Land use in subalpine grasslands affects nitrogen cycling via changes in plant community and soil microbial uptake dynamics

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Summary

1. Nitrogen (N) cycling is a key process determining ecosystem functioning in subalpine grasslands where traditional mowing and manuring are being abandoned. However, the roles of the plant and microbial communities in mediating changes in N availability are still poorly understood.

2. We inoculated 15 subalpine grassland fields with dual-labelled ammonium nitrate ($^{15}NH_4^+$, $^{15}NO_3^-$) during July 2005 and used pool dilutions over 1 month to calculate inorganic N fluxes into the microbial pool and uptake in plant communities by grasses, forbs and legumes. The effects of current land abandonment were assessed by comparing manured and mown terraces (ancient croplands) with other terraces where these practices have ceased, and mown versus unmown unterraced meadows.

3. Rapid cycling of inorganic N and high soil N availability in forb-dominated manured and mown terraces resulted from fast plant N uptake and low microbial C:N ratio. In grass-dominated unmown terraces, N cycling was slower and N retention was greater; microbial N uptake remained similar to that in the other terraces, although a higher C:N ratio suggested a shift towards fungal dominance.

4. In unterraced meadows, pH was low due to reduced mixing of soil with the underlying calcareous rock. Soil $[NH_4^+]$ was high and $[NO_3^-]$ low, but current management had no effect on N pool size, although plant N uptake was greater in the mown than unmown fields. This may be partially explained by high N retention by dominant *Festuca paniculata* tussocks. The microbial N pool and N uptake were both low and the microbial C:N ratio was high, suggesting that fungi slowed N cycling and reduced the influence of mowing on N turnover.

5. *Synthesis*. In these marginal long-term grasslands, with low productivity and high biodiversity value, changes in ecosystem function associated with reduced management intensity were mediated through slower N cycling. This response was expressed as more gradual nutrient uptake but greater retention by unmown plant communities, slower microbial uptake and smaller soil N pools. In contrast to more productive ecosystems, such as north-western European grasslands, reduced management is detrimental to both biodiversity and the maintenance of soil-related ecosystem services. These costs will need to be balanced against potential benefits, such as carbon storage.

Key-words: ^{15}N isotope, marginal grasslands, microbial N, N mineralization, plant functional types, pool dilution, traditional land use

Introduction

Changes in the intensity of grassland management can fundamentally affect ecosystem function by altering plant–community composition and through the manipulation of nutrient cycling (Chapin 1980; Güsewell, Jewell & Edwards 2005; Kahmen *et al.* 2006). This has particular importance in highly valued ecosystems, in both fertile-temperate grasslands where intensification of management reduces plant diversity (Losvik 1999; Bardgett 2005) and contrastingly infertile grasslands

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where the relationship between diversity and fertility is positive as soil nutrient pools are small (Grime 1973). Traditionally farmed subalpine grasslands are of particular interest in this context since the abandonment of mowing and manuring can combine with the legacy of past land use to precipitate a reduction in nitrogen (N) availability (Robson *et al.* 2007). Following the abandonment of mowing and light fertilization, shifts in the community composition of hay meadows and subalpine grasslands, from forb-rich to shrub- or tussock grass-dominated, are usually associated with a decline in fertility (Losvik 1999; Tappeiner *et al.* 1999; Zeller, Bardgett & Tappeiner 2001) and the degradation of ecosystem services such as biodiversity conservation, fodder quality and maintenance of water quality through reduced N leaching (Quétier, Thébault & Lavorel 2007). Identification of the mechanisms driving these shifts in plant communities and associated changes in microbial communities is dependent on our understanding of how both communities interact with ecosystem processes, such as N cycling (Eviner & Chapin 2003; Zak *et al.* 2003; Schimel & Bennett 2004).

Reduced soil fertility as a consequence of land-use change in marginal temperate grasslands, where N is often limiting (Miller & Bowman 2002; Kahmen *et al.* 2006), can alter relative N demand by the plant and microbial communities (Bardgett *et al.* 2005). Beyond direct N inputs, abandonment of mowing and reduction of grazing intensity in grasslands remove the competitive advantage of fast plant growth, early development and a short life cycle (Bullock *et al.* 2001; Louault *et al.* 2005). Furthermore, the encroachment of species with more conservative resource use and greater nutrient retention not only restricts N availability for the existing plant community but can also manipulate the soil microbial community through N-poor litter recycling and root exudates (Lipson & Schmidt 2004; Bardgett 2005; Chapman *et al.* 2006), which favour fungal over bacterial dominance, further slowing N cycling (Zeller *et al.* 2000).

Our study was conducted in a subalpine grassland area in the French Alps where traditionally managed terraced hay meadows, fertilized with manure once a year, were compared with unfertilized and unmown terraces. A similar comparison was made between adjacent mown and unmown unterraced meadows. Previous research in these grasslands has revealed that resin-extractable soil N decreases with abandonment of mowing for hay and manuring (Robson *et al.* 2007), and that these changes are concomitant with a shift in the dominant functional characteristics of the plant community (Quétier, Thébault & Lavorel 2007). Mowing and manuring have favoured fast-maturing N-demanding species, while the cessation of annual mowing has promoted dominance by coarse and tussock grasses in both ancient terraced fields and unterraced meadows, corresponding to reduced N availability. Denitrifying enzyme activities (DEA) were far higher in the manured and mown terraces than in terraces where mowing had ceased (Robson *et al.* 2007). In unterraced fields, denitrification followed a similar pattern of reduction with abandonment of mowing, and was lower overall than in the terraces. A positive correlation between DEA and inorganic soil N

suggested that N substrate availability limited microbial activity (Robson *et al.* 2007).

To understand the dynamic shifts in nutrient cycling that follow reduced management intervention in these subalpine grasslands, we quantified the pools and fluxes of nitrogen, adding a pulse of ^{15}N and measuring pool dilutions to calculate the rate of soil N uptake and to determine the sink strengths of N allocation to the plant and microbial compartments under contrasting management regimes. After mowing is abandoned, phenological development can be extended and nutrient retention is likely to increase in the plant community, leading us to predict slower plant N uptake and release in the less intensively managed plant communities of both the terraced and unterraced grasslands. This would result in slower depletion of ^{15}N from the soil in the unmown fields following inoculation. We also anticipate an increase in the dominance of fungi over bacteria, relative decreases in litter quality and input from the labile fraction of soil organic matter (Bardgett 2005; Booth, Stark & Rastetter 2005; Mulder 2006) reflected in a change from N-rich to N-poor soil microbial pools and contributing to a general depletion of inorganic soil N after the abandonment of mowing. As a result of these modifications we expect greater N retention by the more conservative plant and microbial communities of the unmown fields, and a small soil N pool in both unterraced land uses. Given the previously reported differences in DEAs (Robson *et al.* 2007), we suggest that microbial N uptake should be greater in the mown fields than in the unmown fields.

Materials and methods

STUDY SITE

The field site is located in the upper Romanche valley of the central French Alps (45°02′ N, 6°20′ E, 1650–2000 m a.s.l.) between the village of Villar d'Arêne and the Col du Lautaret. The climate is subalpine with a strong continental influence. Winters are cold and snowy, with a mean February temperature of −7.4 °C and mean annual precipitation of 956 mm. The growing season starts following snow melt in late April to early May and continues until late September, with the highest mean monthly temperature in July of 13 °C.

Fifteen fields (three replications of five land uses) were inoculated with ^{15}N: terraces: (i) fertilized with farmyard manure annually and mown for hay in early August (Fert + Mown), (ii) mown but not fertilized (Mown), or (iii) neither mown nor fertilized but lightly grazed in the autumn (Unmown); and unterraced fields that are (iv) mown for hay in early August (U Mown), or (v) unmown but lightly grazed (U Unmown). These same 15 fields were investigated using resin bags and soil cores during 2004 and examined for microbial enzyme activity in May 2005 (Robson *et al.* 2007); their plant communities and site characteristics have been well described (Quétier, Thébault & Lavorel 2007). The terraced fields are close together and of similar slope, aspect and soil texture, only differing in their current land use (see Table S1 in Supporting Information). The same is true of the unterraced meadows. Typically, inaccessibility for agricultural machinery was the reason for the abandonment of manuring or mowing, and fields with different management are often located side by side.

ISOTOPE ADDITION AND SAMPLING

The ^{15}N pool-dilution experiment was initiated on 30 June and 1 July 2005, at the peak in flowering and biomass in the fields, which allowed us to remove the final samples before the terraces were mown. The soil was inoculated with ammonium nitrate (NH_4^+ NO_3^-) 99% dual-labelled with ^{15}N, to provide available N for both plants and microbes. The labelled NH_4^+ NO_3^- was administered in 100 mL of solution across four 20×40 cm areas within each of the 15 fields. A 5 mL volume was injected into the soil at 0–5 cm depth, spaced 2.5 cm apart over the delimited area. An alloy nail slightly wider than the syringe needle was used to make a conduit for the needle to enter the soil. A concentration of 0.2 g m^{-2} of ^{15}N (7.12 µg N g^{-1} soil) was chosen, equivalent of *c.* 12% of the maximum NH_4^+ NO_3^- pool (0–10 cm) at the site.

Directly prior to inoculation, soil cores and samples of plant biomass were collected and processed to obtain zero-time-control ^{15}N natural abundance measurements. Following inoculation, a time course of ^{15}N remaining in the NH_4^+ and NO_3^- pools was obtained from samples taken after 48 h, 1 week and 1 month.

Each sample of above-ground biomass was harvested from 200 cm^2 of each inoculated area (four subplots) in all 15 fields, allowing a border to avoid any edge effect. Two soil cores of 4.5 cm diameter were removed from all four subplots, in the same place as the biomass harvest. One core was used for soil analyses and the other for root analyses. These cores were divided into three segments corresponding to 0–5, 5–10 and 10–15 cm depths, allowing for compaction of the soil in the corer. The fresh weight of each core was recorded.

Those cores required for the analyses of root mass were carefully dissolved in tepid water and separated by floatation. Grass roots were distinguished from other plants by their morphology and legume roots were removed. All roots were classified as coarse (> 2 mm diameter) or fine (< 2 mm diameter). Immediately following separation, root samples were dried at 65 °C, weighed and ground to a fine powder for analysis of ^{15}N content by mass spectrometry (see next section for details).

Cores for use in soil analyses were kept on ice in the field and maintained at −2 °C on return to the laboratory (within 2 h). Soil was passed through a 2-mm sieve to remove roots and stones. These roots were washed to remove small stones and weighed to give a crude estimate of their mass. The fresh, sieved soil was reweighed and two 10 g subsamples were removed, one for extraction of mineral N in KCl, and the other for chloroform fumigation and subsequent KCl extraction to yield microbial N. Chloroform fumigation extraction was performed for 14 days (following Cabrera & Beare 1993). Subsamples of 10 g sieved soil were shaken mechanically in 30 mL of 2 M KCl for 1 h at 200 r.p.m. then centrifuged at 9500 *g* for 5 min. NH_4^+ and NO_3^- were each removed from the KCl solutions using acid-trap diffusion extraction (Stark & Hart 1996), and prepared for mass spectrometry to determine ^{14}N and ^{15}N concentrations. The respective soil microbial ^{14}N and ^{15}N contents were calculated as the sum of NH_4^+ and NO_3^- from unfumigated soil subtracted from total mineral N from the fumigated soil (NH_4^+ plus NO_3^-; although NO_3^- is approximately zero following fumigation). All samples were analysed for ^{14}N and ^{15}N using a direct-combustion mass spectrometer (Europa Scientific SL-2020 system; Toby Hooker, Stable Isotope Lab, Utah State University, Logan, UT, USA). A subsample of KCl solution was analysed by colorimetry (Fiastar 5012 Flow Injection Analyser, Foss Tecator AB, Sweden) (following Bowman, Bahn & Damm 2003), to provide estimates of NO_3^- and NH_4^+ for the mass spectrometry.

Microbial C : N ratios were estimated using samples taken in July 2008 from the same fields as those compared in the July 2005 experiment. The different year prohibits the direct integration of these data with the 2005 experiment, but they give an approximate indication of the microbial C : N ratio in these fields. The same sampling and fumigation protocol was used in 2005 and 2008, except that extraction by persulphate oxidation in K$_2$SO$_4$ instead of KCl was used to obtain estimates of microbial biomass C (following Lipson *et al.* 1999). Soil microbial biomass C was calculated as the difference between the organic C extracted from fumigated and non-fumigated soil (TOC-5000A; Shimadzu Corp., Kyoto, Japan). A multiplication factor of 0.45 was applied to obtain microbial biomass C (following Wu *et al.* 1990; Joergensen 1996).

The harvested above-ground biomass was divided into litter, forbs, legumes and grasses. Grasses from the unterraced plots were further subdivided into the dominant species *Festuca paniculata* and other species. Each category of above-ground biomass was dried at 65 °C and weighed, and a subsample of green leaf material from non-senescent but fully expanded leaves was removed and ground for ^{15}N analysis by mass spectrometry.

DATA ANALYSIS

All biochemical analyses were performed on pooled samples from four subplots of each field, and replicated across three fields of each land use. All soil and root data were analysed separately at 0–5, 5–10 and 10–15 cm depth, but where land-use effect did not significantly differ with depth, these data are presented together. The N content of above- and below-ground biomass was based on N concentration of leaf and root samples multiplied by above-ground (leaf and stem) and root biomass data for each plant life-form. Thus, the above-ground N data give an overestimate at the community level since only N concentrations in leaf tissue, not stems or flowers, were measured.

The effects of land-use intensity in the terraces and unterraced fields on biomass, N content and N flux in each of the soil, leaf, litter and root compartments were assessed, as was the interaction of land use with plant type, soil depth and root depth, and root diameter class. To test these effects, a multi-level model was fitted to the data. An ANOVA based on a completely randomized split-plot design was performed in SAS using PROC MIXED (SAS V8.2; SAS Institute, Cary, NC, USA). The Repeated statement was used to identify the repeated measurements of N from each plot. A first order autoregressive covariance matrix was specified and Satterthwaite's determination of degrees of freedom was used. Separate analyses were also made to compare pairs of land uses on the terraces. The relative proportion of the added ^{15}N remaining in the soil and in the microbial N fraction was used to calculate the daily rate of microbial N uptake over the period from inoculation until each of the three sampling dates, following Stark (2000, pp. 225–227). Likewise, plant N uptake was calculated from change in plant tissue ^{15}N relative to soil ^{15}N content over the sampling period.

The rate of depletion of the ^{15}N tracer over the entire course of the experiment, i.e. after 48 h, 1 week and 1 month, was fitted to a power function ($y = ax^b$) for each field (see Fig. S2). This function provided a very close fit to the rate of extinction of the ^{15}N tracer.

Results

BIOMASS COMPOSITION OF THE PLANT COMMUNITY

Biomass production in July 2005 was higher than that reported by Quétier, Thébault & Lavorel (2007) for July 2004, but

followed a very similar pattern among land uses (Fig. 1). On the terraces, the manured-mown fields were most productive (679 ± 16 g m^{-2}, mean ± 1 SE), followed by the mown (609 ± 27 g m^{-2}) and unmown (557 ± 31 g m^{-2}) fields. There was a shift from dominance by forbs to dominance by grasses along this gradient of decreasing land use (Fig. 1a). In the unterraced grasslands, *Festuca paniculata* made a higher contribution to the biomass produced in the unmown (overall 743 ± 89 g m^{-2}) than mown (717 ± 21 g m^{-2}) fields, at the expense of the rest of the plant community (Fig. 1c). In both the terraces and unterraced grasslands, litter (together with above-ground necromass) in the mown fields constituted less than half the biomass of that in unmown fields (Fig. 1a,c). Grass fine-root biomass generally exceeded that of forbs ($F_{1,12} = 284$, $P < 0.001$, Fig. 1b; $F_{1,8} = 69$, $P < 0.001$, Fig. 1d). The biomass of both forb and grass roots of all sizes decreased along the gradient from manured-mown through mown to unmown terraced grasslands ($F_{2,4} = 13.6$, $P = 0.016$, Fig. 1b). In the unterraced grasslands, there was no net effect of land use on root biomass ($F_{1,2} = 1.52$, $P = 0.343$, Fig. 1d).

SOIL AND MICROBIAL N POOLS

In each of the terraced grasslands, [NO$_3^-$] and [NH$_4^+$] were approximately equal, and both pools diminished with decreasing land-use intensity (Table 1a). The division of inorganic N differed markedly in the unterraced grasslands, where NO$_3^-$ content was far lower than NH$_4^+$ content, and both pool sizes were unaffected by mowing (Table 1a).

The majority of microbial biomass N was recovered from 0 to 5 cm depth of soil (47 %), and relatively little from 10 to 15 cm depth ($< 20\%$), so (as with soil inorganic N) the amalgamated data from 0- to 15-cm soil depth are presented (Fig. 2). The microbial N pool sizes were similar per g soil across the terraced land uses, but when considered on a per-area basis, microbial biomass N was slightly lower in the unmown terraces than in the manured-mown and other mown terraces due to a difference in soil bulk density (land use, $F_{2,4} = 7.44$, $P = 0.045$, Table 1b,d).

In the unterraced grasslands, microbial biomass N was lower per g soil than in the terraces and was unaffected by land use ($F_{1,2} = 0.29$, $P = 0.646$, Table 1b). The ratios of microbial C : N in each land use were lower in the terraces than in the unterraced fields, and increased with the abandonment of manuring and mowing in the terraces only (Table 1b).

PLANT NITROGEN POOLS

The plant community under each land use differed in overall above-ground N content per field area (Table 1c). Differences with land use (Fig. 2a,b) were due to changes in the composi-
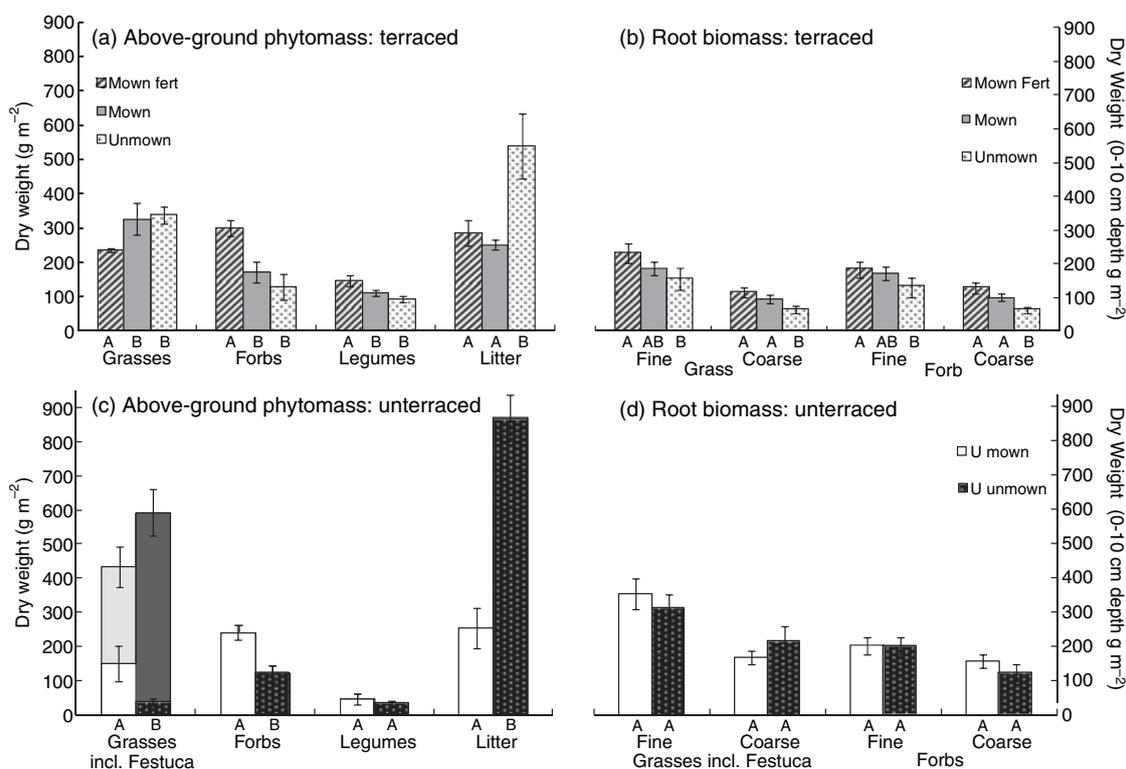

Fig. 1. Biomass of grasses, forbs and legumes in each land use by on 30 June 2005 (max standing biomass). Above-ground biomass in the (a) terraced fields and (c) unterraced fields. Data for *Festuca paniculata* (grey bars) in the unterraced fields are stacked on that for the other grasses. Fine root (< 2 mm diameter) and coarse root biomass, averaged between 0 and 15 cm depth, in the (b) terraced fields and (d) unterraced fields. Data from four 200-cm^2 areas per field were amalgamated to give mean values for three fields ± 1 SE. Different letters within groups represent $P < 0.05$.

Table 1. (a) The soil pools of nitrate (NO_3^-), ammonium (NH_4^+) and (b) microbial biomass N per m^2 area in the upper 10 cm of each field prior to inoculation (mg N m^{-2} area), (c) plant nitrogen (N) and litter pools, and (d) soil characteristics. Data from four 200-cm^2 areas per field were amalgamated to give mean values of three fields per land use $\pm$ 1 SE. Different letters within groups represent $P < 0.05$

	Terraced			Unterraced	
	Fert + Mown	Mown	Unmown	U Mown	U Unmown
(a) Soil N pools (0–10 cm depth)					
N-NO_3^- (μg N g^{-1} soil)	29.1 ± 2.9^A	23.9 ± 2.1^{AB}	21.6 ± 2.0^B	7.0 ± 0.6^C	6.4 ± 1.6^C
N-NO_3^- (g N m^{-2} area)	2.88 ± 0.29^A	2.26 ± 0.20^{AB}	1.82 ± 0.17^B	0.90 ± 0.07^C	0.78 ± 0.20^C
N-NH_4^+ (μg N g^{-1} soil)	29.7 ± 2.0^A	25.3 ± 0.6^B	22.9 ± 0.9^C	45.7 ± 4.8^D	48.7 ± 4.5^D
N-NH_4^+ (g N m^{-2} area)	2.94 ± 0.20^A	2.38 ± 0.05^B	1.93 ± 0.07^C	5.85 ± 0.61^D	5.90 ± 0.54^D
(b) Microbial N pools (0–10 cm depth)					
Microbial biomass N (μg N g^{-1} soil)	165.5 ± 6.5^A	163.2 ± 7.9^A	155.0 ± 9.3^A	99.9 ± 7.3^B	103.3 ± 4.0^B
Microbial biomass N (g N m^{-2} area)	16.37 ± 0.65^A	15.39 ± 0.74^A	13.05 ± 0.78^B	12.79 ± 0.94^B	12.52 ± 0.48^B
Microbial C : N ratio	6.8 ± 0.4^A	8.0 ± 0.3^B	9.5 ± 0.9^C	10.4 ± 0.4^C	10.6 ± 0.6^C
(c) Plant N pools					
Above-ground plant (mg N g^{-1} biomass)	23.3 ± 0.7^A	18.7 ± 0.7^B	18.4 ± 0.6^B	18.8 ± 0.8^B	19.1 ± 0.7^B
Above-ground plant (g N m^{-2} area)	15.85 ± 0.59^A	11.37 ± 0.49^B	10.24 ± 0.31^C	13.47 ± 0.54^D	14.17 ± 1.05^A
Litter and necromass (mg N g^{-1} biomass)	21.6 ± 0.4^A	16.6 ± 1.6^B	14.7 ± 2.1^B	12.0 ± 0.9^B	11.9 ± 0.3^B
Litter and necromass (g N m^{-2} area)	6.18 ± 0.83^A	4.16 ± 0.78^B	7.92 ± 1.95^{AC}	3.06 ± 0.43^B	10.43 ± 0.41^C
Root (mg N g^{-1} biomass)	10.9 ± 0.9^A	9.4 ± 0.4^B	9.0 ± 0.6^B	7.1 ± 0.3^C	7.5 ± 0.4^C
Root (g N m^{-2} area)	7.19 ± 0.77^A	5.23 ± 0.54^B	3.73 ± 0.62^C	6.28 ± 0.61^A	6.39 ± 0.82^A
(d) Soil characteristics (0–10 cm depth)					
pH	7.16 ± 0.29^A	7.63 ± 0.07^B	7.86 ± 0.08^C	5.59 ± 0.28^D	6.33 ± 0.31^E
Soil C : N ratio	10.96 ± 0.79^A	12.03 ± 0.60^A	13.94 ± 0.27^B	11.66 ± 0.29^A	11.52 ± 0.34^A
Bulk density (g cm^{-3})	1.26 ± 0.13^{AB}	1.20 ± 0.13^{AB}	1.31 ± 0.15^B	1.12 ± 0.03^A	1.17 ± 0.02^{AB}
Total N (mg g^{-1})	11.59 ± 2.10^A	11.85 ± 0.67^A	9.82 ± 0.16^{AB}	9.24 ± 0.65^{AB}	7.98 ± 0.10^B
Total N (kg m^{-2})	1.15 ± 0.21^A	1.12 ± 0.06^A	0.83 ± 0.01^B	1.18 ± 0.08^B	0.97 ± 0.01^C
% SOC	14.25 ± 1.91^{AB}	16.38 ± 1.09^B	15.75 ± 0.54^B	12.41 ± 1.00^A	10.58 ± 0.39^C

tion of grasses, forbs and legumes among land uses (Fig. 1a,b), but also enhanced by concomitant differences in N concentration within plant life-forms across land uses (Fig. S1a,b). Along the gradient of decreasing land use in the terraced grasslands, leaf and root N concentration of grasses and forbs, and likewise litter, decreased.

In the terraces, differences in root N pools (Table 1c: land use, $F_{2,4} = 7.1$, $P = 0.047$) coincided with the gradients in soil N among the land uses (Table 1a), and were a product of small differences with land use in both root biomass (Fig. 1b) and root N concentration (land use, $F_{2,4} = 5.5$, $P = 0.074$, Fig. S1b). Above-ground plant N pools also agreed with the ranking of soil N fertility based on the rate of N cycling among the terraced land uses (land use, $F_{1,2} = 18.4$, $P = 0.049$, Table 1c), but differences were life-form specific (Fig. 2a). Furthermore, when the litter-and-necromass N pool was added to the phytomass pool there was no significant difference between the total mown and unmown above-ground N pools (land use, $F_{1,2} = 0.3$, $P = 0.883$, Table 1c). The grass above-ground N pool was smaller in the manured-mown fields than the other terraced land uses but the forb above-ground N pool was significantly greater in the manured-mown terraces than in the other mown and unmown terraces (Fig. 2a), mainly due to relative changes in biomass of the two life-forms across land uses.

In the unterraced fields, there was no effect of mowing on the above-ground or below-ground N pools per area across

the plant community (Table 1c). Neither did tissue N concentration within each plant life-form differ with land use (Fig. S1d,e), but differences in composition meant that total N per field area was affected by mowing (Fig. 2c,d). In the unmown fields, *F. paniculata* held most N at the expense of other grasses and forbs (Fig. 2c). Coarse grass roots, which mainly belonged to *F. paniculata* may have been used for N storage since they held as much N as fine roots in the unmown fields (Fig. 2d).

SOIL AND MICROBIAL N FLUXES AND TRACER DEPLETION

The rates of extinction of the ^{15}N tracer from both NO_3^- and NH_4^+ were faster in the manured-mown than mown terraces and slowest in unmown terraces, and were faster in the mown than unmown unterraced fields (Fig. S2). These data were used to estimate the gross rates of NO_3^- and NH_4^+ consumption from the soil pools (Table 2a). In the terraced grasslands, consumption of NO_3^- was fastest in the manured-mown fields, intermediate in the mown fields and slowest in the unmown fields (Table 2a). The consumption of NH_4^+ was almost twice as fast as that of NO_3^-, and both showed similar trends with land use (Table 2a). In each case, the trends with land use became more difficult to distinguish with time since inoculation as
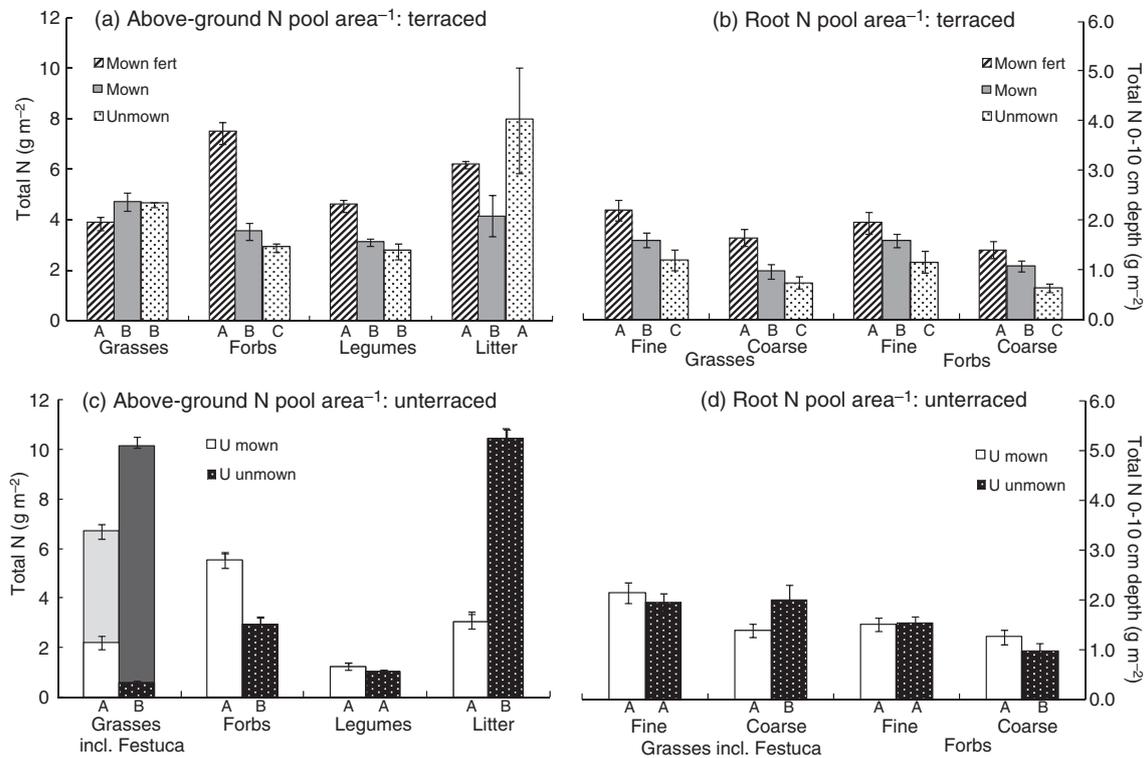
Fig. 2. Nitrogen (N) pool of soil, leaves and roots in each subalpine grassland calculated from leaf N concentration × biomass per m². Above-ground N pool in grasses, forbs and legumes: (a) terraced and (c) unterraced fields. Data for *Festuca paniculata* (grey bars) in the unterraced fields are stacked on that for the other grasses. Fine and coarse grass and forb roots 0–15 cm depth: (b) terraced and (d) unterraced fields. Mean values of three fields ± 1 SE. Different letters within groups represent $P < 0.05$.

Table 2. (a) Soil nitrogen (N) flux per day calculated using the ^{15}N pool dilutions for 48 h after inoculation from the fitted functions for ^{15}N-NO_3^- and ^{15}N-NH_4^+ extinction: (b) Microbial N uptake, and (c) plant N uptake, per day, calculated for the same period also using the ^{15}N pool dilutions. Data from four 200-cm² areas per field were amalgamated to give mean values of three fields per land use ± 1 SE. Different letters within groups represent $P < 0.05$

	Terraced			Unterraced	
	Fert + Mown	Mown	Unmown	U Mown	U Unmown
(a) Soil N flux (0–10 cm depth)					
Gross N-NO_3^- consumption rate (µg N g^{-1} soil day^{-1})	11.42 ± 0.98^A	8.37 ± 0.98^B	5.86 ± 0.48^C	3.13 ± 0.27^D	2.23 ± 0.57^E
Gross N-NO_3^- consumption rate (mg N m^{-2} day^{-1})	1129 ± 97^A	789 ± 93^B	493 ± 40^C	400 ± 35^D	270 ± 69^E
NO_3^- flux : pool ratio	0.39	0.35	0.27	0.44	0.35
Gross N-NH_4^+ consumption rate (µg N g^{-1} soil day^{-1})	20.52 ± 1.42^A	14.42 ± 0.36^B	11.09 ± 0.43^C	15.20 ± 1.47^B	9.24 ± 0.80^D
Gross N-NH_4^+ consumption rate (mg N m^{-2} day^{-1})	2030 ± 140^A	1360 ± 34^B	933 ± 36^C	1945 ± 189^A	1120 ± 97^C
NH_4^+ flux : pool ratio	0.69	0.57	0.48	0.33	0.19
NO_3^- : NH_4^+ loss ratio	0.56	0.58	0.53	0.21	0.24
(b) Microbial N uptake (0–10 cm depth)					
Specific N uptake rate (mg N g^{-1} microbial biomass day^{-1})	92.5 ± 4.99^A	75.1 ± 3.34^B	86.4 ± 3.10^A	36.7 ± 2.83^C	35.5 ± 2.53^C
Gross N uptake rate per g soil (µg N g^{-1} soil day^{-1})	107.6 ± 7.7^A	92.5 ± 5.8^B	99.0 ± 5.7^{AB}	37.4 ± 4.3^C	38.2 ± 3.4^C
Gross N uptake rate per field area (g m^{-2} day^{-1})	10.26 ± 0.76^A	9.36 ± 0.55^A	11.22 ± 0.48^A	4.76 ± 0.54^B	4.59 ± 0.41^B
(c) Plant N translocation to above-ground biomass					
Specific N uptake rate (mg g^{-1} biomass day^{-1})	14.39 ± 1.82^A	7.12 ± 1.17^B	6.90 ± 1.01^B	9.74 ± 2.13^{AB}	8.68 ± 1.93^B
N Uptake rate per field area (mg m^{-2} day^{-1})	3501 ± 453^A	1746 ± 283^B	1576 ± 212^B	1771 ± 428^B	1755 ± 346^B
(d) Plant root N uptake (0–10 cm depth)					
Specific N uptake rate (mg g^{-1} biomass day^{-1})	14.96 ± 2.91^A	7.82 ± 1.38^B	4.36 ± 0.69^C	6.72 ± 1.22^B	5.90 ± 1.38^B
N Uptake rate per field area (mg m^{-2} day^{-1})	2734 ± 862^A	1232 ± 353^B	531 ± 203^C	1578 ± 472^{AB}	1347 ± 527^{AB}

the isotope became more dilute, and presumably recycling of the ^{15}N in the ecosystem made this calculation less valid (Fig. S2).

In the unterraced grasslands, the consumption rates of NH_4^+ were similar to those in the terraces, but consumption of NO_3^- was slower (Table 2a). Despite similar pool sizes among the unterraced fields, there was a large effect of mowing on the consumption of both NO_3^- and NH_4^+ since both rates were far faster in the mown than unmown fields (Table 2a). Relative to NO_3^- pool size, the daily efflux of NO_3^- was similar in the terraced and unterraced fields, in both cases cycling of the NO_3^- pool was faster in the more intensive land uses (Table 2a). Likewise, irrespective of historical terracing, the ratio of NH_4^+ consumption to NH_4^+ pool size was larger in the more intensively managed fields than their unmown counterparts, but unlike NO_3^-, the proportion of the NH_4^+ pool taken up was very low in the unterraced fields compared to the terraces (Table 2a).

The partition of soil cores into three depth bands (0–5, 5–10 and 10–15 cm) generally revealed similar patterns of ^{15}N depletion with depth across the land uses (data not shown). Rainfall was low (9 mm) during the experiment and very little ^{15}N tracer reached 10–15 cm depth (max 5% of that at 0–5 cm), suggesting that loss through leaching was insignificant. Low ^{15}N content in litter confirmed that application of the ^{15}N solution into the soil was effective, that there was little wicking to the surface and that only a small quantity of the tracer was recycled through senescent tissue during the experiment (data not shown).

Accumulation of ^{15}N in the microbial biomass N fraction relative to ^{15}N remaining in the soil pool was used to calculate the daily rate of microbial N uptake over the sampling period (Table 2b). Linear regression of microbial N uptake over the three sampling dates was used to verify whether the rate of update changed with time from inoculation (Fig. S3). The rate of microbial N uptake per g microbial biomass was faster in the manured-mown than the mown terraced fields (Table 2b). It was also faster in the unmown terraced fields (Table 2b), but this rate gradually declined with time after inoculation (decline of 1.78 mg N g^{-1} microbial biomass day^{-1}: slope from linear regression), until it was lower than the other land uses after 1 month (Fig. S3a). When microbial N uptake per field area was considered on the terraces the differences due to land use after 48 h were nullified (Table 2b), partially because of the lower microbial C : N ratio (Table 1a) in the manured-mown and mown fields and partially resulting from differences in bulk density (Table 1c).

The rate of microbial uptake per g microbial biomass in the unterraced fields was less than half of that in the terraces (Table 2b). There was no significant difference in N uptake per g microbial biomass or per area in the mown and the unmown unterraced fields (Table 2b). The uptake rates in both land uses were fairly stable over the 1 month following inoculation (Fig. S3), providing no evidence that addition of the ^{15}N had stimulated microbial uptake in the unterraced fields. Microbial uptake and the microbial biomass N pool were both smaller in the unterraced fields than in the terraces (Tables 1b and 2b), and yet soil NH_4^+ consumption was slow (Table 2a), suggest-
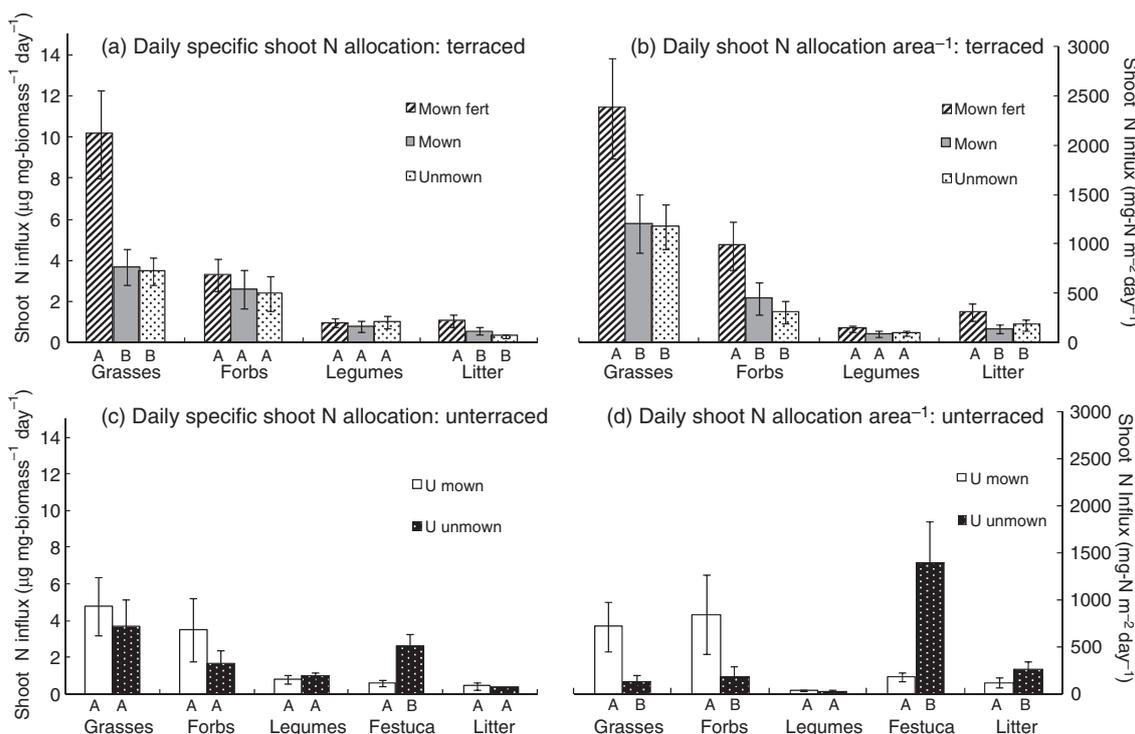

Fig. 3. The flux of nitrogen (N) into above-ground plant tissue per day, on a per-g-biomass basis in the (a) terraced and (c) unterraced fields, and on a per-area basis in the (b) terraced and (d) unterraced fields. Uptake of N is calculated from the ^{15}N concentration and the soil $^{14}N : ^{15}N$ ratio. Mean values of three fields ± 1 SE. Different letters within groups represent $P < 0.05$.
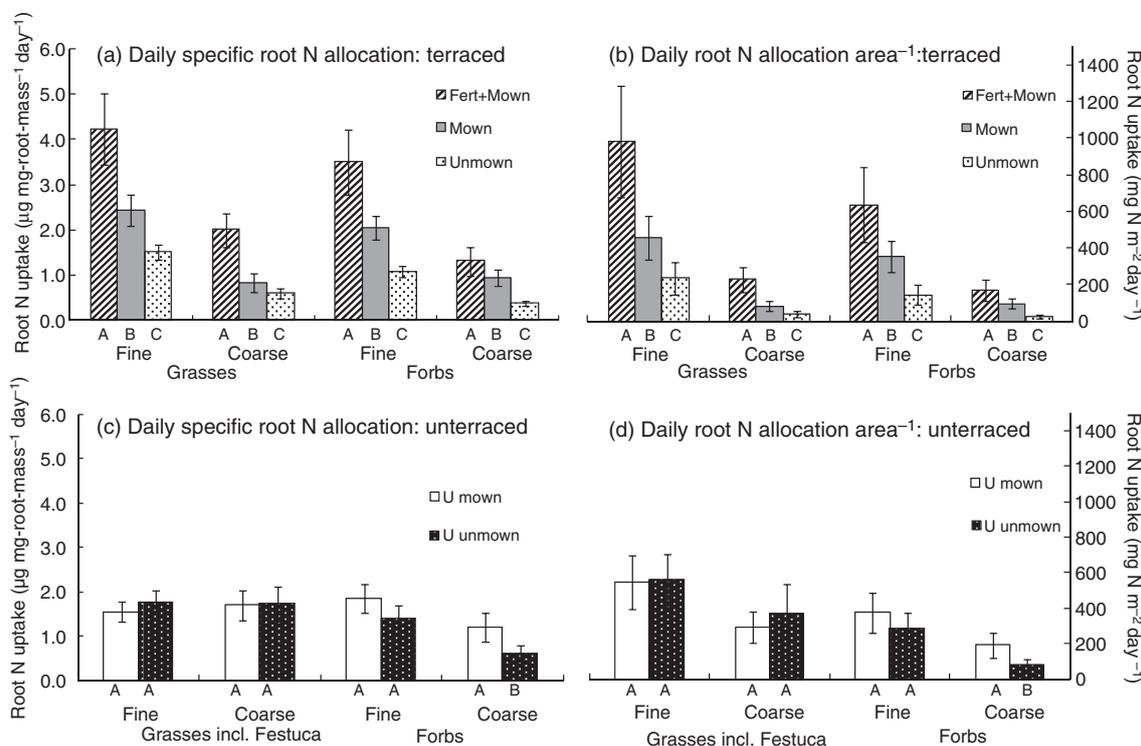
Fig. 4. The flux of nitrogen (N) into fine and coarse, grass (including *Festuca paniculata*) and forb roots per day, on a per-g-biomass basis in the (a) terraced and (c) unterraced fields, and on a per-area basis in the (b) terraced and (d) unterraced fields. Uptake of N is calculated from the ^{15}N concentration and the soil ^{14}N : ^{15}N ratio. Mean values of three fields ± 1 SE. Different letters within groups represent $P < 0.05$.

ing that microbial activity was restricted in the unterraced fields, but not by N availability.

PLANT N UPTAKE

Both root N uptake and above-ground N translocation (henceforth shoot N allocation) were greater in grasses than forbs and legumes (Figs 3 and 4). This may be a phenological effect since it was not reflected in the N pools of the respective plant forms measured at peak biomass (Fig. 2). All plant forms took up more N in the manured-mown and mown terraces than in the unmown terraces, and there was no plant-form × land-use interaction (Figs 3a,b and 4a,b). In the unterraced fields, grass and forb N uptake and subsequent above-ground allocation in the mown fields surpassed that in unmown fields, but *F. paniculata* dominated above-ground N allocation in the unmown fields both per area and per biomass (Fig. 3c,d), negating any difference at the community level. Since we did not distinguish fine roots of *F. paniculata* from other grasses, the below-ground patterns of N uptake between grasses and forbs are equivocal (Fig. 4c,d). However, allocation of N to coarse grass roots in both the mown and unmown unterraced fields was higher than would be expected when compared with fine roots, and the terraced land uses (Fig. 4).

Discussion

Our results revealed that reduced management intensity influenced N cycling through a marked decrease in fluxes (plant

uptake, soil NO_3^- and NH_4^+; although microbial uptake was relatively constant). These changes translated into smaller N pools (plant, microbial, soil NO_3^- and NH_4^+) in the terraced but not in the unterraced fields (Fig. 5).

NITROGEN CYCLING IN THE THREE TERRACED LAND USES

Of the terraced land uses, the soil N pool was largest in the manured-mown fields (Table 1), where the rate of N cycling was also fastest (Table 2). This supports our hypothesis that greater plant and microbial uptake of N in these grasslands does not lower soil N concentration, but on the contrary, stimulates greater N availability as nutrients are recycled more readily back into the soil (Ashton *et al.* 2008; Van der Heijden, Bardgett & van Straalen 2008).

The rates of soil N cycling in the terraces corresponded to the trends in plant and microbial N uptake and allocation per area with land use. The differences in plant N uptake among land uses were much more pronounced than for microbial N uptake (Table 2), and this would suggest that in the terraces plant N demand exerts a greater control over N cycling than microbial N demand, at least during July when the experiment was performed. The prevalence of N hotspots in these fields (Robson *et al.* 2007) may help plants to influence the rates of N cycling through their greater capacity to exploit soil N heterogeneity compared with microbes (Luxhøi, Jensen & Nielsen 2004; Chapman *et al.* 2006). Niche partitioning between plants and microbes on a seasonal basis has been reported for alpine
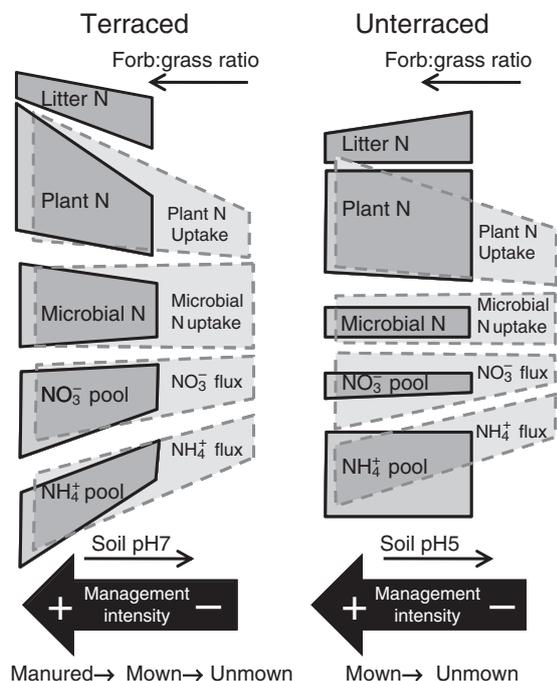
Fig. 5. Schematic diagram showing trends in pool and flux sizes of nitrogen (N) in the two grasslands, terraced and unterraced, along a gradient of management intensity. The box size is proportional to the sizes of the N pools (see Table 1) and fluxes (see Table 2) on a per-area basis, and their change with land use from left to right, relative to others components of the ecosystem.

grasslands where plant uptake dominates early in the season and microbes later in the year (Jaeger *et al.* 1999; Miller & Bowman 2002), and in the Scottish highlands where this seasonal uptake pattern is reversed (Bardgett *et al.* 2002), although the occurrence of similar patterns remains to be tested in our subalpine grasslands.

On the terraces, the soil microbial N pool exceeded soil NO_3^- or NH_4^+ pools in all three land uses (Table 1a), consistent with high rates of N cycling (Table 2a). Fast consumption of NH_4^+ and high uptake into microbial N might suggest that the microbial communities in the terraces were N-demanding or had access to more labile C substrates (Booth, Stark & Rastetter 2005; Uhlířová, Šimek & Šantrůčková 2005). The only exception to the trend of faster N consumption and plant and microbial N uptake under more intensive management was for faster specific microbial N uptake (per g of microbial biomass) in the unmown terraces. This anomaly may be explained as a rapid short-term response to N fertilization by the ^{15}N added to the small soil N pool, if the microbial community in the unmown fields is N-starved, since the trend was no longer evident after 1 month (Fig. S3a,b). Otherwise, the trends in microbial N uptake with land use conform to our expectation based on higher soil DEA in the manured and mown terraces compared to the unmown terraces in May 2005 (Robson *et al.* 2007). The higher soil C : N ratio in the unmown fields supports our assertion that microbes in the unmown terraces are more N-limited than in the manured-

mown or mown terraces (Booth, Stark & Rastetter 2005). The different rates of N cycling across the terraces match our expectations based on resin-extractable N and patterns of soil-pool NO_3^- but not NH_4^+ in 2004 (Robson *et al.* 2007).

Although NO_3^- consumption was not as fast as that of NH_4^+, it still reached a turnover of 30–40% of the NO_3^- pool per day and differed with land use (Table 2). The rapid capture of N by the plants suggests that the shift in plant resource economies from exploitative in the manured-mown grasslands to conservative in the unmown grasslands (Quétier, Thébault & Lavorel 2007) interacts with the rate of N cycling and controls relative N availability among the three land uses. It is not clear from this experiment whether the plant community was competing with microbes for soil N during July (Bardgett, Streeter & Bol 2003) or how much plants were utilizing NH_4^+ and organic N forms in addition to NO_3^- (Miller & Bowman 2002; Weigelt, Bol & Bardgett 2005). Given the calculated rate of soil N consumption, microbial N release back into the soil must also be rapid in the terraced fields, although organic N fluxes would need to be quantified in order to produce an N budget. We propose that the higher microbial C : N ratio in the unmown terraces compared to the mown terraces may be interpreted as an impact of plant uptake influencing microbial community composition in the terraced fields (Zak *et al.* 2003; Kardol *et al.* 2007; Song *et al.* 2007).

NITROGEN CYCLING IN THE MOWN AND UNMOWN UNTERRACED MEADOWS

Different mechanisms appear to govern N cycling in the terraced and unterraced fields since the relationships between changing flux rates and pool sizes with reduced management intensity were not consistent between the two systems. Although in both cases, consumption of NH_4^+ and NO_3^- was consistently higher in the mown than unmown fields (see also Robson *et al.* 2007), there was no effect of mowing on inorganic N pool size in the unterraced meadows.

In contrast to the terraced grasslands where there is greater interplay between changes in the plant and microbial communities, microbial biomass was very low in all the unterraced meadows, so changes in the plant community with abandonment of mowing will be the primary influence on rates of N cycling (Loiseau *et al.* 2005; Smith *et al.* 2008). *Festuca paniculata* may be responsible for slowing the rate of N cycling in the unmown fields, without necessarily lowering the concentrations of NH_4^+ and NO_3^- (Craine *et al.* 2005). Direct plant uptake of NH_4^+ and organic N has been reported for many ecosystems including alpine and subalpine grasslands (Xu *et al.* 2006; Ashton *et al.* 2008; Kahmen, Wanek & Buchmann 2008). Trials under controlled conditions have shown *F. paniculata* to directly uptake more NH_4^+ than NO_3^- when NH_4^+ is more prevalent, since its affinity for NO_3^- is lower than that of co-occurring species (Maalouf 2008). This may provide a plausible explanation for the sustained NH_4^+ uptake given the dominance of *F. paniculata* in the unterraced meadows, but requires further supporting evidence.

Throughout the unterraced meadows, grass coarse root N content was unexpectedly high compared to fine-root N and compared with the terraces (Fig. 4c). Plant N capture for storage has been reported for other alpine species (Kleijn, Treier & Müller-Schärer 2005; Monson *et al.* 2006). Storage within coarse roots and at the base of the stem (F. Baptist, unpublished data) may allow *F. paniculata* to hinder the establishment of exploitative species in the unterraced meadows, and particularly in the unmown fields where it forms large dense tussocks of persistent unpalatable foliage, which senesce to produce recalcitrant litter (Quétier, Thébault & Lavorel 2007). The recalcitrant carbon pool that accumulates in the unmown fields as litter promotes fungal dominance of the microbial community (suggested by higher C : N) (Zeller *et al.* 2000), which in turn lowers the microbial N requirement and is expected to lower microbial N demand in the unterraced fields (Uhlířová, Šimek & Šantrůčková 2005).

EFFECTS OF HISTORICAL TERRACING ON N CYCLING

Patterns of soil N availability and N use by the plant community confirmed previous findings that the terraces are more fertile overall than the unterraced fields (Robson *et al.* 2007). In the unterraced fields, the pool of NO_3^- was small and NO_3^- consumption was slow relative to the terraces, although the ratio of NO_3^- consumption to NO_3^- pool size was similar between the two systems. This, along with similar specific rates of N uptake by grasses and forbs, suggests that regardless of past management and its effect on soil properties, the effect of the cessation of mowing on rates of NO_3^- uptake by the plant community is consistent.

However, a relatively large soil NH_4^+ pool in all unterraced fields was accompanied by high NH_4^+ consumption but very low microbial uptake. This suggests low microbial demand for N, at least during July. Additional causes of the high absolute NH_4^+ turnover may be plant species NH_4^+ uptake, immobilization on clay (Lambers, Chapin & Pons 1998, pp. 240–241; Zeller, Bardgett & Tappeiner 2001), or nitrification, although the latter possibility is not supported by our previous findings of low nitrifying enzyme activity during May in these unterraced fields (Robson *et al.* 2007). In fact, the acidic soil pH of the unterraced fields will limit bacterial activity and, consequently, nitrification and volatilization of NH_4^+ (Bardgett 2005; Zeglin *et al.* 2007), which will in turn increase NH_4^+ and reduce the NO_3^- available for plant uptake. Whereas, in the terraced fields, historical ploughing has mixed underlying calcareous bedrock with the top soil, raising pH to levels more favourable for bacterial activity (Robson *et al.* 2007).

LINKING LAND-USE CHANGE AND N CYCLING THROUGH THE PLANT AND MICROBIAL COMMUNITIES

The trends towards smaller N pools, lower N use and shifts in functional composition with decreased management intensity or abandonment were similar to those reported elsewhere in subalpine and temperate grasslands (Zeller *et al.* 2000; Kahmen *et al.* 2006). These effects can be interpreted within the framework of positive feedbacks between plants and soil fertility (Eviner & Chapin 2003; Wardle 2005). Given that microbial uptake was stable across land uses within the terraced and unterraced grasslands, we assume that these feedbacks are predominantly plant-driven (Chapman *et al.* 2006).

In terraced fields, a decrease in N-rich forbs (and legumes) relative to N-poor grasses following the cessation of manuring resulted in lower overall community N and greater N immobilization in litter (Fig. 5). The relatively greater uptake by grasses (on a per gram basis) in mown and manured plots during July, despite their lower average tissue N content, can be related to their later growth phenology as compared to forbs. Phenological differences across life-forms can alter the timing of N uptake, so that increased fertility associated with a greater plant diversity may extend the growing season (Louault *et al.* 2005; Quétier, Thébault & Lavorel 2007) and thereby total N uptake. Finally, decreased litter decomposability because of decreased leaf N content from manured and mown to mown and unmown fields (Quétier, Thébault & Lavorel 2007; Fortunel *et al.* 2009) is a likely key component of this positive feedback.

A similar feedback mechanism could explain the changes in fluxes in unterraced grasslands, with decreased forb abundance following cessation of mowing, concomitant with a considerable increase in *F. paniculata* abundance. In the unterraced grasslands, the NH_4^+ pool was large and efflux small relative to the pool size (Tables 1 and 2). We suggest that more acidic conditions in these soils compared to the terraced soils inhibited nitrification and led to a high microbial C : N ratio. These factors may have favoured the growth of *F. paniculata,* which may itself affect N cycling by taking up NH_4^+ preferentially and producing fibrous, lignin-rich recalcitrant litter (Fortunel *et al.* 2009). We suppose that the inability of *F. paniculata* to invade the terraced grasslands is due to their higher pH caused by calcareous stones, and the corresponding higher $NO_3^- : NH_4^+$ ratios on the terraces which would negate any competitive advantage of NH_4^+ uptake. The relatively small effect of mowing on N pools in the unterraced fields requires further investigation, but may be related to lower microbial activity than in terraces reducing the impact of plant–microbe interactions, and to low $[NO_3^-]$, prohibiting the invasion of more N-demanding plant species, and possible rhizodeposition within the plant community further lowering soil pH (Uhlířová, Šimek & Šantrůčková 2005). The understanding of such feedback loops is essential in order to articulate what underpins shifts in ecosystem services following land-use change (Quétier *et al.* 2007). Our results highlight how changes in plant composition and associated decreases in plant diversity following decreases in management intensity feed forward to decrease N sequestration as well as soil fertility in terraced grasslands, although not in unterraced meadows (Fig. 5). The reliance of soil services, including not only nitrogen- but also carbon-cycling processes, on plant functional properties is an important issue for future agricultural management (De Deyn, Cornelissen & Bardgett 2008; Fornara, Tilman & Hobbie 2008; Phoenix *et al.* 2008).

Acknowledgements

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Supporting Information

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

Table S1. Extra site and soil characteristics of each land use.

Figure S1. Nitrogen concentrations for leaf, root, soil and microbial N in each subalpine grassland.

Figure S2. The time course of soil ^{15}N loss from each land use.

Figure S3. The nitrogen (N) uptake by soil microbes per day, on a per-g-biomass basis in the (a) terraced and (c) unterraced fields, and on a per-area basis in the (b) terraced and (d) unterraced fields.

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