Plant community controls on short-term ecosystem nitrogen retention

Franciska T. de Vries and Richard D. Bardgett
Faculty of Life Sciences, The University of Manchester, Oxford Road, Manchester, M13 9PT, UK

Author for correspondence:
Franciska T. de Vries
Tel: +44 161 3068091
Email: franciska.devries@manchester.ac.uk

Received: 18 September 2015
Accepted: 27 November 2015

New Phytologist (2016) 210: 861–874
doi: 10.1111/nph.13832

Key words: competition, functional group, functional traits, leaf traits, nitrogen enrichment, plant–soil interactions, root traits, soil microbial community.

Summary
• Retention of nitrogen (N) is a critical ecosystem function, especially in the face of widespread anthropogenic N enrichment; however, our understanding of the mechanisms involved is limited. Here, we tested under glasshouse conditions how plant community attributes, including variations in the dominance, diversity and range of plant functional traits, influence N uptake and retention in temperate grassland.
• We added a pulse of $^{15}$N to grassland plant communities assembled to represent a range of community-weighted mean plant traits, trait functional diversity and divergence, and species richness, and measured plant and microbial uptake of $^{15}$N, and leaching losses of $^{15}$N, as a short-term test of N retention in the plant–soil system.
• Root biomass, herb abundance and dominant plant traits were the main determinants of N retention in the plant–soil system: greater root biomass and herb abundance, and lower root tissue density, increased plant $^{15}$N uptake, while higher specific leaf area and root tissue density increased microbial $^{15}$N uptake.
• Our results provide novel, mechanistic insight into the short-term fate of N in the plant–soil system, and show that dominant plant traits, rather than trait functional diversity, control the fate of added N in the plant–soil system.

Introduction
Humans have irreversibly changed the global nitrogen (N) cycle by doubling the amount of reactive N in the biosphere, which has led to increased greenhouse gas emissions and nutrient enrichment of aquatic and terrestrial ecosystems (Galloway et al., 2008; Schlesinger, 2009). Chronic N enrichment can indirectly affect ecosystem functioning by reducing plant community diversity and encouraging dominance of fast-growing, highly productive species (Stevens et al., 2004; Klumpp & Soussana, 2009), but also through its negative effects on mycorrhizal fungi (Bradley et al., 2006), which promote plant species coexistence and diversity (Van der Heijden et al., 1998; Wagg et al., 2011a,b). Ecosystem N retention is a critical ecosystem function, especially in the light of increased N loads, given that plants and soils together can retain significant amounts of N, thereby preventing it from being released to the surrounding environment. While it is well known that abiotic factors such as pH, soil texture and soil organic matter content affect ecosystem N retention, much less is known about the role of biotic factors, including interactions between plants and soil microbes (De Vries & Bardgett, 2012). This represents a significant gap in understanding, given growing evidence that plant–microbial linkages are likely to play a crucial role in determining the balance between N retained in plant and soils and the amount of N lost as gases or leachates to the surrounding environment (Suding et al., 2008).

Many studies have shown a positive effect of plant species richness on ecosystem N retention, which has been attributed to niche complementarity and overyielding, resulting in greater root uptake of available N (Hooper & Vitousek, 1998; Scherer-Lorenzen et al., 2003; De Deyn et al., 2009; Bingham & Biondini, 2011), but also to the increased chance of diverse mixtures including a highly influential species (Mulder et al., 2002). The number and identity of functional groups present in a plant community can also affect N leaching. For example, legumes can increase N leaching through their positive effect on soil N availability (Scherer-Lorenzen et al., 2003), while grasses can decrease N leaching, presumably through their dense root systems (Phoenix et al., 2008). However, it has also been found in experimental mesocosms that individual legume and forb species can decrease N leaching (De Deyn et al., 2009). To better understand these observed diversity, functional group and individual species effects on N leaching and other ecosystem functions, ecologists are increasingly turning to trait-based approaches (Lavorel & Garnier, 2002; Diaz et al., 2007; De Deyn et al., 2008; Lavorel et al., 2013). These trait-based explanations of plant community effects on ecosystem functioning make use of the leaf economics spectrum, which links leaf traits to plant resource uptake strategies and subsequent growth rates. Here, high specific leaf area (SLA) and high leaf N content (leaf N) are traits directly involved in photosynthesis and are representative of species with exploitative growth strategies, while high leaf dry matter content
(LDMC) is involved in defence and longevity of above-ground plant parts and linked to resource-conservative growth strategies (Diaz et al., 2004; Wright et al., 2004). Recently, the focus of these trait-based approaches has shifted below ground, although root traits are not as easily characterized into conservative or exploitative strategies as leaf traits (Mommer & Weemstra, 2012; Bardgett et al., 2014).

Plant species richness effects on primary productivity (Naeem, 2002), ecosystem carbon (C) fluxes (Milcu et al., 2014) and soil faunal community composition (Milcu et al., 2013) have been attributed to the diversity and divergence of functional traits, rather than species diversity per se. However, as yet, no studies have explicitly tested whether functional traits underlie plant species richness effects on processes of N cycling, although studies have linked plant traits related to N acquisition to microbial communities and their activities in monocultures. For example, exploitative plant traits, such as high SLA and leaf N content, have been linked to high soil N availability (Orwin et al., 2010), but also to greater plant N uptake (Grassein et al., 2015). Also, in pot experiments, plant N-use efficiency (specific root N uptake) was found to reduce the abundance of nitrate-reducing bacteria (Moreau et al., 2015), and root traits were shown to be stronger controls on potential rates of denitrification and nitrification than leaf traits (Cantarel et al., 2015). In addition, a number of field studies have explained N cycling processes using trait-based approaches. A forest study demonstrated that dominance of exploitative plant traits, measured as community-weighted means (CWMs), is linked to high rates of soil N cycling, and potentially high N losses through nitrification and denitrification (Laughlin, 2011). By contrast, grasslands dominated by conservative plant species can have greater ecosystem N retention, both in the field and in laboratory experiments (Suding et al., 2008; De Vries et al., 2012a, 2015; Grigulis et al., 2013). Thus, there is a gap in understanding between individual species and observational plant community effects on N cycling, namely how different components of plant functional diversity mechanistically affect ecosystem N retention or loss.

Although plant community effects on N retention can act directly, through root uptake and subsequent transport to above-ground plant parts (De Vries et al., 2012a), short-term ecosystem N retention mainly depends on N uptake by soil microbes (Zogg et al., 2000b; De Vries et al., 2012a). Especially over short timescales (h to d), microbes are better competitors for N than plant roots and take up most of the soil available N, which can become available for plant uptake after subsequent remineralization (Kaye & Hart, 1997; Bardgett et al., 2003; De Vries et al., 2012a). Previous studies have suggested that competition for N is fiercer in plant communities dominated by conservative plant traits, and that this results in greater microbial immobilization of N (Harrison et al., 2008) and N retention, and lower N loss (De Vries & Bardgett, 2012) than in communities dominated by exploitative traits. However, evidence for this is inconsistent; a few field-based studies have shown that grasslands dominated by conservative plant traits have smaller N leaching loss (De Vries et al., 2012a; Grigulis et al., 2013) through greater N immobilization by fungal-dominated microbial communities (De Vries et al., 2012a), while others did not find evidence that plant growth strategies affected microbial N uptake in a glasshouse experiment (Harrison et al., 2008). In addition, a recent glasshouse experiment found that nitrate-reducing bacteria were reduced under plants with high N-uptake rates, but plant N uptake was not explained by plant traits (summarized in a nitrophily index, Moreau et al. (2015)). Thus, although it is well established that plant functional traits can affect plant N uptake and microbe-mediated processes of N cycling, it is not clear how they influence microbial immobilization of N in soil or how they impact N retention, or the total amount of N retained in plants, microbes and soil.

Here, we used a unique experimental design involving a range of constructed plant communities to experimentally test how plant community attributes affect ecosystem N retention. We focused on dominant leaf traits, trait functional diversity and divergence, and used temperate grassland as a model system. We aimed to contrast the mass-ratio hypothesis, which states that dominant plant species control ecosystem processes (Grime, 1998), and the functional diversity hypothesis (Johnson et al., 1996; Diaz et al., 2007), which states that trait functional diversity, rather than species diversity or dominant plant species per se, controls ecosystem processes. Thus, we compared two specific hypotheses on how plant community composition controls N retention: dominance of conservative plant traits increases N retention through greater plant uptake and through promoting microbial immobilization, thus increasing N retention and reducing N leaching; and trait functional diversity and trait functional divergence enhance N retention through greater above-ground and below-ground N uptake through niche complementarity and overyielding.

Materials and Methods

Experimental setup

We collected soil from mesotrophic grassland at Lancaster University’s Hazelrigg Field Station in northern England (54°11’N, 2°46’W, 94 m above sea level). The soil used was a silt loam of the Brickfield 2 association, %N 0.19, %C 2.35, pH 4.75. Soil was sieved and homogenized (4 mm mesh size) and 2.5 kg field-moist soil was packed in 31 (19 cm diameter, 15 cm depth) pots.

We constructed grassland plant communities representing a range of CWM trait, functional diversity and functional divergence values using a species pool of 24 grassland species. Twelve grasses and 12 herb species representative of mesotrophic grassland were selected and ranked according to their SLA (from Grime et al., 2007; Table 1). We used SLA because this leaf trait is strongly correlated to leaf N, on both a local and a global scale, and these two traits have a strong physiological link with leaf photosynthesis (Wright et al., 2004). Moreover, both SLA and leaf N have previously been linked to soil microbial community composition and carbon (C) and N cycling in grasslands (De Vries et al., 2012b; Garcia-Palacios et al., 2013; Grigulis et al., 2013; Milcu et al., 2014). Ranking the 24 grassland species
resulted in three categories for both grasses and herbs: exploitative grasses and herbs with high SLA (category 1); conservative grasses and herbs with low SLA (category 3); and a group of grasses and herbs with intermediate SLA (category 2; Table 1). These trait categories were used to construct plant communities.

Each plant community consisted of both grasses and herbs from the same trait category, in equal abundances, to correct for functional group effects. We constructed plant communities consisting of species from one trait category only (two and four species mixtures), two trait categories in all possible combinations (two and four species mixtures), and three trait categories (six and 12 species mixtures; Table 2). Thus, our communities represented a range of CWM traits, as well as a gradient of species diversity, trait functional diversity and trait functional divergence. Species were randomly assigned to treatments. Each species occurred in nine plant communities and each of four replicate plant communities was unique, allowing us to investigate functional trait controls on N retention while controlling for individual species and functional group (grass vs herb) influences. This experimental design, including 14 treatments, each with four unique replicates, resulted in 56 different plant communities (Table 2).

Seedlings of all species were germinated and grown for 13 wk (using the same soil that was used for the main experiment), after which they were transplanted into the experimental pots. Each plant community consisted of 12 individuals that were randomly assigned to a planting grid and planted at field densities. Pots were arranged in a randomized block design and kept at constant soil moisture (60% water-holding capacity, which equaled 40% moisture content) in a controlled growth chamber (16 : 8 h, light : dark at 16°C) for 4.5 months.

After 4.5 months, 45 ml of $^{15}$NH$_4$$^{15}$NO$_3$ solution (98.5 atom% enriched, 85 mg $^{15}$N, equivalent to 30 kg ha$^{-1}$, which is the high end of yearly atmospheric N deposition in upland areas in the north of England (Defra, 2011), was injected in the top 5 cm at nine evenly spaced locations (5 ml each) of each pot, as in De Vries et al. (2012a). Forty-eight hours after $^{15}$N addition, pots were leached by slowly adding 850 ml of demineralized water (equivalent to a 30 mm rainfall event), following the approach of De Vries et al. (2012a). We chose to harvest 48 h after N addition because a previous experiment showed that ecosystem N uptake at this time point is representative of longer-term N retention: although initial N retention was predominantly in roots and microbes, this was later transferred to above-ground plant parts, and as a result the total amount of N retained in plants, microbes and soil was the same after 48 h and after 2 months (De Vries et al., 2012a). Leachate volumes were recorded and leachates were kept in the fridge for a maximum of 1 wk until further analysis. Above-ground vegetation was clipped and sorted to species, dried at 60°C for 48 h, weighed and ground. Soil was gently shaken off roots, passed through a 4 mm sieve and homogenized. Fresh soil was kept in the fridge until further analyses, and a subsample was air-dried and ground. Roots were washed, dried at 60°C for 48 h, weighed and ground.

| Species | SLA | Functional group | Category |
|---------|-----|------------------|----------|
| Deschampsia cespitosa (Dc) | 18.5 | Grass | 1 |
| Festuca rubra (Fr) | 17.7 | Grass | 1 |
| Poa pratensis (Poa) | 21.5 | Grass | 1 |
| Trisetum flavescens (Tf) | 20.1 | Grass | 1 |
| Campanula rotundifolia (Cr) | 22.2 | Herb | 1 |
| Filipendula ulmaria (Fu) | 18.6 | Herb | 1 |
| Leucanthemum vulgare (Lv) | 22.1 | Herb | 1 |
| Plantago lanceolata (Pl) | 22.4 | Herb | 1 |
| Anthoxanthum odoratum (Ao) | 27.3 | Grass | 2 |
| Cynosurus cristatus (Cc) | 26.4 | Grass | 2 |
| Dactylis glomerata (Dg) | 27.7 | Grass | 2 |
| Lolium perenne (Lp) | 26.4 | Grass | 2 |
| Centaurea nigra (Cn) | 26.3 | Herb | 2 |
| Geranium sylvaticum (Gs) | NA* | Herb | 2 |
| Hypochaeris radicata (Hr) | 23.3 | Herb | 2 |
| Ranunculus acris (Ra) | 23.8 | Herb | 2 |
| Agrostis capillaris (Ac) | 30.8 | Grass | 3 |
| Holcus lanatus (Hl) | 33.5 | Grass | 3 |
| Phleum pratense pratense (Phlp) | 30.6 | Grass | 3 |
| Poa trivialis (Pt) | 31.3 | Grass | 3 |
| Cerastium fontanum (Cf) | 29.2 | Herb | 3 |
| Leontodon hispidus (Lh) | 28.8 | Herb | 3 |
| Prunella vulgaris (Pv) | 30.3 | Herb | 3 |
| Rumex acetosa (Ruma) | 28.1 | Herb | 3 |

Category number indicates a gradient from conservative (category 1), to intermediate (category 2), and exploitative (category 3).

*SLA data not available for G. sylvaticum in Grime et al. (2007); unpublished data were used for including this species in category 2.

Table 2 The 12 experimental treatments, representing a range of species richness treatments, number of categories (a proxy for functional diversity) and category average (a proxy for the ‘exploitativeness’ of the plant community, which averages the categories present, with 1 indicating dominance of conservative plant traits, and 3 indicating dominance of exploitative plant traits)

| Treatment | No. of species | No. of categories | Categories | Average category |
|-----------|---------------|------------------|------------|-----------------|
| A         | 2             | 1                | 1          | 1               |
| B         | 2             | 2                | 2          | 2               |
| C         | 3             | 3                | 3          | 3               |
| D         | 2             | 1+2              | 1.5        |                 |
| E         | 2+3           | 2.5              |            |                 |
| F         | 1+3           | 2                |            |                 |
| G         | 4             | 1                | 1          | 1               |
| H         | 2             | 2                | 2          | 2               |
| I         | 3             | 3                | 3          | 3               |
| J         | 2             | 1+2              | 1.5        |                 |
| K         | 2+3           | 2.5              |            |                 |
| L         | 1+3           | 2                |            |                 |
| M         | 6             | 3                | 1+2+3      | 2               |
| N         | 12            | 3                | 1+2+3      | 2               |

Each experimental treatment (A to N) had four unique replicates, drawn from the species pool in Table 1.

© 2016 The Authors
New Phytologist © 2016 New Phytologist Trust
C and N analyses

Leachates were analysed for inorganic N (NO$_3^-$ and NH$_4^+$), dissolved organic N (DON), and dissolved organic C (DOC), as described in De Vries et al. (2012a). Above-ground vegetation (all species separately) and a representative subsample of roots were analysed for C and N using an Elementar Vario EL elemental analyzer (Hanau, Germany), and soil microbial biomass C and N were determined by fumigation extraction, as described by Brookes et al. (1985).

Leachate (after freeze-drying), shoot (separated by species), root, soil and microbial biomass $^{15}$N (determined by diffusing microbial-derived N onto an acid trap) were analysed using a Carlo Erba NA2000 analyser (CE Instruments, Wigan, UK) and a SerCon 20–20 isotope ratio mass spectrometer (SerCon Ltd, Crewe, UK) at Rothamsted Research, North Wyke. A dried and ground grass herbage sample labelled with $^{15}$N (2.79 atom% $^{15}$N) or natural abundance wheat flour (0.368 atom% $^{15}$N), both calibrated against IAEA-N-1 by Iso- Analytical (Crewe, UK), were used as the references for enriched or natural abundance samples, respectively. $^{15}$N excess atom% values, $^{15}$N concentrations in samples, total amounts of $^{15}$N in pools and total ecosystem $^{15}$N retention were calculated using the following calculations (De Vries et al., 2012a):

1. atom% excess $^{15}$N = atom% $^{15}$N enriched – atom% $^{15}$N natural abundance

2. $^{15}$N sample (mg g$^{-1}$) = atom% excess $^{15}$N $\times$ N sample (mg g$^{-1}$)/100

3. $^{15}$N pool (kg ha$^{-1}$) = $^{15}$N sample (mg g$^{-1}$) $\times$ pool (g pot$^{-1}$) $\times$ 352,698 (pots ha$^{-1}$)/1 $\times$ 10$^{6}$ (conversion from mg to kg)

4. $^{15}$N retention (kg ha$^{-1}$) = $^{15}$N shoot + $^{15}$N root + $^{15}$N soil + $^{15}$N microbes (all in kg ha$^{-1}$)

Trait analyses

An additional set of monocultures of each plant species was grown under the same conditions (duration, planting density, soil type) in the experimental blocks of the main experiment for the analysis of leaf traits. Leaf trait analyses were not done on the main experiment because this would compromise the accuracy of $^{15}$N analysis, for which all above-ground plant tissue is needed. One healthy leaf was cut under water from five individuals per species and rehydrated overnight below 6°C (Garnier et al., 2001). Leaf N content and C : N ratio, SLA and LDMC were measured using standard protocols (Cornelissen et al., 2003; Perez-Harguindeguy et al., 2013). In addition, intact root systems of five individuals were washed and kept in 10% ethanol until analysis for specific root length (SRL), root diameter and root tissue density (RTD), using WinRhizo$^\text{®}$ root analysis software (Regent Instruments Inc., Sainte-Foy-Sillery-Cap-Rouge, QC, Canada) and an Epson flatbed scanner. After analysis, roots were blotted dry, weighed, dried at 60°C for 48 h, and reweighed for root dry matter content (RDMC). Dry root samples were ground and analysed for C and N content using an Elementar Vario EL elemental analyzer.

Community-weighted means for measured leaf functional traits were calculated using trait values per species and species relative abundance in treatments, assessed as DW (Garnier et al., 2004). In addition, trait functional diversity (Fdiv), trait functional divergence (FDiv), Rao’s quadratic entropy (Rao), functional richness (FRich), and functional evenness (FEve) (Mouchet et al., 2010), were calculated using FDIVERSITY software, as described by Casanoves et al. (2011). Although above-ground plant community composition does not necessarily represent below-ground species abundances, we also calculated CWM root traits based on root trait values per species.

Statistical analyses

All data were checked for assumptions of normality and log-transformed where necessary. We used linear mixed effect models (function lme in the R package nlme) to test species and functional group effects on species-level trait measurements (with species as a nested factor to account for multiple measurements on one species). Species-level root and leaf traits were examined by principal component analysis (PCA) using the R package vegan, and correlations between traits were analysed using Spearman’s rank correlations. Treatment and plant community effects (number of categories, number of species and average category) on plant community attributes and N pools and retention were analysed using linear models (function lm in R). All analyses were done in R 3.2.0 (R Core Team, 2012).

We performed structural equation modelling to test direct and indirect controls of plant community attributes, CWM traits, and trait diversity and divergence on ecosystem N pools and retention. This is a robust statistical method to test how experimental data fit a hypothesized causal structure that is well suited for investigating interactions between multiple traits and ecosystem functioning based on prior knowledge (Grace, 2006; Garcia-Palacios et al., 2013). A priori models were constructed based on our hypotheses and theoretical knowledge of plant–microbe controls on N uptake and retention (Figs 1, 2). We selected plant community properties to be included based on their significance for explaining $^{15}$N pools in regression analyses, as detailed earlier. We first fitted models including only leaf traits, after which we fitted models including both leaf and root traits. Data were rescaled to correct for large differences in variances and we fitted our a priori models to the rescaled data using the lavaan package in R. We used model modification indices and stepwise removal of nonsignificant relationships, and tested the effect of these removals on Akaiake information criterion (AIC) and model fit using a likelihood ratio test. We used a minimum set of parameters to assess model fit, including $\chi^2$, root mean square error of approximation (RMSEA), and comparative fit index (CFI). Adequate model fits are indicated by a nonsignificant $\chi^2$ test
(\(P<0.05\)),
any change of CWM root traits with category rank (Table 5). Still, as intended, our constructed plant communities represented a range of CWM functional trait values, for both leaf and root traits (Fig. S4; Table S2). Both CWM leaf N and CWM root N values were positively correlated with shoot N content and root N content of total above-ground and below-ground vegetation (Fig. S5), but were overestimating actual N content of these pools. This was more apparent for CWM root N, which also had a considerably lower predictive power (Fig. S5). As a result of differences between grasses and herbs for LDMC, leaf N, SRL and root N (Table 3; Fig. S2), CWM values of these traits were strongly affected by the proportion of herb biomass of total above-ground biomass (Fig. S6). The proportion of herb biomass itself was not affected by our treatments (Table 5).

We found that functional diversity increased with both number of species richness ($P = 0.0049, R^2 = 0.14$; Fig. S3b; Table 5) and realized species richness ($P < 0.0001, R^2 = 0.33$; Fig. S3d; Table 5); root biomass increased weakly with species richness ($P = 0.036, R^2 = 0.08$; Fig. S3c; Table 5); and Rao’s quadratic entropy also increased weakly with species richness ($P = 0.010, R^2 = 0.12$; Fig. S3e; Table 5). By contrast, functional richness decreased with species richness ($P < 0.0001, R^2 = 0.30$; Fig. S3f; Table 5), and above-ground biomass was not affected by our treatments (Table 5).

Table 3 Mean trait values ± SE for grasses and herbs and P-values for their difference (grasses, $n = 59$; herbs, $n = 58$)

| Plant trait measure | Grasses | Herbs | $P$-value |
|---------------------|---------|-------|-----------|
| LDMC (g g$^{-1}$)   | 0.28 ± 0.01 | 0.17 ± 0.01 | < 0.001   |
| SLA (mm$^2$ mg$^{-1}$) | 30.6 ± 1.3 | 31.3 ± 1.1 | 0.745     |
| Leaf N (mg g$^{-1}$)  | 13.2 ± 0.4 | 19.3 ± 0.5 | 0.030     |
| RDMC (g g$^{-1}$)   | 0.29 ± 0.03 | 0.21 ± 0.02 | 0.087     |
| SRL (cm g$^{-1}$)   | 29638 ± 772 | 17609 ± 1407 | 0.002     |
| Root N (mg g$^{-1}$) | 7.3 ± 0.1 | 9.7 ± 0.4 | 0.011     |
| RTD (g cm$^{-3}$)   | 0.17 ± 0.01 | 0.20 ± 0.02 | 0.422     |

LDMC, leaf dry matter content; SLA, specific leaf area; leaf N, leaf N content; RDMC, root dry matter content; SRL, specific root length; root N, root N content; RTD, root tissue density.

Table 4 Spearman’s rank correlation matrix of plant traits measured for all 24 species occurring in the experimental treatments ($n = 117$). Values indicate $R$ values; values in bold are $P < 0.05$

| Trait | LDMC | SLA | Leaf N | RDMC | SRL | Root N | RTD |
|-------|------|-----|--------|------|-----|--------|-----|
| LDMC | –0.18 | –0.57 | 0.47 | 0.27 | –0.36 | 0.14 |
| SLA  | 0.46 | 0.34 | –0.02 | –0.02 | 0.53 |
| Leaf N | –0.13 | –0.44 | 0.20 | 0.26 |
| RDMC | –0.03 | –0.40 | 0.68 |
| SRL  | 0.11 | –0.39 | 0.45 |
| Root N | 0.17 | 0.45 |
| RTD  | 0.17 | 0.45 |

LDMC, leaf dry matter content; SLA, specific leaf area; leaf N, leaf N content; RDMC, root dry matter content; SRL, specific root length; root N, root N content; RTD, root tissue density.

Effects of plant traits and community attributes on $^{15}$N pools and retention

The greatest amount of added $^{15}$N was taken up by soil microbes, followed by plant tissue (root and shoot) and soil (Fig. 4). These pools were not affected by the plant community treatments (category rank, number of categories and species richness), although root uptake of $^{15}$N decreased with greater category rank (i.e. communities constructed to be dominated by exploitative traits, $P = 0.004, R^2 = 0.12$; Fig. 5a). Although treatment effects on $^{15}$N pools and retention were limited, several plant and microbial community attributes were related to $^{15}$N pools and retention. Total plant $^{15}$N uptake increased with root biomass ($P < 0.001, R^2 = 0.50$; Fig. 5b). Microbial uptake of $^{15}$N decreased with microbial C:N ratio ($P < 0.001, R^2 = 0.29$; Fig. 5c), and the retention of $^{15}$N in the plant–soil system increased, albeit weakly, with root biomass ($P = 0.07, R^2 = 0.08$; Fig. 5d). Finally, the amount of $^{15}$N leached from the system increased with CWM RDMC and decreased with functional diversity ($P = 0.016, R^2 = 0.10$; Fig. 5e; and $P = 0.05, R^2 = 0.07$; Fig. 5f). Herb biomass increased both root and shoot uptake of $^{15}$N ($P = 0.0010, R^2 = 0.18$ and $P = 0.0014, R^2 = 0.17$, respectively; Fig. 5g).

All inorganic N that was leached consisted of the added $^{15}$N ($P < 0.001, R^2 = 0.84$; Fig. 5h), and the amount of $^{15}$N leached...
was strongly positively linked to the amounts of DON and DOC leached ($P < 0.001$, $R^2 = 0.25$, and $P = 0.006$, $R^2 = 0.13$, respectively; Fig. S8b,c). The amounts of $^{15}$N leached, inorganic N leached and DOC leached all significantly increased with greater CWM LDMC and RDMC (Fig. S9); DON leached was not explained by any plant community properties. The only system $^{15}$N pool that significantly explained the total amount of $^{15}$N retained in the system was soil $^{15}$N ($P < 0.001$, $R^2 = 0.44$), which itself was not explained by any plant community properties (Fig. S10).

Our structural equation models (SEMs) revealed that plant traits and plant community attributes both directly and indirectly controlled $^{15}$N uptake in the various ecosystem pools, and the amount of $^{15}$N retained in the plant–soil system after leaching. The SEM for explaining $^{15}$N leaching and plant and microbial $^{15}$N pools only using leaf traits fitted the data well ($\chi^2 = 11.014$, df = 11, $P = 0.442$; CFI = 1.000; RMSEA < 0.05, $P = 0.563$), and showed that root biomass and the proportion of herbs directly controlled plant $^{15}$N uptake, while CWM SLA indirectly controlled both plant and microbial $^{15}$N uptake through its effect on microbial biomass C:N ratio (Fig. 6). Root biomass strongly increased plant uptake, which subsequently decreased $^{15}$N leached (the standardized indirect effect of root biomass on $^{15}$N leached = $0.774 \times -0.492 = -0.381$). The proportion of herbs in above-ground biomass also decreased N leaching through its effect on plant $^{15}$N uptake, although this indirect effect was weaker than that of root biomass on $^{15}$N leaching (indirect effect of herb proportion on $^{15}$N leached = $0.288 \times -0.492 = -0.142$). Higher CWM SLA decreased the microbial C:N ratio, which in turn increased microbial $^{15}$N uptake (indirect effect of SLA on microbial $^{15}$N leached = $-0.341 \times -0.539 = 0.183$), and decreased plant $^{15}$N uptake (indirect effect of SLA on plant $^{15}$N uptake = $-0.341 \times 0.206 = -0.070$).

When we used both leaf and root traits in our model explaining $^{15}$N leaching and plant and microbial $^{15}$N pools, RTD was retained as a significant predictor, while SLA dropped out (Table S5). However, although RTD was a better predictor for $^{15}$N pools than SLA, still the model including RTD had a higher AIC than our model including only SLA (2546.4 for the model including RTD vs 2516.5 for the model including SLA; Figs 6 and 7). This model included many similar relationships to that including only SLA; however, one major difference was that plant $^{15}$N uptake decreased with a higher RTD (Fig. 7). In addition, apart from a higher RTD decreasing microbial C:N ratio and indirectly increasing microbial $^{15}$N uptake (indirect effect of RTD on microbial $^{15}$N uptake = $-0.365 \times -0.623 = 0.227$),
In addition to testing our *a priori* SEMs explaining plant and microbial $^{15}$N uptake and leaching of $^{15}$N, we used SEM to test which plant community properties explained $^{15}$N retention in the plant–soil system, which is the sum of $^{15}$N retained in plants, microbes and soil (Fig. 2). Our final model for $^{15}$N retention (the sum of plant, microbial and soil $^{15}$N retained in the system after leaching) only included a few predictors (Fig. 8) – this model did not change when including both leaf and root traits. The fit of this model was good ($\chi^2 = 4.309$, $df = 5$, $P = 0.506$; $CFI = 1.000$; $RMSEA < 0.05$, $P = 0.582$), with $^{15}$N retention being strongly linked to the total root N pool, which in turn was affected by LDMC and root biomass; LDMC content was reduced by the proportion of herbs in above-ground biomass. This lower LDMC increased the total root N pool and thus indirectly $^{15}$N retention (indirect effect of herb proportion on $^{15}$N retention $= -0.853 \times -0.399 \times 0.330 = 0.112$, and of LDMC on $^{15}$N retention $= -0.399 \times 0.330 = -0.132$). Greater root biomass indirectly increased $^{15}$N retention through its positive effect on the total root N pool (indirect effect of root biomass on $^{15}$N retention $= -0.800 \times 0.330 \times 0.33 = 0.264$).

**Discussion**

Our experimental treatments created a gradient of CWM leaf traits, functional diversity and divergence representative of those found in the field. For example, in a previous study covering a range of grassland types across England, CWM SLA ranged from 17.6 to 35.1 $\text{mm}^2 \text{g}^{-1}$, CWM LDMC from 0.15 to 0.35 $\text{g} \text{g}^{-1}$, and CWM leaf N from 17.8 to 35.1 $\text{mg} \text{g}^{-1}$ (De Vries *et al.*, 2012b). In comparison, in our treatments, CWM SLA ranged from 19.8 to 41.8 $\text{mm}^2 \text{g}^{-1}$, CWM LDMC ranged from 0.14 to 0.33 $\text{g} \text{g}^{-1}$, and CWM leaf N ranged from 7.57 to 20.89 $\text{mg} \text{g}^{-1}$. Given this, the gradients in CWM leaf traits produced in our study allowed us to test our contrasting hypotheses on plant community controls on ecosystem N retention. We hypothesized that either the dominance of conservative leaf traits controls plant and microbial N uptake and hence N leaching loss and ecosystem N retention, or that trait functional diversity or divergence enhanced N retention through greater plant N uptake. We found that root biomass, the proportion of herbs in communities, dominant leaf traits and, to a lesser extent, dominant root traits controlled $^{15}$N uptake by plants and microbes, and $^{15}$N leached. Thus, our results support the mass-ratio hypothesis, rather than the diversity hypothesis.

Although root biomass only increased marginally with higher species richness, greater root biomass significantly increased plant $^{15}$N uptake and indirectly increased microbial $^{15}$N uptake, reducing the amount of $^{15}$N leached and increasing $^{15}$N retention in the plant–soil system. The proportion of herbs in our plant communities increased plant $^{15}$N uptake, while a higher CWM SLA indirectly increased microbial $^{15}$N uptake, and a higher CWM RTD decreased plant $^{15}$N uptake. These results confirm the central role of roots in ecosystem N retention (De Vries *et al.*, 2012a), and corroborate findings that plants with exploitative growth strategies have the highest rates of N uptake (Grassein *et al.*, 2015). However, they contradict the notion of lower plant N uptake with higher species richness.

**Table 5** Statistics for linear models of treatment effects on plant community properties

| Predictor | Response variable | $R^2$ | $P$-value |
|-----------|------------------|------|----------|
| Species richness | Above-ground biomass | 0.006 | 0.565 |
| Root biomass | 0.079 | 0.036 |
| Herb proportion | 0.008 | 0.507 |
| Functional diversity | 0.334 | < 0.001 |
| Functional divergence | 0.014 | 0.383 |
| Functional richness | 0.295 | < 0.001 |
| Rao’s quadratic entropy | 0.117 | 0.010 |
| Evenness | 0.027 | 0.228 |
| Shannon’s diversity | 0.719 | < 0.001 |
| Nr of categories | Above-ground biomass | < 0.001 | 0.998 |
| Root biomass | 0.026 | 0.232 |
| Herb proportion | 0.010 | 0.446 |
| Functional diversity | 0.138 | < 0.0049 |
| Functional divergence | < 0.001 | 0.975 |
| Functional richness | 0.048 | 0.105 |
| Functional evenness | 0.006 | 0.648 |
| Rao’s quadratic entropy | 0.030 | 0.201 |
| Evenness | 0.022 | 0.279 |
| Shannon’s diversity | 0.266 | < 0.001 |
| Category average | Above-ground biomass | 0.001 | 0.861 |
| Root biomass | 0.001 | 0.771 |
| Herb proportion | 0.010 | 0.463 |
| CWM SLA | 0.363 | < 0.001 |
| CWM LDMC | 0.028 | 0.211 |
| CWM leaf N | 0.004 | 0.662 |
| CWM SRL | 0.004 | 0.644 |
| CWM RDMC | 0.067 | 0.055 |
| CWM root N | 0.021 | 0.286 |
| CWM RTD | 0.042 | 0.131 |

For minimum, maximum and average values for these properties see Supporting Information Table S2.

CWM, community-weighted mean; LDMC, leaf dry matter content; SLA, specific leaf area; leaf N, leaf N content; RDMC, root dry matter content; SRL, specific root length; root N, root N content; RTD, root tissue density.

Fig. 4 Uptake of $^{15}$N in the various ecosystem pools. The size of $^{15}$N pools was not affected by the number of categories. Bars represent treatment means ± 1 SE ($n = 24$ for one and two categories, $n = 8$ for three categories). A higher RTD also directly decreased microbial $^{15}$N uptake. Still, root biomass was the best predictor for plant $^{15}$N uptake, and indirectly for $^{15}$N leaching (indirect effect of root biomass on $^{15}$N leached $= 0.680 \times -0.480 = -0.326$; Fig. 7).
(De Vries & Bardgett, 2012), and field observations (Laughlin, 2011; De Vries et al., 2012a; Grigulis et al., 2013), that plant communities dominated by slow-growing, resource-conservative species and their associated microbial communities have the greatest N retention.

Our treatments created a wide gradient in CWM SLA, which was also the trait included in our SEM for explaining plant and microbial $^{15}$N uptake and $^{15}$N leaching. CWM SLA indirectly increased microbial $^{15}$N uptake through modifying the microbial C : N ratio. Greater CWM SLA decreased the microbial C : N ratio, apparently alleviating microbial N limitation and potentially indicating a shift towards more bacterial-dominated microbial communities, which are characterized by a lower C : N ratio than fungal-dominated communities (Van Veen & Paul, 1979; Bloem et al., 1997). This link between exploitative plant traits and C-limited, bacterial-dominated microbial communities supports similar findings from field observations (Orwin et al., 2010; De Vries et al., 2012b; Grigulis et al., 2013). These linkages between plant traits and microbial communities are often attributed to the quality and quantity of plant litter inputs (Bardgett & Wardle, 2010), but the duration of our experiment was too short to allow for significant litter inputs. Therefore, it is more likely that root processes influenced microbial communities. We found that CWM RTD affected microbial C : N ratio and microbial $^{15}$N uptake in the same direction as CWM SLA. This follows the positive correlation we found between these two traits, but is contrary to our expectation, as higher RTD indicates a greater investment in tissue longevity and efficient C use, and would thus be placed towards the conservative end of the root economics spectrum. By contrast, the decreased plant $^{15}$N uptake with higher CWM RTD suggests that this trait is associated with conservative growth strategies.

In contrast to our expectation, lower microbial C : N ratio was associated with greater microbial $^{15}$N uptake, indicating that despite an alleviation of N limitation, these microbes had the greatest affinity for N. This might indicate a shift to greater relative abundance of bacteria, which have been suggested to be able to use larger amounts of readily available N than fungi (Myrold & Posavatz, 2007), despite many studies reporting greater $^{15}$N immobilization in fungal-dominated microbial communities (De Vries et al., 2011, 2012a). Our results therefore indicate that soil microbial communities that are not N-limited have the greatest affinity for available N and can increase N retention in the plant–soil system, especially given that the $^{15}$N immobilized in microbes exceeded that in plants (Fig. 5). Our SEM including CWM SLA shows that a lower microbial C : N ratio decreased plant $^{15}$N uptake, indicating an intensified competition for N between plants and microbes, as also found by Moreau et al. (2015). Although we found no direct competition between plant and microbial $^{15}$N pools, this is supported by our finding that...
CWM RTD increased microbial $^{15}$N, but decreased plant $^{15}$N uptake.

The proportion of herbs in our plant communities was an important determinant of plant $^{15}$N uptake, and thus indirectly of $^{15}$N retention. Total plant $^{15}$N uptake was higher with an increased proportion of herbs, and on an individual plant level, herbs had higher shoot $^{15}$N uptake than grasses. This is in contrast to previous findings of greater N allocation into root and shoot biomass in grasses compared with herbs (Robson et al., 2010), and of reduced N leaching with higher grass abundance, which has been attributed to their thin and dense root systems (Phoenix et al., 2008; De Vries et al., 2015). Indeed, we found that herbs had higher SRL than grasses, but this was not the trait that explained plant $^{15}$N uptake on a community or individual species level. Herbs also had lower LDMC and higher root and leaf N than grasses, of which leaf N might underlie their higher uptake of $^{15}$N, as this trait best explained individual species $^{15}$N uptake. In addition, higher LDMC reduced the root N pool in our plant communities, which in turn reduced ecosystem $^{15}$N retention. These findings are in line with the findings by Grassein et al. (2015), who found that the uptake and affinity for N of individual grasses increased with exploitative leaf traits. Importantly, although herbs differed from grasses in most traits and thus affected CWM values of these traits in our mixtures, they did not differ in SLA and RTD, which were the traits that best explained plant and microbial $^{15}$N uptake in our SEMs.

Greater species richness did not result in greater above-ground biomass, but it did result in overyielding below ground (Ravenek.
et al., 2014), and this greater root biomass was associated with a reduced microbial C:N ratio, which in turn increased microbial 15N uptake. Greater root biomass also strongly increased plant 15N uptake (Figs 3b, 5, 6) and the total root N pool (Fig. 8), and hence 15N retention (Fig. 3d). Root N was increased with lower CWM values of the conservative trait LDMC, and this greater root N pool increased 15N retention (Fig. 8). These results corroborate previous studies that show the importance of root N uptake for ecosystem N retention (Zogg et al., 2000; De Vries et al., 2012a, 2015). Moreover, they point to the dominance of exploitative plant traits, namely low CWM LDMC and high root N content, enhancing ecosystem N retention. This is in line with results from Garcia-Palacios et al. (2013), who found, in a pot experiment similar in scale and duration to ours, that CWM SLA reduced soil N availability. Our results suggest that greater root and shoot uptake in plant communities dominated by conservative species, as reported in field observations (De Vries et al., 2012a; Hoefl et al., 2014; but see Bingham & Biondini, 2011), might be a result of low nutrient availability, rather than of a greater affinity for N of slow-growing, resource-conservative plant species.

The total amount of 15N retained in our system consisted of the sum of 15N in plant, microbes, and soil. Although 15N uptake in plants and microbes, as well as the amount of 15N leached from the system, were well-explained by root biomass, the proportion of herbs, and CWM leaf and root traits, we struggled to find adequate predictors of ecosystem 15N retention. We found that the total amount of 15N retained was best explained by the amount of 15N that was retained in soil (Fig. S10), which in itself was a highly variable pool that was not related to any plant community attributes. In addition, there was much unexplained variation in many of our measured 15N pools. Since our soil was sieved, homogenized, and packed to the same bulk density across all pots, we do not believe that this unexplained variation was caused by differences in soil texture, density, pH, or organic matter content, which all play a role in the adsorption of positively charged ions such as ammonium (Six et al., 1998; Denef et al., 2002; Gonod et al., 2006). However, this unexplained variation might point to the importance of particular groups of microbes and their activities, which can be linked to plant functional traits, for explaining variation in 15N pools (e.g. Cantarel et al., 2015; Moreau et al., 2015).

Despite the need for caution in calculating CWM root traits based on above-ground species abundances, we found that several CWM root traits affected plant and microbial 15N uptake, but our SEM including CWM SLA was superior to that including CWM RTD. This might be a result of the discrepancy between above-ground and below-ground community composition rather than root traits actually being worse predictors for these pools, as several studies have found that root traits have a stronger control on ecosystem N dynamics and retention than above-ground functional traits, which were the focus of our study (Grigulis et al., 2013; Bardgett et al., 2014; but see Grassey et al., 2015). The correlations we found between leaf and root traits support previous work (Craine et al., 2005; Tjoelker et al., 2005; Roumet et al., 2006; Freschet et al., 2010); however, they do not support the existence of a root economics spectrum, as above-ground exploitative traits correlated strongly with below-ground traits considered to be conservative.

Collectively, our results show that root biomass, herb abundance and the dominance of exploitative leaf traits, namely high SLA and leaf N and low LDMC, directly and indirectly increase short-term ecosystem N retention. However, caution is needed when interpreting these results: plant communities dominated by fast-growing, resource-exploitative species and their associated microbial communities might rapidly take up available N, but high rates of nutrient cycling also mean that N is remineralized (Bengtson & Bengtsson, 2005) and potentially lost quickly from ecosystems. We did not measure this process, but it can be relevant at longer timescales. Nevertheless, our results show that N addition increases N uptake by exploitative plants and microbes, thereby possibly favouring their dominance in the longer term. In sum, we show that dominant plant traits, rather than trait functional diversity, contribute to the fate of added N in the plant-soil system.

Acknowledgements

This work was supported by the EU 7th Framework project SOILSERVICE, and F.T.d.V. is additionally funded by a BBSRC David Phillips Fellowship (BB/L02456X/1). We thank Victor van Velzen and Louise Walker for help in the laboratory, and Liz Dixon of Rothamsted Research North Wyke for 15N analyses.
Author contributions
F.T.d.V and R.D.B planned and designed the research; F.T.d.V. performed the experiment and analysed the data; and F.T.d.V. and R.D.B. wrote the manuscript.

References

Bardgett RD, Mommer L, De Vries FT. 2014. Going underground: root traits as drivers of ecosystem processes. Trends in Ecology & Evolution 29: 692–699.

Bardgett RD, Streeter TC, Bol R. 2003. Soil microbes compete effectively with plants for organic-nitrogen inputs to temperate grasslands. Ecology 84: 1277–1287.

Bardgett RD, Wardle DA. 2010. Aboveground—belowground linkages. Biotic interactions, ecosystem processes, and global change. New York, NY, USA: Oxford University Press.

Bengtsen P, Bengtsson G. 2005. Bacterial immobilization and remineralization of N at different growth rates and N concentrations. FEMS Microbiology Ecology 58: 13–19.

Berendse F, Moller F. 2009. Incorporating plant functional diversity effects in ecosystem service assessments. Proceedings of the National Academy of Sciences, USA 104: 20684–20689.

De Vries FT, Van Groenigen JW, Hofland E, Bloem J. 2011. Nitrogen losses from two grassland soils with different fungal biomass. Soil Biology & Biochemistry 43: 997–1005.

De Vries FT, Bloem J, Quirk H, Stevens CJ, Bol R, Bardgett RD. 2012a. Extensive management promotes plant and microbial nitrogen retention in temperate grassland. PLoS ONE 7: e51201.

De Vries FT, Bracht Jørgensen H, Hedlund K, Bardgett RD. 2015. Disentangling plant and soil microbial controls on carbon and nitrogen loss in grassland mesocosms. Journal of Ecology 103: 629–640.

De Vries FT, Manning P, Tallowin JRB, Mortimer SR, Pilgrim ES, Harrison KA, Hobbs PJ, Quirk H, Shipley B, Cornelissen JHC et al. 2012b. Abiotic drivers and plant traits explain landscape-scale patterns in soil microbial communities. Ecology Letters 15: 1230–1239.

De Vries FT, Van Groenigen JW, Hofland E, Bloem J. 2011. Nitrogen losses from two grassland soils with different fungal biomass. Soil Biology & Biochemistry 43: 997–1005.

Defra. 2011. UK pollutant deposition. URL: http://pollutantdeposition.defra.gov.uk [accessed 31 December 2015]

Denef K, Six J, Mercx R, Paustian K. 2002. Short-term effects of biological and physical forces on aggregate formation in soils with different clay mineralogy. Plant and Soil 246: 185–200.

Diaz S, Hodgson GJ, Thompson K, Cabido M, Cornelissen JHC, Jalili A, Montserrat-Martí G, Grime JP, Zarrínkamar F, Asy I et al. 2004. The plant traits that drive ecosystems: evidence from three continents. Journal of Vegetation Science 15: 295–304.

Diaz S, Lavelle P, de Bello F, Quétier F, Grigulis K, Robson M. 2007. Incorporating plant functional diversity effects in ecosystem service assessments. Proceedings of the National Academy of Sciences, USA 104: 20684–20689.

Freschet GT, Cornelissen JHC, van Logtestijn RSP, Aerts R. 2010. Evidence of the ‘plant economics spectrum’ in a subarctic flora. Journal of Ecology 98: 362–373.

Fujita Y, Robroek BJM, de Ruiter PC, Heil GW, Wassen MJ. 2010. Increased N affects P uptake of eight grassland species: the role of root surface phosphatase activity. Oikos 119: 1665–1673.

Galloway JN, Townsend AR, Erisman JW, Bekunda M, Cai Z, Frenney JR, Martinelli LA, Seitzinger SP, Sutton MA. 2008. Transformation of the nitrogen cycle: recent trends, questions, and potential solutions. Science 320: 889–892.

Garcia-Palacios P, Maestre FT, Milla R. 2013. Community-aggregated plant traits interact with soil nutrient heterogeneity to determine ecosystem functioning. Plant and Soil 364: 119–129.

Garner E, Cortez J, Billes G, Navas ML, Roumet C, Debussche M, Laurent G, Blanchard A, Aubry D, Bellmann A et al. 2004. Plant functional markers capture ecosystem properties during secondary succession. Ecology 85: 2630–2637.

Garner E, Shipley B, Roumet C, Laurent G. 2001. A standardized protocol for the determination of specific leaf area and leaf dry matter content. Functional Ecology 15: 688–695.

Gonod LV, Jones DL, Chenu C. 2006. Sorption regulates the fate of the amino acids lysine and leucine in soil aggregates. European Journal of Soil Science 57: 320–329.

Grace JB. 2006. Structural equation modeling and natural systems. Cambridge, UK: Cambridge University Press.

Grassein F, Lemauviel-Lavenant S, Lavelle P, Bahn M, Bardgett RD, Desclous-Theveniau M, Laine P. 2015. Relationships between functional traits and inorganic nitrogen acquisition among eight contrasting European grass species. Annals of Botany 115: 107–115.

Grigulis K, Lavelle P, Krawiec U, Legay N, Baxendale C, Dumont M, Kastl E, Arnoldi C, Bardgett RD, Poly F et al. 2013. Relative contributions of plant traits and soil microbial properties to mountain grassland ecosystem services. Journal of Ecology 111: 47–57.

Grime JP. 1998. Benefits of plant diversity to ecosystems: immediate, filter and founder effects. Journal of Ecology 86: 902–910.

Grime JP, Hodgson JG, Hunt R. 2007. Comparative plant ecology: a functional approach to common British species. Colvent, Dalbeattie, UK: Castlepoint Press.

Harrison KA, Bardgett RD. 2010. Influence of plant species and soil conditions on plant–soil feedback in mixed grassland communities. Journal of Ecology 98: 384–395.

Harrison KA, Bol R, Bardgett RD. 2008. Do plant species with different growth strategies vary in their ability to compete with soil microbes for chemical forms of nitrogen? Soil Biology & Biochemistry 40: 228–237.
Hodge A, Fitter AH. 2010. Substantial nitrogen acquisition by arbuscular mycorrhizal fungi from organic material has implications for N cycling. Proceedings of the National Academy of Sciences, USA 107: 13754–13759.

Hofert I, Keuter A, Quinones CM, Schmidt-Walter P, Veldkamp E, Corde MD. 2014. Nitrogen retention efficiency and nitrogen losses of a managed and phytodiverse temperate grassland. Basic and Applied Ecology 15: 207–218.

Hooper DU, Vitousek PM. 1998. Effects of plant composition and diversity on nutrient cycling. Ecological Monographs 68: 121–149.

Johnson KH, Vogt KA, Clark HJ, Schmitz OJ, Vogt DJ. 1996. Biodiversity and the productivity and stability of ecosystems. Trends in Ecology & Evolution 11: 372–377.

Kaye JP, Hart SC. 1997. Competition for nitrogen between plants and soil microorganisms. Trends in Ecology & Evolution 12: 139–143.

Klumpp K, Soussana JF. 2009. Using functional traits to predict grassland ecosystem change: a mathematical test of the response-and-effect trait approach. Global Change Biology 15: 2921–2934.

Laughlin DC. 2011. Nitrification is linked to dominant leaf traits rather than functional diversity. Journal of Ecology 99: 1091–1099.

Lavorel S, Garnier E. 2002. Predicting changes in community composition and ecosystem functioning from plant traits: revisiting the Holy Grail. Functional Ecology 16: 545–556.

Lavorel S, Storey J, Bardgett RD, de Bello F, Le Roux X, Moretti M, Mulder C, Pakeman RJ, Diaz S et al. 2013. A novel framework for linking functional diversity of plants with other trophic levels for the quantification of ecosystem services. Journal of Vegetation Science 24: 942–948.

Legay N, Baxendale C, Grigulis K, Krauter U, Kastl E, Schloter M, Bardgett RD, Arnoldi C, Bahn M, Dumont M et al. 2014. Contribution of above- and below-ground plant traits to the structure and function of grassland soil microbial communities. Annals of Botany 114: 1011–1021.

Mäder P, Vierheilig H, Steerwalt-Engel R, Boller T, Frey B, Christie P, Wiemken A. 2000. Transport of 15N from a soil compartment separated by a polytetrafluoroethylene membrane to plant roots via the hyphe of arbuscular mycorrhizal fungi. New Phytologist 146: 155–161.

Milcu A, Allan E, Roscher C, Jenkins T, Meyer ST, Veldkamp E, Schmitz OJ, Schmidt-Walter P, Veldkamp E, Corre MD. 2015. The role of roots in the resource economics spectrum. New Phytologist 195: 725–727.

Moreau D, Picat B, Bru D, Busset H, Deau F, Faivre C, Matejicka A, Srzik B, Philippot L, Mougel C. 2015. Plant traits related to nitrogen uptake influence plant-microbe competition. Ecology 96: 2300–2310.

Mouchet MA, Villeger S, Mason NW, Mouillot D. 2010. Functional diversity measures: an overview of their redundancy and their ability to discriminate community assembly rules. Functional Ecology 24: 867–876.

Mulder CPH, Jumpponen A, Hogberg P, Huss-Danell K. 2002. How plant diversity and legumes affect nitrogen dynamics in experimental grassland communities. Oecologia 133: 412–421.

Myrold DD, Posavatz NR. 2007. Potential importance of bacteria and fungi in nitrate assimilation in soil. Soil Biology & Biochemistry 39: 1737–1743.

Naem S. 2002. Disentangling the impacts of diversity on ecosystem functioning in combinatorial experiments. Ecology 83: 2925–2935.

Orwin KH, Buckland SM, Johnson D, Turner BL, Smart S, Oakley S, Bardgett RD. 2010. Linkages of plant traits to soil properties and the functioning of temperate grassland. Journal of Ecology 98: 1074–1083.

Perez-Harguindeguy N, Diaz S, Garnier E, Lavorel S, Poorter H, Jauregiberry P, Bret-Harte MS, Cornell WK, Craine JM, Gurvich DE et al. 2013. New handbook for standardised measurement of plant functional traits worldwide. Australian Journal of Botany 61: 167–234.

Phoenix GK, Johnson D, Grime JP, Booth RE. 2008. Sustaining ecosystem services in ancient limestone grassland: importance of major component plants and community composition. Journal of Ecology 96: 894–902.

Pugesek BH, Tomer A, von Eye A. 2003. Structural equation modelling. Cambridge, UK: Cambridge University Press.

Ravenek JM, Bessler H, Engels C, Scherer-Lorenzen M, Gessler A, Gockele A, De Luca E, Temperton VM, Ebeling A, Roscher C et al. 2014. Long-term study of root biomass in a biodiversity experiment reveals shifts in diversity effects over time. Oikos 123: 1528–1536.

R Core Team. 2012. R: a language and environment for statistical computing. Vienna, Austria: R Foundation for Statistical Computing.

Robson TM, Baptist F, Clement JC, Lavorel S. 2010. Land use in subalpine grasslands affects nitrogen cycling via changes in plant community and soil microbial uptake dynamics. Journal of Ecology 98: 62–73.

Roscher C, Schumacher J, Gubisch M, Lipowsky A, Weigelt A, Buchmann N, Schmid B, Schulze E-D. 2012. Using plant functional traits to explain diversity-productivity relationships. PLoS ONE 7: e36760.

Roumet C, Urcelay C, Diaz S. 2006. Suitability of root traits differ between annual and perennial species growing in the field. New Phytologist 170: 357–368.

Scherer-Lorenzen M, Palmborg C, Pinz A, Schulze ED. 2003. The role of plant diversity and composition for nitrate leaching in grasslands. Ecology 84: 1539–1552.

Schlesinger WH. 2009. On the fate of anthropogenic nitrogen. Proceedings of the National Academy of Sciences, USA 106: 203–208.

Six J, Elliott ET, Paustian K, Doran JW. 1998. Aggregation and soil organic matter accumulation in cultivated and native grassland soils. Soil Science Society of America Journal 62: 1367–1377.

Stevens CJ, Disb MB, Mountford JO, Gowing DJ. 2004. Impact of nitrogen deposition on the species richness of grasslands. Science 303: 1876–1879.

Suding KN, Ashton JW, Bechtold H, Bowman WD, Mobley ML, Winklemann R. 2008. Plant and microbe contribution to community resilience in a directionally changing environment. Ecological Monographs 78: 313–329.

Tilman D, Wedin D, Knops J. 1996. Productivity and sustainability influenced by biodiversity in grassland ecosystems. Nature 379: 718–720.

Tjoelker MG, Craine JM, Wedin D, Reich PB, Tilman D. 2005. Linking leaf and root trait syndromes among 39 grassland and savannah species. New Phytologist 167: 493–508.

Van der Heijden MGA, Kilonomos JN, Ursic M, Moutoglis P, Streitwolf ER, Boller T, Wiemken A, Sanders IR. 1998. Mycorrhizal fungal diversity determines plant biodiversity, ecosystem variability and productivity. Nature 396: 69–72.

Van Veen JA, Paul EA. 1979. Conversion of biovolume measurements of soil organisms, grown under various moisture tensions, to biomass and their nutrient content. Applied and Environmental Microbiology 37: 688–692.

Wagg C, Jansa J, Schmid B, van der Heijden MGA. 2011a. Belowground biodiversity effects of plant symbionts support aboveground productivity. Ecology Letters 14: 1001–1009.

Wagg C, Jansa J, Studler M, Schmid B, van der Heijden MGA. 2011b. Mycorrhizal fungal identity and diversity relaxes plant–plant competition. Ecology 92: 1303–1313.

Wright IJ, Reich PB, Westoby M, Ackerly DD, Baruch Z, Bongers F, Cavender-Bares J, Chapin T, Cornelissen JHC, Diemer M et al. 2004. The worldwide leaf economics spectrum. Nature 428: 821–827.

Zogg GP, Zak DR, Pregitzer KS, Burton AJ. 2000. Microbial immobilization and the retention of anthropogenic nitrate in a northern hardwood forest. Ecology 81: 1858–1866.

Supporting Information

Additional supporting information may be found in the online version of this article.

Fig. S1 PCA biplots for leaf traits and root traits for the 24 species used in the experiment.

Fig. S2 PCA biplots for leaf traits and root traits for the 24 species used in the experiment.
**Fig. S3** Treatment (average trait category, number of trait categories, and species richness; see Tables 1 and 2) effects on plant community attributes and $^{15}$N pools.

**Fig. S4** Histograms showing frequency distributions for community-weighted mean (CWM) leaf and root traits for the experimental communities.

**Fig. S5** The relationship between community-weighted mean (CWM) leaf N and root N content calculated from individual abundances and species-averaged traits and measured total community shoot and root N content.

**Fig. S6** The effect of the proportion of herb biomass of total community biomass on values for CWM traits, for leaf and root traits.

**Fig. S7** Relationships between above-ground $^{15}$N uptake and herb biomass.

**Fig. S8** Relationship between $^{15}$N leached and the amounts of inorganic N, dissolved organic N (DON), and dissolved organic C (DOC) leached.

**Fig. S9** Amounts of $^{15}$N, DON, inorganic N, and DOC leached as explained by leaf dry matter content (LDMC) and root dry matter content (RDMC).

**Fig. S10** Relationships between individual $^{15}$N pools and the amount of $^{15}$N retained in the system.

**Table S1** Leaf and root trait values per species

**Table S2** Minimum, maximum and mean values for plant community attributes in our experiment

**Table S3** Model selection procedure and statistics for the structural equation model (SEM) explaining $^{15}$N pools and leaching, only including leaf traits

**Table S4** The effect on $R^2$ of the removal of individual parameters from regressions containing multiple predictors in the final SEM for $^{15}$N pools and leaching, only including leaf traits

**Table S5** Model selection procedure and statistics for the structural equation model (SEM) explaining $^{15}$N pools and leaching, including leaf traits as well as root traits

**Table S6** The effect on $R^2$ of the removal of individual parameters from regressions containing multiple predictors in the final SEM for $^{15}$N pools and leaching, including leaf and root traits

**Table S7** Model selection procedure and statistics for the structural equation model (SEM) explaining $^{15}$N retention, including leaf traits as well as root traits

**Table S8** The effect on $R^2$ of the removal of individual parameters from regressions containing multiple predictors in the final SEM for $^{15}$N retention

Please note: Wiley Blackwell are not responsible for the content or functionality of any supporting information supplied by the authors. Any queries (other than missing material) should be directed to the *New Phytologist* Central Office.