). Also, consistent with our second hypothesis, we showed that plant traits best explained the variation in ecosystem properties, such as fodder production and quality, in N-rich soils, which again confirms previous field-based findings at the three sites (Grigulis et al. 2013, Legay et al. 2014). We showed the limited relationships (e.g., covariation) between abiotic soil parameters, plant traits, and microbial properties within and across all the sites, and we identified common variables associated with ecosystem nutrient turnover for plant traits (root C/N ratio and LDMC), microbial properties (Mycor and DEA), and abiotic soil parameters (soil porosity and total N).

These results support the generally accepted concept that abiotic soil parameters are major controls of N turnover in grasslands (Qian and Cai 2007, Sundqvist et al. 2011). However, our study also demonstrated that this paradigm only holds in N-limited grasslands, but not in grasslands of higher soil N availability. In fact, at the British site, where original soil inorganic N availability was highest, and after the significant fertilizer input at the less N-rich Austrian and the French sites, the contribution of biotic variables (together or individually) to ecosystem properties increased and was greater than abiotic soil parameters. These findings support the notion that plants and microbes strongly influence the ecosystem functioning in N-rich grasslands (De Deyn et al. 2009, Legay et al. 2014, De Vries et al. 2015). Moreover, the switch from abiotic soil parameters to biotic (microbial properties and plant traits) as the dominant controls of N turnover with fertilizer addition is slightly different with in situ

field observations at these sites, which showed a switch from microbial to plant control on the same ecosystem properties with increasing nutrient availability (Grigulis et al. 2013). Hence, collectively, our results suggest that there is a switch from belowground abiotic and biotic (abiotic soil parameters and microbial properties) to biotic ones (microbial properties and plants traits) controlling ecosystem properties related to N turnover when soil N availability increases (Fig. 1). Interestingly, our observations also suggest non-linear behaviors of the three groups of variables. First, the contribution of abiotic soil properties, including soil moisture, to the variation in nutrient turnover showed a bell-shaped response and was nonsignificant only at the highest level of soil N availability, suggesting a stronger control at moderate N availability and high influence on nutrient mineralization in the ecosystem. Second, microbial effects on these EPs showed an inverse bell-shaped response to N availability, resulting in a switch among biotic variables from predominantly microbial to predominantly plant functional effects at moderate N availability. Under high N availability, plant functional traits became a major driving variable along with microbial properties to processes of soil N turnover, and completely excluded the contribution of abiotic soil parameters (Figs. 1 and 2).

Plant and microbial control on N turnover

The switch from microbial properties to plant traits as the dominant determinants of EP at higher levels of soil N availability might be related to a change in microbe–plant competition for N. At low levels of N availability, it is likely that the microbial community competes more effectively than plants for N resources (Bardgett et al. 2003, Kuzyakov and Xu 2013, Legay et al. 2013, Thebault et al. 2014), thereby possibly explaining their dominant role in the variation of N-related EPs at lower levels of N. Denitrifiers could be a major control of EPs under nutrient-poor conditions because they compete with plant communities for nitrate acquisition under oxygen-limited conditions either at the microsite level or after rain events (Le Roux et al. 2013), and influence the rate of N losses (Boyer et al. 2006). At the moderate levels of N availability, such as in unfertilized mesocosms at the British site and in fertilized mesocosms at the Austrian

site, the increase in plant influence on N turnover was likely related to higher rates of N cycling and microbial turnover favoring plant N acquisition (Bardgett et al. 2003). Moreover, roots could be the major contributor in the variation in N turnover under higher N availability. Indeed, through their ability to take up N, roots can influence the plant N content with cascading positive effects on digestibility and biomass production, microbial communities as well as N turnover (Mokany et al. 2008, Klumpp et al. 2009, Legay et al. 2014). At higher levels of N availability (i.e., fertilized mesocosms at French and British sites), the co-influence of microbial properties and plant traits on N turnover suggested that an excess in N availability limited competition.

Mechanisms of nitrogen turnover in grassland soils

We have identified a series of potential mechanisms explaining the contribution of plant traits, microbial properties, and abiotic soil parameters to the variation in the EP linked to N turnover. First, the contribution of abiotic soil parameters declined especially as N availability increased. Indeed, in nutrient-poor ecosystems, biomass production and PNM were strongly dependent on low nutrient turnover (Robson et al. 2007, Freschet et al. 2013). In ecosystems with moderate nutrient availability and faster N cycling, water availability and N availability were strong limiting abiotic variables (Gross et al. 2008, Attard et al. 2011) and drivers of key EP such as plant digestibility (Duru 2003, Dumont et al. 2015), biomass production (Hawkins et al. 2003, Chollet et al. 2014), or N mineralization (Parker and Schimel 2011). Finally, in nutrient-rich ecosystem, variation in EP was no longer dependent on N availability because a fast N turnover allowed for the accumulation and recycling of more N (Bardgett et al. 2014), but at the risk of high nutrient leaching (Qian and Cai 2007, Cameron et al. 2013).

Second, our results highlight the importance of belowground traits in understanding ecosystem functioning (Bardgett et al. 2014, Legay et al. 2014). Consistent with other studies, we showed that root traits were strongly related to ABM and RM (Mokany et al. 2008, Schumacher and Roscher 2009) and plant digestibility. Although contributions were weaker, root traits were also related to belowground EPs including nutrient cycling (Denef et al. 2009). This supports the

assumption that root traits (e.g., root C/N ratio, SRL, and RDMC) are closely related to many ecosystem processes (Klumpp et al. 2009). This also highlights the role of root functional traits as markers of EP in grasslands and more broadly as key determinants of the functioning of terrestrial ecosystems (Jackson et al. 2000, Legay et al. 2014, Bardgett et al. 2014).

Interestingly, the intensity of arbuscular mycorrhizal colonization as well as nitrification and denitrification parameters emerged as the main variables related to the variation in N turnover. Arbuscular mycorrhizas were always retained as drivers of EP except in fertilized mesocosms at the French site. This supports commonly accepted understanding of the key roles of arbuscular fungi in plant nutrient uptake influencing the primary productivity (van der Heijden et al. 1998, 2008) or digestibility, but also recent findings on the role of arbuscular fungi effects on nutrient cycling such as leaching (Bender et al. 2015). Denitrification was retained as generic driver for SOM and PNM and was related to MCOA, PNM, plant digestibility, and N leaching for any level of soil N availability. These results highlight the importance of denitrification for N availability in soil. On the one hand, the link between denitrification and PNM was probably due to the fact that many microbes that are able to mineralize nitrogen are also denitrifiers (Redondo-Nieto et al. 2013). The major negative relationships between N leaching and denitrification was easily attributable to the fact that nitrate is the substrate for denitrification. Hence, taken together, these results confirm that DEA is a relevant marker for N availability for plants in upland grassland soils.

CONCLUSION

Overall, the results of our cross-site study confirmed that ecosystem properties in perennial grasslands were related to abiotic soil parameters, plant functional traits, and microbial properties. We showed how their relative contributions switched along a gradient of soil N availability. In relatively N-poor soils, N turnover was mainly controlled by microbial properties and abiotic soil parameters, whereas in the relatively N-rich soils, N turnover was mainly controlled by microbial properties and plant functional traits.

Beyond confirming the overall patterns observed in the field, our experimental results provide mechanistic insights disentangling the role of abiotic and biotic parameters in the variation of ecosystem properties associated with N cycling. Finally, our results point toward the important role of root functional traits and arbuscular mycorrhizal colonization, supporting the growing view that they are key determinants of aboveground and belowground linkages and of the functioning of terrestrial ecosystems.

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