

# Plant response traits mediate the effects of subalpine grasslands on soil moisture

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## Summary

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- In subalpine grasslands, changes in abiotic conditions with decreased management intensity alter the functional composition of plant communities, leading to modifications of ecosystem properties. Here, it is hypothesized that the nature of plant feedbacks on soil moisture is determined by the values of key traits at the community level.
- As community functional parameters of grasslands change along a gradient of land uses, those traits that respond most to differences in abiotic conditions produced by land use changes were identified. A vegetation removal experiment was then conducted to determine how each plant community affected soil moisture.
- Soil moisture was negatively correlated with community root length and positively correlated with canopy height, whereas average leaf area was associated with productivity. These traits were successfully used to predict the effects on soil moisture of each plant community in the removal experiment. This result was validated using data from an additional set of fields.
- These findings demonstrate that the modification of soil moisture following land use change in subalpine grasslands can be mediated through those plant functional traits that respond to water availability.

**Key words:** community functional parameters, land use, leaf area, response and effect traits, root length, soil moisture, subalpine grasslands, vegetation removal.

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## Introduction

Plant functional traits can be considered in terms of their response to environmental factors ('response traits'), or from the perspective of the effect that they have at a community scale on ecosystem properties ('effect traits') (Chapin *et al.*, 2000; Lavorel & Garnier, 2002). As the relationship between the plant community and environmental factors is dynamic, certain traits may be implicated as mediators if they both respond to environmental factors and affect ecosystem properties. Using these traits it should be possible to predict the ecosystem-level consequences of environmental changes (changes in land use or climate) for plant communities (Lavorel & Garnier, 2002; Suding *et al.*, 2008). There is evidence to

support this hypothesis at both species and community levels (reviewed by Lavorel *et al.*, 2007). At the species scale, some leaf traits that respond to nutrient or water availability (e.g. leaf dry matter content (LDMC) and leaf nitrogen content (LNC)) also affect leaf palatability and litter decomposability (Cornelissen *et al.*, 1999; Diaz *et al.*, 2004). The same relationship can be extended to the community scale. For example, the community-level means of some leaf traits (e.g. specific leaf area (SLA), LDMC or LNC), also referred to as community functional parameters (CFPs: the average species trait values weighted by their relative abundance in the community; Violle *et al.*, 2007), both respond to land use change in European grasslands and affect nutrient cycling (Garnier *et al.*, 2004; Quétier *et al.*, 2007). In this paper, we

will refer to those traits that can be identified as both response and effect traits as 'response-effect traits'. The examples mentioned above illustrate how the response-effect traits framework can apply to components of the nutrient cycle. In contrast, few studies have explicitly identified mediating response-effect traits for water availability, especially at the community level (Eviner & Chapin, 2003; Lavorel *et al.*, 2007). Whilst numerous studies have focused on traits that can predict individual species response to water availability (e.g. Reich *et al.*, 1999, 2001; Schwinning & Ehleringer, 2001; Wright *et al.*, 2001; Schwinning & Sala, 2004; Ackerly, 2004), much less is known about those traits that affect water availability, whether at species or community level (Eviner & Chapin, 2003).

The most likely candidate response-effect traits are those continuous traits that reflect species strategies in response to environmental gradients (Grime, 2001; Suding *et al.*, 2003). Response traits usually co-vary along an axis of specialization (Grime, 2001; Suding *et al.*, 2003; Ackerly, 2004; Diaz *et al.*, 2004). For example, SLA and leaf area (LA) are often closely positively correlated with photosynthesis and transpiration rate (Reich *et al.*, 1999; Westoby *et al.*, 2002). Plants with a conservative strategy (low SLA and transpiration rate) may tolerate low water availability, whereas plants with an exploitative strategy (high SLA and transpiration rate) are often drought intolerant (Reich *et al.*, 1999; Diaz *et al.*, 2004). Because plants with low SLA tend to deplete soil moisture more slowly than those with high SLA, SLA can be considered an effect trait.

Similarly, plant stature and root-related traits have been proposed to behave as both response and effect traits. Short plants are considered to be more drought tolerant than tall plants (Westoby *et al.*, 2002; Ackerly, 2004), whereas increased canopy height can reduce incident radiation and limit evaporation by modifying the albedo and vegetation boundary-layer thickness (Fliervoet & Werger, 1984; Luo & Dong, 2002), promoting soil moisture retention. Finally, high root allocation is likely to improve plant uptake during a pulse of water following a rain event, especially when roots are located close to the soil surface (Schwinning & Ehleringer, 2001; Schwinning & Sala, 2004). Between pulses, deeply rooted, drought-intolerant plants are able to avoid drought because they have access to deep moist layers (Sala *et al.*, 1989; Eviner & Chapin, 2003; Ackerly, 2004). Root traits are, thus, likely to determine plant water uptake zones and capacity, and thereby the extent of soil moisture depletion (Eviner & Chapin, 2003). Overall, there is sufficient evidence to suggest a large overlap between response and effect traits relating to soil moisture, making this ecosystem property a candidate for the exploration of response-effect linkages (Lavorel & Garnier, 2002).

In this study, we investigate how soil moisture is affected by plant CFPs in neighbouring subalpine grasslands under different land uses. Ongoing land-use extensification affects the functional composition of subalpine grasslands (Tasser & Tappeiner, 2002; Quétier *et al.*, 2007). At the same time, key

ecosystem properties are modified, in particular nutrient availability (Robson *et al.*, 2007), and this has been directly linked to community-level trait responses to management (Diaz *et al.*, 2007). We consider whether prospective response-effect traits mediate the relationship between the plant community and a gradient of water availability provided by fields under differing land uses. (1) To this end, we investigated how fertility and those soil physical characteristics determining plant-water relations affect the distribution of CFPs (leaf, stature and root traits) across grasslands under different land uses. (2) Then, we conducted a removal experiment to determine the vegetation effect on soil moisture in each community, that is, we identified effect traits. (3) By matching response (step 1) and effect (step 2) traits, we identified those (response-effect) traits that mediate between the plant community and soil moisture. (4) Lastly, we proposed a statistical model of soil moisture as a function of soil properties and community-level response-effect traits, and validated this model using data from other subalpine grasslands at our field site.

## Materials and Methods

### Study site

The study site is located on the south-facing aspect of the upper catchment of the Romanche River, in the central French Alps (Villar d'Arène, 45.04°N, 6.34°E), close to the Lautaret Pass. All fields were situated between altitudes of 1850 and 1950 m within 5 km of each other. The climate is subalpine with a strong continental influence. The mean monthly temperatures range between a minimum of -7.4°C in February and a maximum of 19°C in July. Mean annual precipitation is 956 mm, most of which falls as snow during the cooler months, and there is a pronounced summer drought (only 18% of annual rainfall occurs during the summer).

We studied four types of grasslands representing different combinations of past and present land use (Quétier *et al.*, 2007). In the Alps, the traditional practices of hay making, manure fertilization and grazing are being increasingly abandoned, resulting in succession towards tussock-grass communities (Tasser & Tappeiner, 2002; Quétier *et al.*, 2007). Land use types differ in species composition (Tasser & Tappeiner, 2002) and are also characterized by contrasting functional composition (Quétier *et al.*, 2007) (Supporting Information Table S1). At our site, all grassland types are dominated by grass species with a fibrous root architecture, which make up from 50% (fertilized grasslands) to 80% (unmown grasslands) of total cover, and shrubs are absent (Quétier *et al.*, 2007). Two grassland types were formerly arable fields (> 50 yr ago) on terraced slopes that are now mown annually for hay. Some of these hay meadows are periodically fertilized using farmyard manure. These fertilized grasslands (terraced fertilized mown (TFM)) are characterized by tall vegetation

dominated by exploitative species such as *Dactylis glomerata* and *Trisetum flavescens*. By contrast, those mown terraced grasslands that are no longer fertilized (terraced mown (TM)) are characterized by shorter vegetation dominated by conservative species (Table S1) such as *Bromus erectus* and *Sesleria caerulea*. The remaining two grassland types were never ploughed, and are lightly grazed in summer and either mown for hay or unmown. The unterraced hay grasslands (mown (M)) are characterized by medium-size vegetation with conservative traits, and are dominated by *Festuca paniculata*, *Meum athamanticum* and *Trifolium alpinum*. Grasslands where mowing has ceased (unmown (U)), are characterized by tall vegetation with conservative traits, where *Festuca paniculata* is highly dominant (> 80% of the community biomass) (Quétier *et al.*, 2007). For each land use type, we selected three replicate fields with similar past and current land use (Quétier *et al.*, 2007). In total, 12 fields were studied where aspect, soil depth and slope were measured.

### Community functional parameters

**Community aboveground traits** We used published data for the aboveground CFP calculated for the same 12 fields by Quétier *et al.* (2007) using the trait values of each species in the corresponding management treatment weighted by the species relative abundance in each field. CFPs for each grassland type are summarized in Table S1.

**Community root traits** Roots were sampled using six randomly distributed soil cores (4 cm diameter), removed from each field during June 2005, and community-level root traits were estimated using standardized methodology (Fitter, 1991). Cores were divided into sections of 0–15 and 15–30 cm depth from the surface in the terraced fields, and additionally 30–50 cm depth in the unterraced fields. Roots were extracted from these cores by flotation in tepid water, and were stored in 2% alcohol until analysis. Root-length analysis was performed on a subsample of fine roots (< 2 mm diameter) from each field using WINRHIZO (Regent Instrument Inc., Quebec, Canada). All root samples were subsequently dried at 65°C and weighed to determine root biomass and calculate root length per gram of soil. Community root length was calculated as the mean of all samples for each field, and similarly the percentage root length in the upper 15 cm.

**Community biomass** A sample representing the peak green and dead aboveground biomass was harvested in each field on 15 July 2005 from four randomly placed 50 × 50-cm quadrats (methods are described in Quétier *et al.*, 2007). Plant material was dried at 65°C and weighed. These data were used to determine aboveground biomass. Plant allocation to roots was calculated for each field as the ratio between belowground biomass and total above- plus belowground biomass. Sward height and light interception in each field

were recorded throughout the growing season (from 15 May 2005 to 31 July 2005) as indicators of plant community growth. Photosynthetically active radiation (PAR) at three randomly selected points above and below the canopy was recorded using a 1-m-long rod (LI-191SA Line Quantum Sensor; Li-Cor, Lincoln, NE, USA). Light interception was the relative difference in light intensity above and below vegetation.

### Characterization of environmental variables

The variables linked to productivity have already been published in Quétier *et al.* (2007), including annual net primary productivity (ANPP), disturbance intensity and the nitrogen nutrition index (INN), which reflects limitation of vegetative growth by nitrogen availability and can be used as an indicator of fertility (Duru *et al.*, 1997; see Quétier *et al.*, 2007 for details on this index). To determine soil physical properties related to water availability in each of the 12 fields, 10 random soil cores of 15-cm depth were collected during late spring (1 June 2005). Cores were mixed, sieved and analysed for physical properties including soil stoniness, organic matter and texture, in addition to gravimetric and volumetric soil moisture.

Soil moisture within each field was measured directly using time domain reflectometry (TDR) probes (mini TRASE system 1; Soil Moisture Equipment Corporation, Santa Barbara, CA, USA). After snowmelt (15 May 2005), six TDR probes (15-cm depth) were randomly distributed in the core 10 × 10-m area of each field. We measured soil moisture on 18 occasions during the growing season (one to three measurements per week from 29 May to 15 September 2005; Supporting Information Fig. S1). Measurements were made between 11:00 and 15:00 h. Rainfall was collected daily throughout this period at the nearby Station Alpine Joseph Fourier (Lautaret Pass, 2100 m). We assume that rainfall did not vary among the 12 fields. Overall, 1152 soil moisture measurements were made on the 12 fields during the growing season 2005.

### Quantification of vegetation effects

To determine the effect of vegetation on soil moisture, a removal experiment was performed in one field of each grassland type. Three blocks (each 16 m<sup>2</sup>) were randomly delimited within each field. The vegetation was removed from one half of each block using a systemic nonselective herbicide (Glyphosate, Roundup; Monsanto, St Louis, MO, USA). We located vegetated and unvegetated treatments side by side at the same level on the field slope, in order to avoid water flux between them. Dead vegetation was removed by hand and we severed any roots around the edge of the cleared area (to 25-cm depth). We inserted three pairs of TDR probes (15-cm depth) in both treatments at the centre of each half-block in order to avoid edge effects.

## Calculations and data analysis

Soil water properties were estimated for the 12 fields indirectly from the soil texture, organic matter and stoniness, using equations from Soil Plant Air Water (SPAW; US Department of Agriculture; Saxton, 1982) to determine available water content (AWC), wilting point and field capacity. We conducted ANOVA type 3 to test the effect of land use on soil water properties. For all analyses, normality and homogeneity of variance were assessed.

The effect of vegetation on soil moisture in the removal experiment was calculated using the log response ratio (LNRR) for each measurement date in each block (Suding *et al.*, 2003):

$$\text{LNRR}_{\text{vegetation}} = \log_e (\text{SMV}/\text{SMB})$$

(SMV, soil moisture with vegetation; SMB, soil moisture without vegetation.) A LNRR > 0 signifies that vegetation causes an increase in soil moisture and a LNRR < 0 denotes a negative effect of vegetation on soil moisture.

**Identification of traits responding to soil moisture** Response traits analysis was conducted on the 12 fields. A repeated-measures ANOVA was performed using a split-plot design on the TDR data for a depth of 0–15 cm for the 12 fields, to test the consistency throughout the growing season of differences in soil moisture across land use types. Land use effect was considered at the main plot level and each land use repetition (three fields per land use type) was considered as a block in the analysis. Time was considered at the subplot level. Field repetitions were used as random effects.

To identify traits (root, leaf and stature traits) responding to environmental factors, we first conducted a principal component analysis (PCA) on trait data to identify covariation among traits and potential axes of specialization. Data were centred before analysis as we used noncommensurate variables. We then conducted a linear regression between the first two axes of the PCA and two environmental variables: productivity (ANPP) and available water content (AWC). ANPP and AWC were selected for this regression based on a PCA showing them to be the major environmental variables segregating the 12 fields. Productivity is related to soil fertility (INN) (Quétiér *et al.*, 2007) and AWC is negatively linked to soil stoniness. However, productivity was not related to AWC (data not shown).

**Vegetation effects on soil moisture** Vegetation effect analyses were performed on data from the four plant communities used for the vegetation experiment. Hence, pseudo-repetitions of land use type were considered for this analysis (three blocks within each field site and one field per land use type). We assessed vegetation effects on soil moisture over the season in a split-plot design using two repeated-measures ANOVAs. We tested the effect of (1) site (one site per land use type) on

measured soil moisture with and without vegetation, and the individual effects of (2) vegetation on soil moisture as measured by  $\text{LNRR}_{\text{vegetation}}$ . For analyses (1) and (2), the site effect was considered at the main plot level, and time effects were considered at the subplot level. Block was used as a random effect. For analysis (1), the vegetation effect on soil moisture was analysed at the subplot level. We excluded samples from 27 May and 7 July as sampling on those dates was not complete. Post hoc analyses were conducted for each repeated-measures ANOVA using Student's *t*-tests. We tested whether LNRR was significantly different from zero at each date for each grassland type using an independent simple *t*-test.

We conducted different regression analyses for the vegetation effect on soil moisture (LNRR) depending on time following each rain event, with three separate analyses for the beginning (from 27 May to 15 June), the middle (from 15 June to 30 July) and the end (30 July to 15 September) of the growing season. We tested whether the rate of drying differed during these three periods using general linear models (GLMs). If no difference among periods during the season was found, we grouped data and conducted regressions between vegetation effect and time since rainfall throughout the season.

## Identification of traits with an effect on soil moisture

Multiple regressions were performed to model the effect of vegetation on soil moisture (LNRR) in each of the four grassland types as a function of time since the last rain event (Fig. S1), peak aboveground standing biomass and CFP for previously identified response traits (response traits analysis). Uncorrelated response traits were chosen for this analysis. Based on the outcome of the analysis of drying rates, we conducted two separate analyses for the early and mid–late seasons.

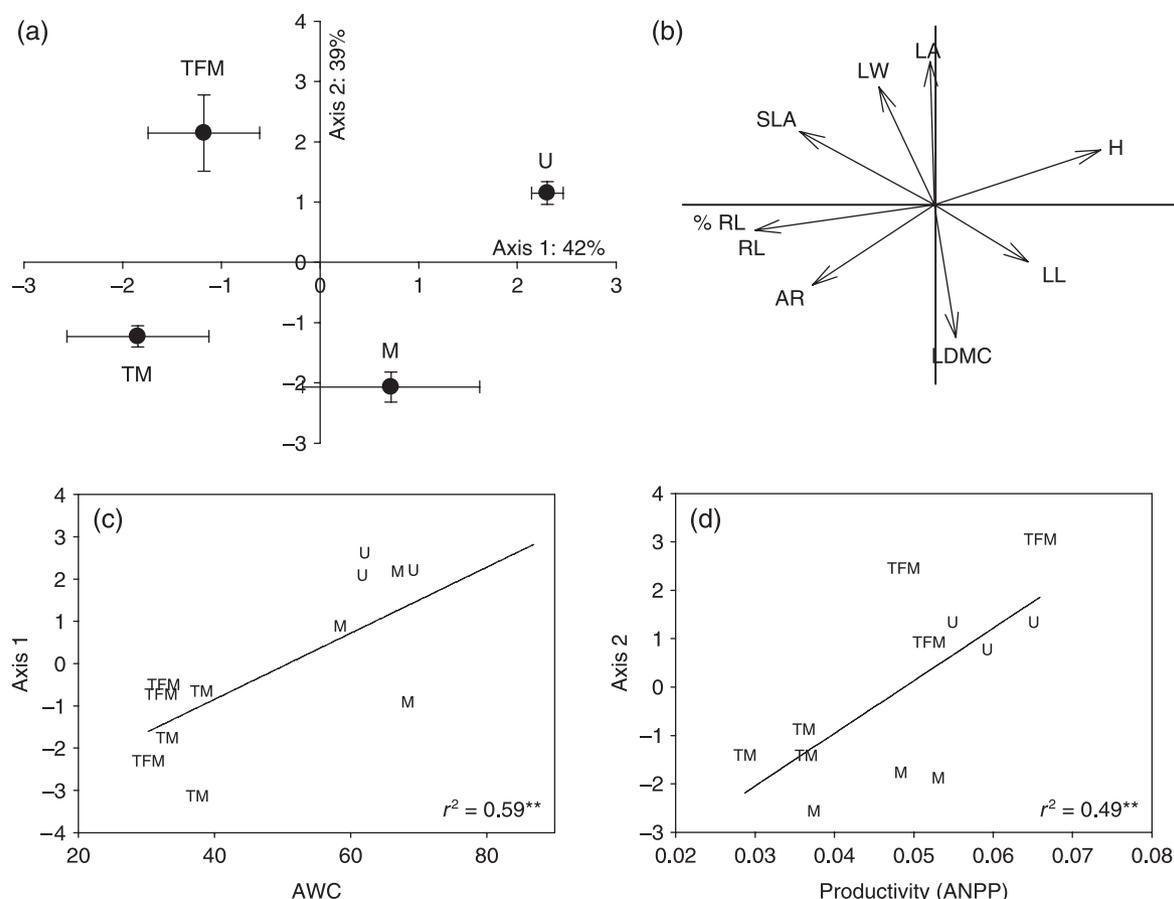
## Validation of experimental results: statistical model construction

The design of the vegetation effect experiment was based on pseudo-replication (i.e. within but not among fields), but we constructed a statistical model based on experimental results to validate the impact of effect traits on soil moisture and thus avoid pseudo-replication (Oksanen, 2001). We used the equations generated by multiple regressions to build the full model. SMV was estimated as the sum of soil moisture without vegetation (SMB) and the modelled vegetation effect. SMB was estimated by regression with time and AWC. There was a significant negative correlation between soil moisture without vegetation and AWC ( $r^2 = 0.98$ ,  $P < 0.0001$ ), indicating that bare soil moisture throughout the season was determined by soil properties.

Hence:

$$\begin{aligned} \text{SMV} &= \text{SMB} + \text{LNRR}, \text{ where } \text{SMB} = f(\text{AWC}, \text{time}) \text{ and} \\ \text{LNRR} &= f(\text{CFP}, \text{biomass}, \text{time}) \end{aligned} \quad (\text{model equation})$$

All components of the model were recorded in all 12 fields. To test the robustness of the model for more general



**Fig. 1** Analysis of response traits: (a, b) principal component analysis (PCA) using aggregated traits, and (c, d) the relationship between PCA axes and abiotic conditions ((c) available water content (AWC) and (d) annual net primary productivity (ANPP;  $\text{kg m}^{-2} \text{d}^{-1}$ )). Two orthogonal axes explain 42% and 39% of the variance. The end of the arrows indicates component loading. Land use (a): T, terraced; F, fertilized; M, mown; U, unmown grasslands. Traits (b): H, vegetative height; LDMC, leaf dry matter content; SLA, specific leaf area; AR, allocation to root; RL, root length per 100 g of soil; % RL, percentage of root length in the upper 15 cm of soil; LL, leaf length; LW, leaf width; LA, leaf area. \*\*,  $P < 0.001$ .

application to grasslands, it was validated using supplementary data from those eight fields not used for the removal experiment. Soil moisture in the eight fields for each date during the mid-late season (when vegetation effects were apparent) was calculated using the model equation, with the AWC, peak biomass and CFP values of each field. We then calculated the seasonal mean of predicted SMV and conducted a linear regression with mean observed soil moisture in the eight fields for the same period. All statistical analyses were performed using the software JMP 5.0.1 (SAS Institute, Cary, NC, USA) and the freeware R.

## Results

### Soil characteristics and vegetation response

CFPs were segregated along two main axes of variation by PCA, one corresponding to community root traits (root

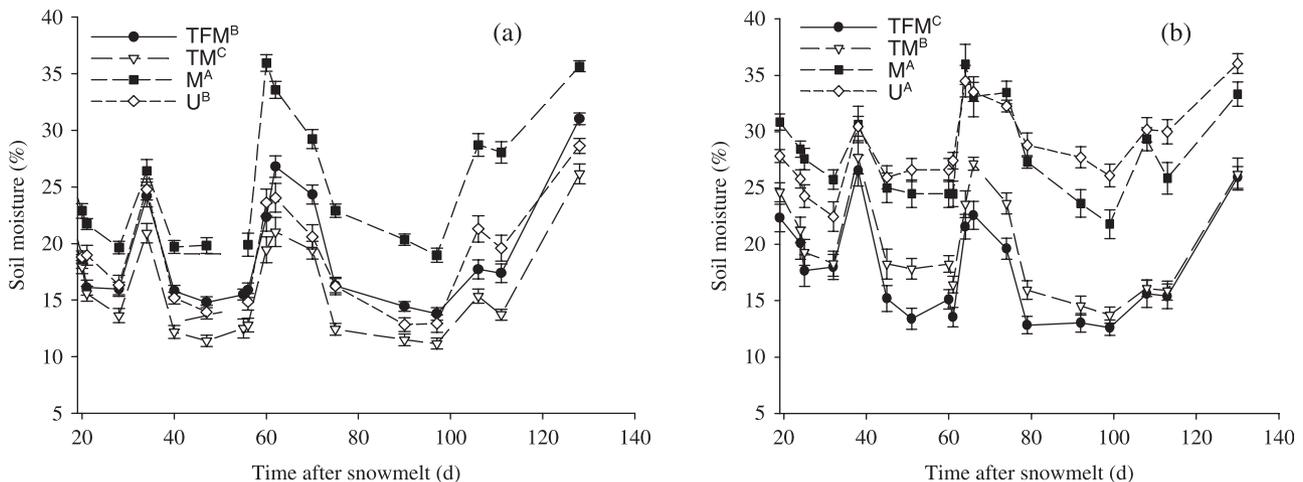
length and percentage of root length) and community height (42% of the total variance) and the other to community leaf traits (leaf area and LDMC) (39% of the total variance) (Fig. 1a,b). Hence, two independent axes of specialization were identified in our field site. The first axis was significantly correlated with AWC (Fig. 1c), whereas the second axis was linked to ANPP (Fig. 1d). As such, traits linked to axis 1 (community root traits and community height) can be considered as traits responding to soil water availability. Leaf traits associated with axis 2 are traits responding to productivity, as demonstrated in Quétier *et al.* (2007).

The segregation between terraced and unterraced grasslands reflected large differences in soil characteristics between terraced and unterraced grasslands (Table 1). The soil was shallower and stonier in the terraced than in the unterraced grasslands (Table 1). Calculations for soil texture indicated higher AWC in the unterraced than in the terraced grasslands: both wilting point and field capacity were higher in terraced

**Table 1** Soil characteristics for the different vegetation types, including soil depth, exposition, slope, stoniness, wilting point (1500 kPa), field capacity (33 kPa) and the available water content (AWC) at a depth of 0–30 cm

Grasslands		Soil characteristics						
Land use	Dominance	Soil depth (cm)	Aspect (°)	Slope (%)	Stoniness (%)	Wilting point (%)	Field capacity (%)	AWC (mm)
TFM	<i>Dactylis glomerata</i>	< 30	207.33	15.33	39 ± 3 <sup>b</sup>	25 ± 1.1 <sup>a</sup>	39 ± 0.9 <sup>a</sup>	30 ± 0.8 <sup>a</sup>
TM	<i>Bromus erectus</i>	< 30	207.67	16.33	48 ± 2 <sup>a</sup>	23 ± 0.9 <sup>a</sup>	40 ± 1 <sup>a</sup>	36 ± 1.9 <sup>a</sup>
M	<i>Festuca paniculata</i>	> 50	150.00	20.00	15 ± 1 <sup>c</sup>	20 ± 1.1 <sup>b</sup>	36 ± 0.9 <sup>b</sup>	71 ± 8.4 <sup>b</sup>
U	<i>Festuca paniculata</i>	> 50	176.67	20.00	15 ± 1 <sup>c</sup>	20 ± 0.6 <sup>b</sup>	34 ± 0.6 <sup>b</sup>	64 ± 2.3 <sup>b</sup>

T, terraced; M, mown; F, fertilized; U, unmown. The results of *t*-tests, which compared each land use type for each variable, are indicated by different letters when values are significantly different.



**Fig. 2** Soil moisture through the growing season (a) with and (b) without vegetation. T, terraced; F, fertilized; M, mown; U, unmown grasslands. Letters represent the results from post-hoc analysis using the *t*-test; when letters are different, fields are significantly ( $P < 0.05$ ) different.

grasslands (Table 1). Additionally, TDR measurements from the 12 fields at 0–15 cm depth (Fig. 2a) revealed a pronounced difference in soil moisture among the four land use types (Table 2a). Mown unterraced grasslands (M) retained the greatest soil moisture through the growing season compared with all other grassland types. Fertilized mown terraces (TFM) and unmown unterraced grasslands (U) were intermediate. Unfertilized mown terraces (TM) were the driest.

#### Effects of vegetation removal on soil moisture

These results concern only the experiment conducted in the four experimental fields (one field per land use type). The removal of vegetation had a large effect on soil moisture (Table 2b,c, Fig. 2) which differed across sites (Fig. 2, Table 2) and changed through the growing season (Table 2b; vegetation × site × time,  $P < 0.0001$ ). On average, the moisture content of bare soil was higher in the unterraced than in the terraced grasslands (Fig. 2b). After vegetation removal, the unmown grassland (U) remained drier than the mown grassland (M).

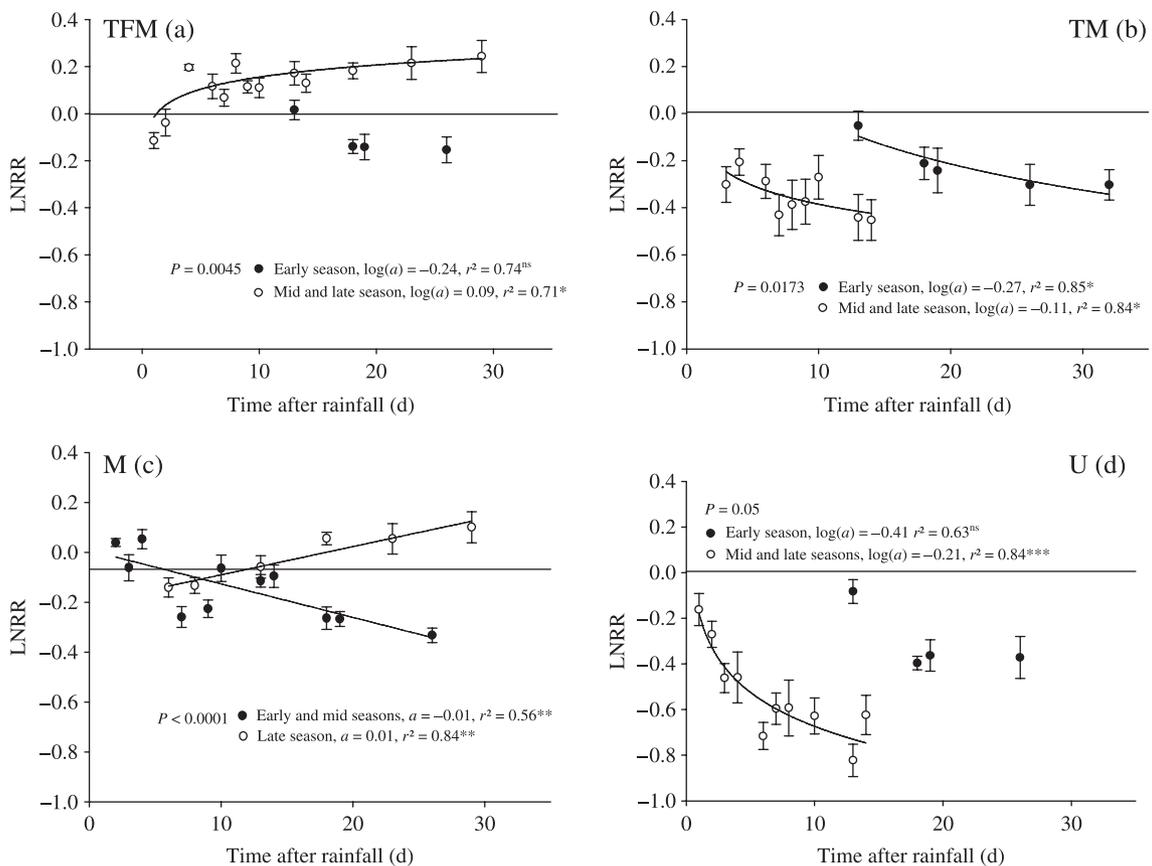
However, bare soil tended to be wetter in the TM grassland than in the TFM grassland. These results indicate that plant community type, as modified by land use, can change the soil moisture ranking among land use types (Table 2b; vegetation × site,  $P < 0.0001$ ).

Seasonal trends in soil moisture were affected by vegetation differently depending on land use (Table 2c). At the beginning of the season, all communities had a similar negative effect on soil moisture (Fig. 3). However, during the season there were large differences in the vegetation effect among grasslands (Fig. 3, Table 2; site,  $P < 0.0001$ ). Despite a negative effect on soil moisture at the beginning of the season (Fig. 3a), vegetation in the fertilized mown grassland (TFM) had a positive effect through the rest of the season. This positive effect started 40 d after snowmelt (c. 15 June). Vegetation in the mown terraced grassland (TM) (Fig. 3b) had a negative effect on soil moisture throughout the growing season. In contrast, vegetation in the mown unterraced grassland (M) had a small initial negative effect on soil moisture at both depths, which, towards the end of the growing season (115 d

**Table 2** Effects of land use (a) and vegetation removal (b and c) on soil moisture analysed with repeated-measures ANOVA in a split-plot design

(a) Soil moisture				(b) Soil moisture				(c) LNRR (vegetation)			
12 sites				Four experimental sites				Four experimental sites			
Effect	d.f.	F	P	Effect	d.f.	F	P	Effect	d.f.	F	P
Plot level				Plot level				Plot level			
LU	3	37.72	< 0.0001	Site	3	186.05	< 0.0001	Site	3	143.68	< 0.0001
Error	8			Error	8			Error	8		
Subplot level				Subplot level				Subplot level			
Time	1	16.47	< 0.0001	Vegetation	1	201.01	< 0.0001	Time	1	44.49	< 0.0001
Time × LU	3	8.10	< 0.0001	Time	1	19.51	< 0.0001	Time × site	3	14.6	< 0.0001
Error	1142			Vegetation × site	3	57.11	< 0.0001	Error	524		
				Time × site	3	8.41	< 0.0001				
				Vegetation × time	1	9.22	< 0.0001				
				Vegetation × time × site	3	10.35	< 0.0001				
				Error	1116						

(a) Effect of land use (LU) on soil moisture using the data set for 12 grassland sites with vegetation; (b) effect of vegetation and its removal on soil moisture using four grasslands (one site per land use type – different grasslands from those in a); (c) effect of vegetation on soil moisture quantified with the log response ratio (LNRR(vegetation)) using four grasslands (one site per land use type); main plot and subplot errors are indicated.



**Fig. 3** Relationship between days after rainfall and the effect of vegetation on soil moisture (log response ratio (LNRR)), in the early (end of May to mid June 2005), mid (mid June to end of July 2005) and late (early August to mid September 2005) season. (a) Terraced fertilized mown (TFM), (b) terraced mown (TM), (c) mown (M) and (d) unmown (U) grasslands. *a* is the regression slope, within each vegetation type; *P*-values indicate the probability that the slopes of regressions are significantly different. When LNRR > 0 vegetation increased soil moisture; when LNRR < 0 vegetation decreased soil moisture. T, terraced; F, fertilized; M, mown; U, unmown grasslands. ns, not significant. \**P* < 0.05; \*\**P* < 0.001; \*\*\**P* < 0.0001.

**Table 3** Relationship between traits and the vegetation effect on soil moisture in the early and mid–late season

	d.f.	Early season		Mid–late season	
		Parameter	Probability	Parameter	Probability
Overall $r^2$		0.69	***	0.83	***
Intercept		0.009	ns	0.89	***
Biomass (kg m <sup>-2</sup> )	1	-0.027	ns	-0.14	***
Time	1	-0.014	***	0.002	ns
Leaf area (mm <sup>2</sup> )	1	0.00002	ns	7.046E-05	***
Root length (cm per 100 g of soil)	1	0.000017	ns	-7.29E-05	***
Error		23		37	

Values shown are multiple regression parameters. ns, not significant ( $P > 0.05$ ); \*\*\*,  $P < 0.0001$ .

after snow melt), became positive (Fig. 3c). In the unmown grassland (U) (Fig. 3d) vegetation had a strong negative effect on soil moisture. All the effects of vegetation increased with time after each rainfall event (Fig. 3).

### Relating the effects of vegetation on soil moisture to plant traits

We selected two candidate independent effect traits among response traits identified in the PCA analysis (Fig. 1). Community root length, which responded to AWC, was considered for the first axis as this trait can influence water uptake by vegetation (Wahl *et al.*, 2001; Eviner & Chapin, 2003). Community leaf area, which responded to productivity, was selected as a candidate effect trait related to axis 2 because it was strongly correlated with community light interception ( $r^2 = 0.80$ ,  $P < 0.0001$ , d.f. = 1, 11). Vegetation effects on soil moisture ( $\text{LNRR}_{\text{vegetation}}$ ) were modelled by multiple regression including time after each rain event (time), above-ground biomass of the community (biomass), and traits responding to productivity and soil moisture, leaf area (LA) and root length (RL) (Table 3). Two multiple regression analyses were conducted depending on the phase of the growing season, testing the regression equation  $\text{LNRR}_{\text{vegetation}} = a \text{ biomass} + b \text{ time} + c \text{ community-LA} + d \text{ community-RL} + e$ . In the early season (1), the effect of vegetation on soil moisture was only dependent on time since rainfall (Table 3,  $r^2 = 0.69$ ), and CFPs did not have any significant effect, that is, water uptake and evaporation from the soil had the same net effect across all communities. During the mid–late season (2), in addition to time since rainfall, total aboveground biomass and the two CFPs strongly influenced the effect of vegetation on soil moisture (Table 3;  $r^2 = 0.83$ ), with effects of a similar magnitude for these three variables. Increases in aboveground biomass and community root length both increased soil moisture depletion (negative  $\text{LNRR}_{\text{vegetation}}$ ). In contrast, an increase in community leaf area ameliorated soil moisture depletion (positive  $\text{LNRR}_{\text{vegetation}}$ ). Leaf area and root length both responded to field abiotic conditions

(fertility and water availability, respectively) and affected soil moisture, and thus could be considered as response-effect traits.

### Statistical model validation

To validate our experimental findings, we used this regression model obtained from experimental data for the four fields representative of the four land use types to predict soil moisture with vegetation in the mid-season for the other eight of our 12 fields (excluding the four experimental fields). If:

$$\text{LNRR}_{\text{vegetation}} = \ln(\text{SMV}/\text{SMB}), \text{ then}$$

$$\text{SMV} = \exp[(\text{LNRR}) + \text{LN}(\text{SMB})] + k, \text{ with}$$

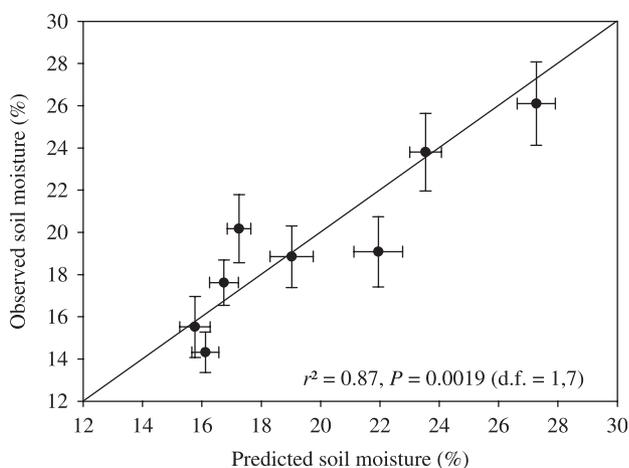
$$\text{LNRR} = f(\text{CFP})$$

and  $\text{SMB} = d \text{ AWC} + e \times t + m$ , where AWC is the available water content of each field,  $t$  is the number of days since rainfall and  $k$  and  $m$  are constant.

Coefficients for  $\text{LNRR}_{\text{vegetation}}$  are given in Table 2. For SMB,  $d = 1.8034$ ,  $e = -0.19957$ ,  $m = -12.89$ , and overall  $r^2 = 0.53$  ( $P < 0.001$ ). Therefore, soil moisture under vegetation was a function of both soil properties (SMB) and vegetation effect ( $\text{LNRR}_{\text{vegetation}} = f(\text{CFP})$ ). There was a strong relationship between mean predicted and observed values of soil moisture in all eight fields during the mid–late season (Fig. 4).

### Discussion

In this study, we investigated plant community effects on soil moisture and concurrent changes in CFP and abiotic factors under different land uses. We combined an analysis of functional composition under different land uses and the experimental manipulation of communities (vegetation removal). By using continuous and quantitative traits, we were able to generalize and validate our results from a few experimental sites to a larger data set and thus avoid pseudo-



**Fig. 4** Relationship between average predicted data and average observed data for soil moisture in the eight fields during the mid-late seasons. The value of the regression test ( $r^2$ ) is indicated in the figure with degree of freedom (d.f.). Error bars represent the standard error across measurements or estimated values for different dates.

replication (Oksanen, 2001). We found that subalpine grassland communities characterized by contrasting CFPs had different effects on soil moisture (Table 1, Fig. 2). This result illustrates the importance of community composition in regulating water fluxes (McLaren *et al.*, 2004) and demonstrates that some response traits can also be considered as effect traits which can be used to estimate changes in ecosystem properties such as soil moisture (Lavorel & Garnier, 2002; Suding *et al.*, 2008).

### Response traits: evidence for two independent axes of specialization

Response traits at the community level were distributed along two axes of specialization (Fig. 1) which were consistent with trait correlation patterns previously identified for individual dominant grass species from the study area (Gross *et al.*, 2007). These two axes of specialization were generally consistent with current plant strategy schemes (Grime, 1977; Westoby, 1998), and reflected different strategies for responses to fertility and water availability (Ackerly, 2004), the two ecological gradients that drive functional composition in the studied communities (Fig. 1).

The primary axis of specialization is linked to vegetation height and community root traits. These response traits were related to soil moisture but not to soil fertility (Fig. 1). Communities with deep roots but short root length and tall canopies were associated with high water availability. These results are consistent with previous studies which have demonstrated that tall plants with deep roots can be associated with drought intolerance resulting from high transpiration rates (Schwinning & Ehleringer, 2001; Wahl *et al.*, 2001; Schwinning & Sala, 2004), whereas small plants with greater

root allocation to the upper layers of the soil are often drought tolerant (Schwinning & Sala, 2004).

The second axis, corresponding to leaf traits, was associated with soil fertility (Fig. 1 and Quétier *et al.*, 2007). It separated conservative and exploitative plant trait syndromes (Diaz *et al.*, 2004). Contrary to our hypothesis, this axis was not related to water availability and provided no evidence that high transpiration rates associated with an exploitative syndrome (Reich *et al.*, 1999; Westoby *et al.*, 2002) could be linked to drought intolerance (Reich *et al.*, 1999; Wright *et al.*, 2001). However, this result is consistent with several other studies where community SLA and associated traits did not vary most closely with soil moisture (Fonseca *et al.*, 2000; Fernandez *et al.*, 2002; Diaz *et al.*, 2004) but were primarily affected by soil fertility (Quétier *et al.*, 2007).

### Traits with an effect on soil moisture

Combination of independently varying traits has been adopted as a practical way to identify different strategies of resource use (Grime, 1977; Lavorel & Garnier, 2002; Ackerly, 2004) and to describe the relationship between the plant community and ecosystem properties (Eviner & Chapin, 2003; Eviner *et al.*, 2006). Here, the same response traits linked to the two independent axes of specialization determined vegetation effects on soil moisture, and were thereby identified as response-effect traits. These traits, leaf area and root length, were combined with community aboveground biomass (Table 2, Fig. 3) to model vegetation effects on soil moisture.

High green and dead aboveground biomass had a negative effect on soil moisture, consistent with a high community transpiration rate. The strongest drying effect occurred in the unmown (U) grassland. This community not only had high biomass production, but also had the greatest litter accumulation. Although the litter layer often reduces soil drying (Suding & Goldberg, 2001), at our field site litter accumulation probably contributed to the dryness of the soil by increasing the interception and absorption of rainfall (Gross, 2007).

Communities with high leaf area had a positive effect on soil moisture. Phenological data indicate that these community types had already finished their growth before the summer drought (Quétier *et al.*, 2007), suggesting that their requirement for water and their transpiration rate might be low by that time (Schwinning & Ehleringer, 2001). The positive effect of high leaf area might be linked to the increased shade causing lower incident radiation and soil temperature and thus reduced evaporation (Rosset *et al.*, 2001). Indeed, in our study, leaf area and not vegetation height was directly linked to light interception. Alternatively, large shade-leaves associated with a tall canopy might have been able to capture and siphon water as dew (Brewer & Smith, 1997; Weathers, 1999).

At the community level, different root trait syndromes led to contrasting drying effects on soil moisture. High root length, which was associated with roots predominantly in the

top layer of the soil (Table S2), had a strong negative effect on soil moisture (consistent with Sala *et al.*, 1989; Schwinning & Ehleringer, 2001; Wahl *et al.*, 2001; Eviner & Chapin, 2003). Contrary to our hypothesis, root allocation seemed to be weakly linked to the vegetation depletive effect. For instance, unterraced and mown grassland vegetation had a small negative effect on soil moisture despite the fact that these community types had the highest root allocation (Table S1). Hence, our results confirm that root length might be a better indicator than root allocation of the vegetation effect on water availability (Gordon & Rice, 1993; Craine, 2006).

### Using response-effect traits to understand the consequences of land use change for ecosystem properties

Links between response and effect traits can provide a mechanistic basis for understanding concurrent changes in ecosystem functioning and land use (Chapin *et al.*, 2000; Lavorel & Garnier, 2002; Lavorel *et al.*, 2007), and this approach has proved applicable in subalpine grasslands (this study; Quétier *et al.*, 2007). As we used a correlative approach for response trait analysis at our field site (Fig. 1), we did not explore all combinations of land use in this study. Indeed, this study (and others carried out at our field site) was based on land use trajectories (Quétier *et al.*, 2007) and not on a factorial design. The only complete comparisons are based on terraced vs unterraced field sites. The effect of mowing or fertilization can be considered only within unterraced fields or terraced fields, respectively. However, based on response-effect traits found in this study, ecosystem functioning under land use change can be estimated from observed changes in response traits at the community level (Suding *et al.*, 2008).

Current land use change at our site and throughout similar mountain and other marginal grasslands across Europe includes the cessation of both mowing and fertilization (Quétier *et al.*, 2007). These land use changes have severely modified CFPs (Louault *et al.*, 2005; Quétier *et al.*, 2007). In this study, we showed that these changes are associated with a decrease in soil moisture, in addition to the depression of other ecosystem properties demonstrated by companion studies, such as decreased nutrient cycling (Robson *et al.*, 2007), delayed litter decomposition (Quétier *et al.*, 2007), and loss of plant diversity (Moog *et al.*, 2002; Quétier *et al.*, 2007). Here, the cessation of fertilization in water-limited grasslands decreased community leaf area (response), increased community root length (response) and thereby switched the effect of the community from positive in fertilized grasslands characterized by high community leaf area to negative in unfertilized grasslands characterized by low community leaf area. Similarly, the cessation of mowing in unterraced grasslands, by increasing green and dead aboveground biomass, is

likely to increase the transpiration rate and water interception and lead to a strong negative effect on soil moisture.

In this study, we successfully modelled the effect of vegetation on soil moisture using a simple trait-based approach. Our findings showed that multiple traits can predict ecosystem properties and that overlapping/coinciding response-effect traits can provide a mechanistic link mediating changes in ecosystem function and land use change. For this purpose, the combination of correlative analysis and experimental manipulations should be encouraged to disconnect response and effect traits.

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## Supporting Information

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

**Fig. S1** Rain events in the growing seasons.

**Table S1** Grassland characteristics for each land use type

**Table S2** Matrix of correlations between community traits

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