SPECIAL FEATURE

PLANT FUNCTIONAL EFFECTS ON ECOSYSTEM SERVICES

Relative contributions of plant traits and soil microbial properties to mountain grassland ecosystem services

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Summary

1. Plant functional diversity and soil microbial community composition are tightly coupled. Changes in these interactions may influence ecosystem functioning. Links between plant functional diversity, soil microbial communities and ecosystem functioning have been demonstrated in experiments using plant monocultures and mixtures, using broad plant and microbial functional groups, but have not been examined in diverse natural plant communities.

2. We quantified the relative effects of plant and microbial functional properties on key ecosystem functions. We measured plant functional diversity, soil microbial community composition and parameters associated with nitrogen (N) cycling and key nutrient cycling processes at three grassland sites in different parts of Europe. Because plant structure and function strongly influence soil microbial communities, we determined relationships between ecosystem properties, plant traits and soil community characteristics following a sequential approach in which plant traits were fitted first, followed by the additional effects of soil micro-organisms.

3. We identified a continuum from standing green biomass and standing litter, linked mostly with plant traits, to potential N mineralization and potential leaching of soil inorganic N, linked mostly with microbial properties. Plant and microbial functional parameters were equally important in explaining % organic matter content in soil. A parallel continuum ran from plant height, linked with above-ground biomass, to plant quality effects captured by the leaf economics spectrum, which were linked with the recycling of carbon (C) and N.

4. More exploitative species (higher specific leaf area, leaf N concentrations and lower leaf dry matter content) and taller swards, along with soil microbial communities dominated by bacteria, with rapid microbial activities, were linked with greater fodder production, but poor C and N retention. Conversely, dominance by conservative species (with opposite traits) and soil microbial communities dominated by fungi, and bacteria with slow activities, were usually linked with low production, but greater soil C storage and N retention.

5. *Synthesis* – Grassland production, C sequestration and soil N retention are jointly related to plant and microbial functional traits. Managing grasslands for selected, or multiple, ecosystem services will thus require a consideration of the joint effects of plant and soil communities. Further understanding of the mechanisms that link plant and microbial functional traits is essential to achieve this.

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Introduction

The hypothesis that plant traits responding to resource and disturbance gradients, especially those describing plant nutrient economics (*sensu* Freschet *et al.* 2010) and size, can be used as predictors of ecosystem functioning (Lavorel & Garnier 2002) has been supported by an increasing number of studies (e.g. Minden & Kleyer 2011; Pakeman 2011; Laliberté & Tylianakis 2012; Lavorel & Grigulis 2012). However, in accordance with theoretical developments on trait–function relationships (Chapin 1993; Reich, Walters & Ellsworth 1997; Garnier & Navas 2012), the majority of quantitative assessments of plant trait effects on ecosystem functioning have focused on above-ground primary productivity and decomposition and do not address how plant traits may influence carbon (C) and nitrogen (N) cycling through their impacts on soil communities (Eviner & Chapin 2003).

Such a 'black box' approach to ecosystem functioning ignores growing evidence that links between plants and soil microbial communities can act as drivers for a wide range of community properties and ecosystem processes (van der Heijden, Bardgett & Straalen 2008; Bardgett & Wardle 2010; Bever *et al.* 2010). It is now well established that individual plant species (Grayston *et al.* 1998; Wardle *et al.* 2003; Harrison & Bardgett 2010), and even different genotypes (Schweitzer *et al.* 2008; Weinert *et al.* 2010), can influence the diversity and activity of microbial communities in soil. Such impacts have been attributed to differences in the amount and quality of resources entering the below-ground compartment in the form of litter and root exudates (Bardgett & Wardle 2010). At the plant community level, it is also evident that changes in plant diversity and community structure can have a cascade effect on soil micro-organisms and their functioning, again through altering the amount and quality of resources entering the soil (e.g. Hedlund *et al.* 2003; De Deyn *et al.* 2004; Eisenhauer *et al.* 2010; De Deyn, Quirk & Bardgett 2011a). In turn, this can have consequences for ecosystem services, such as carbon storage and retention of nutrients in soil (e.g. Spehn *et al.* 2005; Fornara & Tilman 2008; Steinbeiss *et al.* 2008; De Deyn *et al.* 2009). However, our understanding of how plant traits influence C and N cycling through their impacts on soil microbial communities remains limited.

To bridge this gap in understanding, an increasing number of studies have sought to quantify the relationship between plant functional traits, soil microbial communities and the functions that they catalyse (De Deyn, Cornelissen & Bardgett 2008; Bardgett & Wardle 2010). For example, studies in temperate grasslands have shown that landscape-scale variation in soil microbial community composition can be explained by certain plant traits, along with other climatic and soil factors (De Vries *et al.* 2012), and that concomitant variations in leaf economics traits and the relative abundance of fungi and soil bacteria (F : B ratio) are associated with biomass quality and turnover (Pakeman 2011). Moreover, certain root traits have been shown to be strongly correlated with the F : B ratio, as well as the cycling of C (Klumpp *et al.* 2009) and soil nutrients (Orwin *et al.* 2010). Also, Laughlin (2011) demonstrated that in ponderosa pine (*Pinus ponderosa*) forest, understorey leaf economics traits contributed to explaining nitrification potential through litter quality. Despite this growing understanding, most detailed studies that have explored the way in which plant traits influence below-ground communities, and their effects on C and N cycling, have been performed at an individual plant species or functional group level (e.g. Wardle *et al.* 1998; Orwin *et al.* 2010; but see Laughlin 2011). As a result, our understanding of how changes in plant traits impact upon soil microbial communities and ecosystem functioning in natural communities is limited. Moreover, the relative importance of plant traits and soil microbial properties as drivers of ecosystem processes, both above- and below-ground, has not been quantified. Eviner & Chapin (2003) proposed that simple plant traits are likely to be poor predictors of ecosystem functions involving soil processes; this is because of the involvement of soil organisms in these processes, which not only affect biogeochemical cycling directly, but also respond to plant traits (Lavorel *et al.* 2009). However, this idea has not yet been tested due to the paucity of trait-based studies which have addressed specific below-ground properties relevant to C and nutrient cycling (Garnier *et al.* 2004; Klumpp & Soussana 2009; Lavorel *et al.* 2011; Laliberté & Tylianakis 2012; Lienin & Kleyer 2012).

In this study, we quantified, for the first time in the field, the relative contributions of plant and microbial properties, including measures of the abundance and activity of the microbial community and specific microbial functional groups related to N cycling, to a range of above-ground and below-ground ecosystem processes. We hypothesized that (i) above-ground plant traits have stronger effects on above-ground ecosystem processes in comparison with below-ground ecosystem processes and (ii) nutrient retention is more closely related to certain microbial properties than to plant traits. At each of three grassland sites located in the French and Austrian Alps, and in northern England, each with a range of grassland management systems representative of that area, we quantified (i) plant community composition and functional traits; (ii) soil properties, including microbial community composition and function in relation to N cycling, and (iii) key ecosystem properties related to the C and N cycles, including above-ground biomass, standing litter, soil % organic matter, potential N mineralization (PNM) and potential leaching of inorganic N from soil. We determined the relative effects of plant traits and microbial properties on these ecosystem proper-

ties by analysing, sequentially, direct effects of plant traits and then additional effects of soil micro-organisms.

Materials and methods

STUDY SITES

Grasslands were selected at three long-term research sites in the Austrian Tyrol ('Stubai' henceforth; two grasslands; Schmitt *et al.* 2010), northern England ('Yorkshire Dales' henceforth; three grasslands; De Deyn *et al.* 2011b) and the French Alps ('Lautaret' henceforth; three grasslands; Lavorel *et al.* 2011), which represented a range of climatic, geomorphological and land-use conditions typical of western European temperate mountains. Within each site, individual grasslands (*c.* 1000 m^2 each) were representative of current management trends, including abandonment, grazing and/or mowing, with varying levels and frequencies of fertilization (Table 1).

PLANT, SOIL AND ECOSYSTEM PARAMETERS

All plant and soil sampling was carried out in 12 50 × 50 cm quadrats within each grassland when above-ground biomass was at its peak between July and August 2010. Total above-ground biomass and standing litter were estimated in these quadrats using a calibrated visual approach (Lavorel *et al.* 2008).

Surveys of vegetation composition were performed using the BOTANAL method to estimate species relative biomass (Lavorel *et al.* 2008). Plant vegetative traits (vegetative height – VH; specific leaf area – SLA; leaf dry matter content – LDMC; leaf C and N concentrations – LCC and LNC), assumed to be relevant to ecosystem processes and the provision of ecosystem services (Quétier, Thébault & Lavorel 2007; Lavorel *et al.* 2011), were measured following standard protocols for each of the species that collectively made up 80% of the cumulated biomass (Garnier *et al.* 2007). For each plant trait, we calculated community-weighted mean (CWM; Garnier *et al.* 2004) and functional divergence (FD; Mason *et al.* 2003) using the F-Diversity package (Casanoves *et al.* 2011).

Subsequently, four upper soil cores per quadrat were sampled down to 5 or 10 cm depth depending on site specificity. These soil cores were pooled to obtain a composite soil sample, which was passed through a 5.6-mm sieve. Subsamples of soil were stored at 4 °C for soil chemical analyses and measurements of enzymatic activities, or at −20 °C until DNA extraction for molecular analysis of microbial communities. A fifth soil core per quadrat was taken to measure bulk density. This core was also used to study potential leaching of soil nitrate (NO_3^--N) and ammonium (NH_4^+-N; see next section for details).

ECOSYSTEM PROPERTIES

Peak green biomass, standing litter, microbial biomass N, PNM, soil % organic matter content (SOM) and potential leaching of NO_3^--N and NH_4^+-N, were selected as key ecosystem properties likely to be related to above-ground plant and soil microbial traits.

Above-ground phytomass was sorted to living and dead material, dried (60 °C, 7 days), weighed, ground and analysed for total C and N contents using a Flash EA1112 (Thermo Fischer Scientific Inc., Waltham, MA, USA) or a Vario EL III (Elementar Analysensysteme GmbH, Hanau, Germany) elemental analyser. Standardized soil methods for long-term ecological research were used to estimate soil-related parameters (Robertson *et al.* 1999). PNM rates were

determined using anaerobic incubations of fresh soil subsamples (dark, 7 days, 40 °C), during which organic N was mineralized and accumulated as NH_4^+-N (Waring & Bremner 1964; Wienhold 2007). The difference between NH_4^+ contents in a given sample before (*t*1) and after the anaerobic incubation (*t*2) gave PNM = [(NH_4^+-N)$_{t2}$−(NH_4^+-N)$_{t1}$]/dw/7 days. SOM % was obtained by loss on ignition. Soil microbial biomass N was determined using the chloroform fumigation–extraction procedure (Brookes *et al.* 1985; Voroney, Winter & Beyaert 1993). Finally, potentially leached NH_4^+-N and NO_3^--N were calculated from percolates of the fifth soil core/quadrat leached with a given volume of distilled water (de Vries *et al.* 2011).

POTENTIAL ENZYMATIC ACTIVITIES OF NITRIFIER AND DENITRIFIER MICRO-ORGANISMS

Potential rates of nitrification were assessed according to Dassonville *et al.* (2011). Briefly, 3 g dw from each composite fresh soil sample from each quadrat was incubated under aerobic conditions (180 rpm, 28 °C, 10 h) in a solution of $(NH_4)_2SO_4$ (0, 0.5, 1, 2, 5 and 15 mg N L^{-1}). Rates of NO_2 and NO_3 production were measured after 2 h, 4 h, 8 h and 10 h by ionic chromatography (DX120; Dionex, Salt Lake City, UT, USA). Maximal nitrification rate (V_{max}) and NH_4 affinity ($1/K_m$) were assessed by plotting nitrification rates along the gradient of NH_4 concentrations (Lineweaver & Burk 1934). Potential denitrification enzyme activity (DEA) was measured according to Attard *et al.* (2011). Briefly, *c.* 10 g dw soil was placed at 28 °C under anaerobic conditions using 90 : 10 He : C_2H_2 mixture inhibiting N_2O-reductase activity. Each flask was supplemented with *c.* 3 mL KNO_3 (50 μg N-NO_3^- g^{-1} dw), glucose (0.5 mg C g^{-1} dw) and sodium glutamate (0.5 mg C g^{-1} dw), completed with distilled water to reach the water-holding capacity. N_2O was measured at 2, 4, 5 and 6 h using a gas chromatograph (microGC RS3000; SRA instruments, Marcy l'Etoile, France).

QUANTIFICATION OF MICROBIAL COMMUNITIES INVOLVED IN NITRIFICATION AND DENITRIFICATION

Soil fungi and bacteria biomasses were determined using phospholipid fatty acid analysis (PLFA) using the extraction, fractionation and quantification of microbial phospholipids (Bardgett, Hobbs & Frostegard 1996). The fatty acids i150 : 0, a150 : 0, 15 : , i16 : 0, 17 : 0, i17 : 0, cy17 : 0, cis18 : 1ω7 and cy19 : 0 were chosen to represent bacterial fatty acids and 18 : 2ω6 to represent fungal fatty acids (Bardgett & McAlister 1999). Finally, the ratio of fungi : bacteria PLFA (F : B) was calculated.

To measure abundance of nitrifiers, ammonia-oxidizing bacteria and archaea were quantified based on the gene copy numbers of the ammonia monooxigenase gene *amoA* present in soil (*amoA*-AOB *amoA*-AOA), and nitrite-oxidizing bacteria were quantified based on the copy numbers of two targeted genes: *nrx*A of *Nitrobacter* and 16S rRNA of *Nitrospira* (Ns). Denitrifying microbes were quantified using the abundance of both types of nitrite reductase genes (*nir*S and *nir*K). Soil DNA was extracted from 0.5 g of fresh soil using the FastDNA® SPIN Kit for Soil (MP Biomedicals, Irvine, CA, USA) and the Precellys24 Instrument (Bertin Technologies, Montigny-le-Bretonneux, France). Quantity and quality of extracted DNA were tested by spectrophotometry (Nanodrop; PeqLab, Erlangen, Germany). Quantitative Real-Time PCR was carried out using SYBR green as fluorescent dye. Protocol efficiency, levels of qPCR and inhibition levels of DNA extracts are detailed in Appendix S1 (Supporting information).

Table 1. Study site characteristics

	Austria Stubai Valley		England Wensleydale in the Yorkshire Dales			French Alps Lautaret		
Location	47°7′ N, 11°18′ E		54°18′ N −2°5′ W			45.04°N −6.34°E		
Grassland	Meadow	Abandoned grassland	Improved meadow	Semi-improved meadow	Unimproved meadow	Fertilized & mown terrace	Unmown terrace	Unmown meadow
Altitude	1850 m	1970 m	220 m			1700 m		1900 m
Soil/Bed Rock	Dystric Cambisol soils on Granite bedrock		Brown-earth soils over limestone carboniferous limestone bedrock			Brown soils with a sandy-loam texture originating from a mixture of colluviums dominated by calshists with eolian material		
Mean minimum T° of coldest month – mean maximum of the warmest month	−2.4 °C (December–February) to 14.0 °C (July–August)		0 °C (January) to 20 °C (August)			−7.4 °C (February) to 19.5 °C (July)		
Mean annual precipitation	1097 mm		1620 mm			956 mm		
Dominant plant species	Agrostis capillaris, Festuca rubra, Ranunculus montanus, Trifolium pratense, Trifolium repens 2.44% Legumes	Sesleria varia, Erica carnea, Carex sempervirens, Poa alpina, Crocus vernus, Dryas octopetala 1.83% Legumes	Alopecurus pratensis, Poa trivialis, Lolium perenne, Holcus lanatus, Ranunculus acris 0.6% Legumes	Holcus lanatus, Poa trivialis, Lolium perenne, Anthoxanthum odoratum, Filipendula ulmaria, Ranunculus acris 1.6% Legumes	Anthoxanthum odoratum, Agrostis capillaris, Sanguisorba officinalis, Plantago lanceolata, 0.8% Legumes	Dactylis glomerata, Trisetum flavescens, Heracleum sphondylium, Gentiana lutea, Agrostis capillaris 14.6% Legumes	Bromus erectus, Sesleria caerulea, Onobrychis montana 14.1% Legumes	Festuca paniculata 0.1% Legumes
Management regime	Cut once a year, grazed in late summer, manured every 2–3 years	Abandoned since 1983	Cut once or twice a year, high-intensity grazing, manured once a year	Cut annually, medium-intensity grazing, manured every 2 years	Cut annually, low-intensity grazing, no fertilizer	Fertilized & mown	Unmown & grazed in spring & autumn	Unmown & summer-grazed – dominated by large perennial grasses

DATA ANALYSES

We used correlative modelling to quantify the respective contributions of plant and microbial traits to variations in measured ecosystem processes. We used linear mixed models with residual maximum likelihood (REML) estimations. Linear mixed models are a powerful class of models that are, in particular, appropriate for the analysis of grouped or clustered data, with grasslands considered as replicates grouped within each of the three experimental sites (Stubai, Lautaret, Yorkshire Dales). The REML algorithm, by associating common random effects with observations within the same group, allows for the specification of the covariance structure induced by the grouping of the data and provides estimations of parameter effects and variance components for both the fixed and random effects in the model. The general structure of our analyses involved each of the ecosystem process response variables (peak green biomass, standing litter, SOM %, potential leached soil NO_3-N and NH_4-N, microbial biomass N, PNM) being modelled as a function of the fixed effect plant trait parameters SLA (CWM and FDvar), vegetative height (CWM and FDvar), LDMC (CWM and FDvar), LNC (CWM and FDvar), LCC (CWM and FDvar), C/N (CWM and FDvar) and microbial properties (V_{max}, $1/K_m$, DEA, F : B ratio, abundances of *nir*S, *nir*K, *amo*A-AOA, *amo*A-AOB, Ns, *nxr*A *genes and nir*K : *nir*S, AOA : AOB, Ns : *nxr*A ratios), with site (Stubai, Lautaret, Yorkshire Dales) specified as a random effect. Analyses were carried out using the average information (AI) algorithm to estimate variance parameters within the software package Genstat 11th edition (VSN International, Hempstead, UK). All variables were tested for normality, and log transformations applied as required, prior to analysis.

To provide an objective methodology for the selection of the most parsimonious model (Díaz *et al.* 2007), analyses were conducted in two steps. First, we determined the effect of each of the plant and microbial trait parameters in isolation on each of the ecosystem processes using a series of single explanatory variable REML linear models. These analyses yielded, for each ecosystem process, a list of those plant and microbial parameters significantly correlated with the ecosystem process, as well as the percentage of variation in the ecosystem process they explained.

Second, to develop multivariable models for each ecosystem process, a hierarchical approach was used in which plant trait parameters were fitted as fixed factors first, with microbial effects being fitted afterwards (following Lavorel *et al.* 2009). This bottom-up approach to ecosystem functioning was based on the assumption that plant traits have a large influence on microbial traits (Orwin *et al.* 2010), thus considering residual microbial effects after plant effects have been accounted for. Within the set of plant trait parameters, CWM parameters were fitted first, followed by FDvar parameters following Díaz *et al.* (2007), assuming that functional variance effects are only of interest beyond those main effects of functional mean effects (with which they are often correlated; Dias *et al.* 2013). To develop the most explanatory, parsimonious models, for each ecosystem process, only those variables significant from the single variable analyses were used, and these were fitted in the order of the percentage variation in the ecosystem process they explained individually. Each response variable was added to the model in a stepwise manner, with those variables significant in the presence of previously fitted variables being retained in the model and variables no longer significant in the presence of other variables, due to colinearity, being removed from the model. This was especially the case for multiple correlated leaf traits that form the leaf economics spectrum, but which we decided not to select *a priori* or to combine into a single multivariate proxy in order to preserve interpretability based on individual traits. We verified the relevance of this approach by running all alternative models for colinear variables with an adjusted R^2 of at least 0.3. Convergence upon a final multiple variable model occurred once all the plant and microbial trait parameters significant individually for a given ecosystem process had been fitted, and either retained or discarded from the model. These final models allowed us to calculate the percentage of variation in the ecosystem process explained by the retained fixed factors, the breakdown of this variation among plant traits and microbial traits, and the effect of each of the retained parameters on the ecosystem process.

Results

Results from the single variable models are presented as Supporting information (Table S2). The stepwise multiple variable model fitting procedure was designed to produce the most biologically meaningful and parsimonious model possible from the wide range of potential explanatory variables, by adding explanatory variables in the order of their perceived biological importance (first CWM plant traits, followed by FDvar plant traits and then microbial variables), and amount of variation explained (Table 2). We acknowledge, however, that in the case of colinearity between variables used in the final model, it is possible that other combinations of explanatory variables could explain similar amounts of variation and that this could have implications for our conclusions. Table S3 presents, for each ecosystem parameter, those explanatory variables not retained in the final models that had significant colinearity (a significant adjusted R^2 of > 0.3) with the variables retained in the final 'best' model (Table 2). Each of the possible alternative multiple variable models stemming from the replacement of the originally retained variables by these collinear ones is then presented. As none of the alternative models tested explained as much variance as the 'best' models retained through the hierarchical stepwise model fitting procedure (Table 2), we now focus on the latter.

The results of the multiple variable REML models revealed a shift in importance from plant to microbial traits along a gradient from vegetation properties, such as peak green biomass and standing litter, to soil processes, such as potential leaching of soil inorganic N (NO_3-N or NH_4-N) and N mineralization potential (Table 2; see also Table S3 for alternative models with collinear variables). The overall amounts of variation explained in the multivariable models by the retained fixed effects (over and above variation in these variables between the sites, removed as a random factor) ranged from 40% to 87%.

Variation in peak green biomass and soil microbial biomass N were overwhelmingly explained by functional properties of the vegetation (95% and 85% of variation explained due to plant traits, respectively; Table 2). Strong positive relationships between peak green biomass and CWM vegetative height (Fig. 1a), and soil microbial biomass N and CWM vegetative height (Table 2), were found at all sites. Both variables were also correlated with CWM SLA, but across the three sites this relationship was positive for peak green bio-

Table 2. Fixed effect plant and microbial trait variables retained within the multivariable REML models for each of the ecosystem properties

Response variable	Retained fixed effects	% Variation explained by fixed effects	Proportion of % variation explained due to plant traits	Proportion of % variation explained due to microbial traits	*P*	Wald	Standard effect
Peak green biomass	CWM_height	87	95	5	< 0.001	59.8	0.008
	CWM_SLA				< 0.001	53.4	0.007
	FDvar_height				< 0.001	26.7	−0.185
	Fungi : Bacteria				0.014	7.44	−1.165
Microbial biomass N	CWM_SLA	83	85	15	< 0.001	50.6	−0.020
	CWM_height				< 0.001	21.4	0.032
	FDvar_height				0.004	11.6	0.535
	*nir*K				0.003	12.7	−0.560
Litter mass	CWM_height	78	83	17	< 0.001	37.17	0.0197
	CWM_SLA				< 0.001	23.35	−0.0333
	K_m				0.002	12.71	−0.2604
Soil organic matter %	CWM_LDMC	83	50	50	< 0.001	40.5	1.024
	CWM_SLA				< 0.001	15.4	−0.020
	DEA				< 0.001	45.1	0.507
Potential N Mineralization	CWM_LDMC	61	18	82	0.008	8.77	1.916
	DEA				< 0.001	26.2	1.024
Potential leached NO₃-N	FDvar_C : N	61	25	75	0.002	16.3	−0.206
	V_{max}				< 0.001	19.7	0.926
Potential leached NH₄-N	CWM_SLA	40	16	84	0.011	7.77	−0.032
	DEA				0.001	13.7	0.807

CWM, community-weighted mean; DEA, denitrification enzyme activity; FD, functional divergence; LDMC, leaf dry matter content; REML, residual maximum likelihood; SLA, specific leaf area.

Also presented is the % variation in each ecosystem parameter explained by the retained fixed effects (calculated by the reductions in the residual variance due to the addition of the fixed effects to the model after accounting for variation due to the random effect of site), the proportion of explanation afforded by the fixed effects due to plant traits and microbial traits (in italics), respectively, and the significance (P), Wald statistic and the direction and magnitude of the standardized effect for each of the retained fixed effects. Results for tests of individual plant and microbial parameters are presented in Table S2.
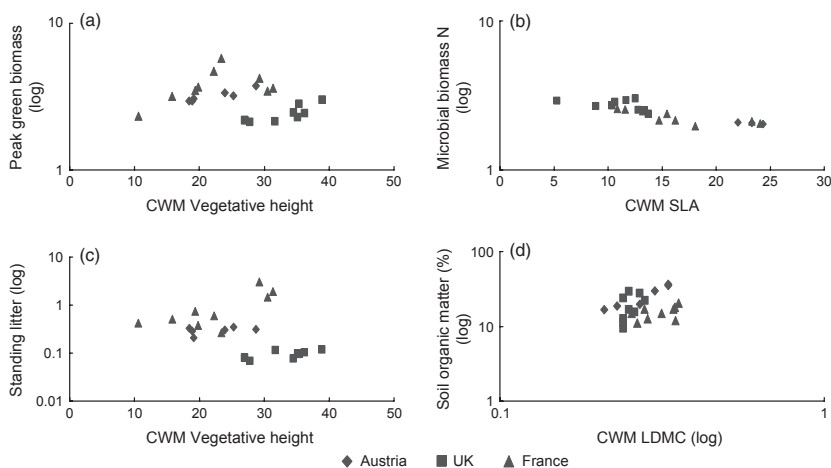
Fig. 1. Selected key plant traits important in controlling (a) peak green biomass, (b) microbial biomass N, (c) standing litter and (d) soil % organic matter content, showing the form of these relationships between the different sites.

mass (Table 2) and negative for soil microbial biomass N (Fig. 1b). In addition, FD in vegetative height had a minor negative effect on peak green biomass, but a minor positive effect on soil microbial biomass N (Table 2). The relationship between peak green biomass or soil microbial biomass N, respectively, with microbial functional parameters (5% and 15% of variation explained, respectively; Table 2) was considerably less than plant trait effects.

Litter mass was mostly explained by plant trait parameters describing the quantity and quality of plant biomass (83% of variation explained due to plant traits). Across the three sites, litter mass was negatively correlated with CWM SLA and positively correlated with vegetative height (Table 2), although this relationship was not detected at the Stubai site (Fig. 1c). Microbial parameters explained only 17% of variation in litter mass, which was positively correlated the affinity of nitrifiers for ammonia (negative correlation with Km, the inverse of affinity).

Soil % organic matter content was equally influenced by both plant functional traits and soil microbial communities

(50% of the variance explained each by these groups of parameters; Table 2). The significant plant trait parameters describe the quality of the vegetation being input into the soil, with increasing SOM content being positively correlated with increasing CWM LDMC (Fig. 1d) and decreasing CWM SLA (Table 2). Overall, potential denitrification activity (DEA) was positively related to SOM content, although this relationship did not hold at the French site (Fig. 2a).

Variations in potential inorganic N (NO_3-N or NH_4-N) leaching and PNM were mainly explained by parameters describing N-cycling soil microbial communities (75–84% of variation). Increases in the V_{max} of potential nitrification were strongly linked to increases in potential leaching of NO_3-N in soils from the Yorkshire Dales and Lautaret, but not from Stubai (Fig. 2b). Similarly, increases in potential denitrification activities (DEA) were positively correlated with higher N mineralization and increased ammonia leaching potentials at all three sites (Table 2 and Fig. 2c,d). For potential soil NO_3-N and NH_4-N leaching, a minor effect was also exerted by plant trait parameters linked with the quality of plant biomass. Decreased FD of leaf C : N was positively correlated with potential NO_3-N leaching, while CWM SLA was negatively related to NH_4-N leaching. Furthermore, plant CWM LDMC showed a positive relationship with PNM (Table 2).

Discussion

ADDITIVE EFFECTS OF PLANT AND MICROBIAL FUNCTIONAL PARAMETERS ON ECOSYSTEM FUNCTIONING

To our knowledge, this is the first study to provide a direct quantification, in the field, of the relative roles of plant traits and microbes in explaining a comprehensive and complementary set of ecosystem properties. Our models, combining plant traits and soil microbial properties, explained high amounts of variance (60–90%) in ecosystem properties (Table 2), demonstrating the value of combining plant functional traits and soil

microbial properties to quantify ecosystem functioning (Wardle *et al.* 2004; De Deyn, Cornelissen & Bardgett 2008). Because our objective was not to obtain a mechanistic causal model (see, e.g. Laughlin 2011; Laliberté & Tylianakis 2012), nor to incorporate plant–soil feedback (Ehrenfeld, Ravit & Elgersma 2005), our analyses did not explicitly incorporate abiotic effects. However, as abiotic variation occurred largely between sites, the use of site as a random factor accounted for much of the direct effects on ecosystem properties.

Our results are consistent with previous studies which show that leaf and size traits explained 60‒80% of variance in ANPP (Garnier *et al.* 2004; Ansquer *et al.* 2009; Schumacher & Roscher 2009; Lavorel *et al.* 2011; Minden & Kleyer 2011; Laliberté & Tylianakis 2012; Lienin & Kleyer 2012). Whilst, overall, 40‒50% of variance in leaf litter decomposition was explained by leaf economics traits (meta-analysis by Cornwell *et al.* 2008; Fortunel *et al.* 2009; Lienin & Kleyer 2012). Eviner & Chapin (2003) suggested that plant trait effects should be weak for processes involving soil biodiversity. Indeed, Laughlin (2011) found that leaf economics spectrum traits explained only 37% of variation in nitrification potential in *Pinus ponderosa* forest understorey. Here, we demonstrated that the incorporation of soil microbial parameters into models significantly improved our ability to explain below-ground ecosystem processes, typically from < 30% with plant traits alone (Table S1) to 60‒80% (Table 2).

Further, by portioning variance in ecosystem properties between plant traits and soil microbial properties associated with N turnover, we identified a continuum of variation from ecosystem properties associated mostly with vegetation traits (> 80% of the explained variance represented by plant traits), such as peak green biomass and standing litter, to ecosystem properties associated mostly with microbial processes, such as soil potential leaching of inorganic N or PNM (> 74% of the explained variance represented by microbial traits). Although we chose a bottom-up approach to quantify direct microbial effects on ecosystem properties beyond those effects already accounted for plant traits, such microbial effects appeared considerable, as suggested by Eviner & Chapin (2003).
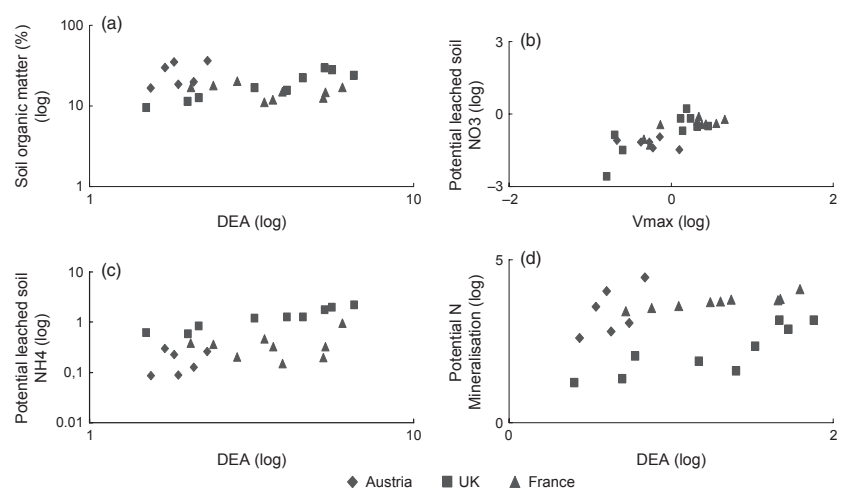
Fig. 2. Selected key microbial traits important in controlling (a) soil % organic matter content, (b) potential leached NO_3-N, (c) potential leached NH_4-N and (d) potential N mineralization, showing the form of these relationships between the different sites.

MECHANISMS UNDERPINNING EFFECTS OF PLANT FUNCTIONAL PARAMETERS ON ECOSYSTEM PROPERTIES

Plant trait effects were overwhelmingly attributed to CWM, supporting the biomass ratio hypothesis and suggesting that traits of the dominant species drive biogeochemical cycling (Grime 1998; Mokany, Ash & Roxburgh 2008; Laughlin 2011; Lavorel *et al.* 2011). Some non-negligible effects of FD were also retained in the models, though their mechanistic explanation is more tentative. Smaller variance in vegetative height was associated with increased peak biomass, which suggests that plant communities strongly dominated by tall plants were most productive (see Laliberté & Tylianakis 2012). This could feed forward to a greater input of resources and/or diversity of habitats for soil micro-organisms and thus explain the positive relationship between FDvar_height and microbial biomass N. A greater diversity in root architectures and quality (FDvar C : N) may also promote better nitrate uptake and thereby lower potential nitrate leaching.

Parallel to the continuum from plant traits to microbial properties, there was within-plant traits, a continuum from plant quantity, linked more with peak biomass, to plant quality parameters, linked more with the recycling of organic matter (Fig. 3). First, community mean plant height was strongly and positively related to peak green biomass and standing litter, as fast growing, taller species produced more biomass over the season (Ansquer *et al.* 2009; Lavorel *et al.* 2011) and thus more litter (Lavorel *et al.* 2011; Laliberté & Tylianakis 2012; Lavorel & Grigulis 2012). In turn, greater litter inputs to soil, and possibly greater root biomass, might stimulate microbial biomass N by providing more resources for microbes in the rhizosphere (Zak *et al.* 1994; Paterson 2003; Tscherko *et al.* 2004).

Secondly, effects on ecosystem processes of leaf economics spectrum traits that reflect the quality of plant material entering the soil (Freschet *et al.* 2010) were consistent with expectations of nutrient turnover being faster in communities dominated by exploitative species (high SLA and LNC, low LDMC) and, conversely, slower in communities dominated by conservative species (opposite traits; Chapin 1993, 2003; Lavorel & Grigulis 2012). The positive effect of CWM SLA on peak biomass is expected to result from faster growth (Reich, Walters & Ellsworth 1997; Garnier *et al.* 2004; Pontes Da Silva *et al.* 2007), while its negative effect on litter mass resulted from poor decomposability of conservative species (Cornwell *et al.* 2008; Mokany, Ash & Roxburgh 2008; Minden & Kleyer 2011). Indeed, at the Lautaret and Stubai sites, the annual rate of litter decomposition was positively correlated with CWM LNC (data not shown; see also Quétier, Thébault & Lavorel 2007; Rubatscher 2008). The observed association between more conservative plants and greater soil organic matter content is consistent with both theory (De Deyn, Cornelissen & Bardgett 2008) and evidence from monoculture and mesocosm studies (De Deyn *et al.* 2009; Orwin *et al.* 2010).

In contrast to above-ground processes, effects of leaf economics spectrum traits on the soil microbial compartment are
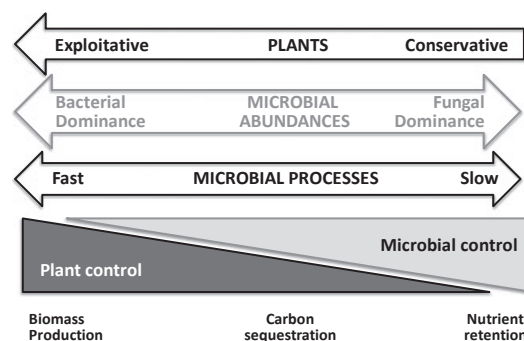
Fig. 3. Schematic overview of simultaneous variations in plant functional strategies, microbial functional composition and activities, and ecosystem processes and services.

poorly understood. The strong negative relationship between CWM SLA and microbial biomass N suggests that the amount of N immobilized by the microbial biomass increases where conservative plants dominate. This finding is broadly consistent with ^{15}N labelling studies which show that soil microbial communities compete more effectively with plants for N in nutrient poor grasslands where conservative species dominate, than in nutrient-rich grasslands dominated by N rich plants, leading to greater microbial immobilization and retention of N in the former (Bardgett, Streeter & Bol 2003; Harrison, Bol & Bardgett 2007; Robson *et al.* 2010).

Collectively, these results strongly support the relevance of the leaf economics spectrum not only to above-ground biomass and litter decomposition or litter pools, but also to soil processes as expected from theory (De Deyn, Cornelissen & Bardgett 2008) and recent empirical studies (Laughlin 2011; Pakeman 2011; see also Craine *et al.* 2002). As such, they support the plant economics spectrum hypothesis and its implications for soil processes (Freschet *et al.* 2010). To complement this systemic view of plant economics, analyses would need to incorporate root traits (Craine *et al.* 2002; Klumpp *et al.* 2009; Orwin *et al.* 2010), even though it has been argued that their effects can be captured through leaf traits (Craine *et al.* 2001; De Deyn, Cornelissen & Bardgett 2008; Laliberté & Tylianakis 2012).

MECHANISMS UNDERPINNING ASSOCIATIONS OF MICROBIAL PROPERTIES WITH ECOSYSTEM PROPERTIES

As expected, we observed strong links between microbial functional properties and below-ground processes such as potential mineral N leaching, PNM (84% variance explained) and SOM content (50% variance explained). Microbial properties measured under optimal conditions, such as potential denitrification activity (DEA), nitrification affinity ($1/K_m$) and nitrification maximum rate (V_{max}), may be considered as microbial functional traits that directly or indirectly influence ecosystem properties. Our correlative approach should only be interpreted as statistical associations, rather than demon-

strating likely complex, causal links between microbial activities and ecosystem services.

Specifically, we focused on two different functional groups, which catalyse different processes of N turnover: nitrifiers and denitrifiers. Nitrification is a two-step aerobic microbial process fuelled by N mineralization and performed by autotrophic ammonia and nitrite-oxidizing microbes (Schauss *et al.* 2009). First, lower litter mineralization decreases NH_4^+ availability and may thus increase plant–microbe competition for available NH_4^+ (Skiba *et al.* 2011). As higher standing litter mass results from poor mineralization, this mechanism could explain its positive correlation with nitrifiers' affinity for NH_4^+ ($1/K_m$). Second, the observed positive effect of nitrification rate (V_{max}) on potential nitrate leaching directly reflects the production by nitrifiers of NO_3^- that is not retained by negatively charged surfaces in soil. In contrast, denitrifiers are heterotrophic facultative anaerobic microbes that reduce NO_3^- to gaseous N forms. They strongly depend on plant-derived C, and their activity is highly influenced by redox conditions in soil and hence by water content or soil texture. As for nitrifiers' affinity for NH_4^+, litter mass was also negatively correlated with denitrifying microbes harbouring the nitrite reductase gene *nir*S, confirming previous reports that *nir*S-harbouring bacteria are out-competed by other denitrifiers in habitats with high C availability (Bannert *et al.* 2011). This strong dependency of denitrifiers on available C sources may also explain the close link of DEA to SOM, which is mainly formed from plant-derived C. Although nitrifiers and denitrifiers exhibit different life histories and occupy different niches in soil (Keil *et al.* 2011), denitrifiers strongly depend on the activity of nitrifiers. This is because NO_3^-, the substrate for denitrification, is exclusively formed by nitrifiers from NH_4^+ (Prosser 1989), itself being sourced from N mineralization. Consequently, PNM was positively and indirectly correlated with DEA. Also, when N mineralization is high, NH_4^+ leaching may occur (Qian & Cai 2007), which may support the positive, but indirect, correlation between DEA and potential NH_4^+ leaching.

The future investigation of other functionally important microbes, such as arbuscular mycorrhizal fungi and N-fixing microbes, or of microbial communities with reduced functional redundancy, such as those that are involved in the degradation of highly polymeric substances like lignin or other phenolic compounds, may show an even more significant contribution of microbial properties to abiotic soil properties.

A SYNTHETIC FRAMEWORK FOR THE PROVISION OF MULTIPLE ECOSYSTEM SERVICES

Our analyses provide a synthetic hypothesis for the control of the provision of multiple ecosystem services by grasslands (Fig. 3). More exploitative plant strategies (high SLA, high LNC, low LDMC) and taller swards are expected to result in greater fodder production (peak green biomass), but at the cost of poor C and nutrient retention. This is most probably because they are associated with soil microbial communities dominated by procaryotes that perform rapid rates of mineralization and nitrification. Conversely, dominance by plants with conservative strategies (high LDMC and C : N ratio) usually results in low fodder production, but provides benefits for soil C sequestration and nutrient retention, which is potentially linked to the dominance of microbial communities dominated by fungi, co-occurring with procaryotes with slow activities. Because conservative and exploitative plants are likely to impact differently on microbial subgroups, further studies should explore whether changes in microbial activity levels relate to specific groups among nitrifiers/denitrifiers or other microbial functional groups harbouring contrasted activity levels.

Such a framework could have important implications for the management of ecosystem services trade-offs. Our results demonstrate that the effects of plant traits and microbial properties on ecosystem functioning underpin critical trade-offs between production and regulation ecosystem services. Thus, we suggest that management intensity and associated changes in soil fertility can steer the provision of multiple ecosystem services, through their effects on plant and soil microbial community composition.

Conclusion

This is the first trait-based study, to the best of our knowledge, providing a direct quantification, in the field, of the relative roles of plant and microbial functional traits for a comprehensive set of above- and below-ground ecosystem properties. By demonstrating a continuum from tight coupling with plant traits for above-ground processes to strong linkages with microbial traits for below-ground processes, we confirm the need and refine the scope for the incorporation of soil functional diversity into analyses of biodiversity effects on ecosystem functioning. Although we used a correlative approach, our results highlighted functional markers of ecosystem functioning, including the leaf economics spectrum and a range of soil microbial properties related to nutrient cycling, such as denitrification potential. Such markers need to be tested over a wider range of ecosystems, and their variations with key environmental gradients need to be documented and better understood. In particular, the lack of data and limited understanding of both plant and microbial trait effects on below-ground processes needs to be addressed. Furthermore, in the future, such knowledge should support the management of ecosystem services trade-offs by selecting desired plant and soil functional properties.

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Supporting Information

Additional Supporting Information may be found in the online version of this article:

Table S1. Quantitative PCR conditions used to estimate abundances of N-related microbial functional groups.

Table S2. Results of REML tests of individual plant and microbial parameters as explanatory factors for each ecosystem parameter.

Table S3. Alternative multivariable REML models for each of the ecosystem properties resulting from the replacement of plant and microbial traits retained in the original final models (Table 2) by alternatively significant collinear traits with an adjusted R of > 0.3.

Appendix S1. Detailed experimental protocol for the quantification of the abundance of nitrifiers, ammonia-oxidizing bacteria and archaea.