

Using functional traits to predict grassland ecosystem change: a mathematical test of the response-and-effect trait approach

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Abstract

The role of plant community structure and plant functional traits for above- and belowground carbon (C) fluxes was studied for 2 years in a mesocosm experiment with grassland monoliths, using continuous gas exchange measurements and soil analyses. Here we test the response-and-effect trait hypothesis, by applying a mathematical framework used to predict changes in C fluxes after a change in disturbance through the community response (R) and effect (E) traits. Monoliths were extracted from two contrasted long-term field treatments (high vs. low grazing disturbance) and exposed to both low and high (simulated grazing) disturbance during a 2 years experiment. Carbon dioxide exchanges were measured continuously in an open flow system. Net ecosystem productivity and ecosystem C balance were positively correlated at low disturbance with plant species richness. Aboveground net primary productivity (ANPP) and soil C sequestration were, however, unrelated to these variables. Community aggregated leaf (specific leaf area, leaf dry-matter content) and root and rhizome (specific length, tissue density, diameter) traits responded (R) significantly to changes in disturbance, indicating an increased dominance of conservative plant growth strategies at low compared with high disturbance. Applying the mathematical framework, ANPP was predicted by distribution of leaf traits within the community (functional divergence), while mean root and rhizome traits had significant effects (E) on soil C sequestration, irrespective of the experimental disturbance and of the year. According to highly significant linear regression models, between 6% and 61% of the transient changes in soil C sequestration resulted from community root and rhizome (response-and-effect) traits after a change in disturbance.

Keywords: biodiversity, carbon cycle, disturbance, ecosystem services

Received 20 November 2008 and accepted 16 January 2009

Introduction

Predicting ecosystem responses to global change has become a major challenge in ecology (Chapin *et al.*, 1998; Loreau *et al.*, 2001), particularly as terrestrial ecosystems contribute to the mitigation of global climate change through carbon (C) sequestration in the soil and vegetation (IPCC, 2007). Grasslands are one of the major biomes worldwide and temperate grasslands contribute to ca. 10% of the global organic C content in soils (IPCC, 2000). Changes in C fluxes were suggested to be greatest where disturbance (e.g. fire, grazing) has

induced shifts in grassland communities, that alter the use and availability of key resources (Chapin *et al.*, 2002). Thus, a better understanding of the coupling between plant diversity and C cycling is essential for predicting ecosystem services under global change.

Biodiversity experiments have shown causal relationships between species number, ecosystem productivity (e.g. Tilman *et al.*, 1997; Hector *et al.*, 1999; Röscher *et al.*, 2005) and C sequestration (Tilman *et al.*, 2006). Despite an increasing agreement on the role of plant species diversity for ecosystem processes and resulting ecosystem services (Hooper *et al.*, 2005), very little is known about the role of plant traits which are mediated by plant species (Chapin *et al.*, 2000; Lavorel & Garnier, 2002; Diaz *et al.*, 2007a).

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In the mass ratio hypothesis, Grime (1998) suggested that the extent to which the trait of a species affects ecosystem properties strongly depends on the relative contribution to the total biomass of that species. Accordingly, ecosystem processes (e.g. C cycling, De Deyn *et al.*, 2008) would be determined by aggregated traits of dominant species. The distribution of trait values within the community, the functional divergence represents the degree of overlap in trait values within a community (Mason *et al.*, 2005). The role of community-aggregated plant functional traits and functional divergence (Diaz *et al.*, 2007b; Mokany *et al.*, 2008) has been shown for aboveground net primary productivity (Garnier *et al.*, 2004; Da S. Pontes *et al.*, 2007), shoot litter decomposability (Aerts *et al.*, 2003; Kazakou *et al.*, 2006; Diaz *et al.*, 2007a) and C-cycling (see Wardle *et al.*, 2004; Bardgett *et al.*, 2005; De Deyn & Van der Putten, 2005), but has never been evidenced for soil C sequestration.

It has been shown that ecosystem responses to environmental changes occur as a result of (i) sorting processes among individuals with appropriate response traits (Weiher, 1998; Grime, 2006) and (ii) changes in ecosystem-effect-traits (Diaz & Cabido, 2001; Lavorel & Garnier, 2002; Diaz *et al.*, 2007b; Violle *et al.*, 2007). Suding *et al.* (2008) proposed a framework incorporating functional traits to predict effects of environmental change on ecosystem.

This framework differentiates between environmental response (response traits) and effects of environmental change on ecosystem processes (effect traits) (e.g. Lavorel & Garnier, 2002). The relationship between response (*R*) and effect (*E*) traits, while a system undergoes a change, plays an important role in predicting ecosystem functioning. For example, traits that are co-related are presumably both predictive of response and effect (Gross *et al.*, 2008). Therefore, changes in ecosystem functioning after environmental change will be partly mediated by community dynamics (Suding *et al.*, 2008). Given the paucity of empirical tests to validate this approach, more experiments are needed where species contribution to ecosystem processes are related to environmental changes and effect traits of a certain species or group (e.g. Reich *et al.*, 2001; Craine *et al.*, 2002; Garnier *et al.*, 2004).

The impact of environmental changes on C sequestration has been shown for a range of European grassland sites (Soussana *et al.*, 2007), where net ecosystem C storage was found to decline with disturbance by grazing and cutting. A mesocosm study with a seminatural grassland showed that net ecosystem productivity (NEP) and belowground net C storage (BNCS) did not vary 2 years after changes in disturbance regime, but were significantly higher for monoliths acclimated for 14 years to a low, compared with a high, pre-experi-

mental (field) disturbance (Klumpp *et al.*, 2007a). Moreover, the decay of soil organic matter fraction (>0.2 mm) was slower at low compared to high pre-experimental disturbance (Klumpp *et al.*, 2007b). The disturbance regime in the field (before mesocosm experiment) had carry-over effects on NEP and soil C storage [calculated by both gas exchange balance and soil organic carbon analyses, Klumpp *et al.*, 2007a], presumably due to plant community dynamics (Paterson, 2003).

Here we analyzed the role of plant community structure and traits for C fluxes in the same mesocosm experiment. Initially we test the effects of field (pre-experimental) and experimental disturbance on plant community structure and community-scale traits. We provide for the first time a mathematical method to test Suding's (Suding *et al.*, 2008) hypothesis, that changes in C fluxes after environmental change can be predicted from the community response (*R*) and effect (*E*) traits, and we assess the role of aggregated traits and of functional divergence for shoot traits, following the framework recently proposed by Diaz *et al.* (2007b).

Materials and methods

Mesocosm experiment with grassland monoliths

The study was carried out at Clermont Ferrand, France, using monoliths (each $0.5\text{ m} \times 0.5\text{ m} \times 0.4\text{ m}$) from an upland grassland site. The grassland studied (vegetation, soil) and the procedures used to select and extract grassland monoliths have been previously described by Klumpp *et al.* (2007a). In June 2002, 56 pasture monoliths were sampled from two permanent (14 years) seminatural grassland plots, of contrasting sheep grazing regimes, without fertilizer applications (Loiseau *et al.*, 2005; Louault *et al.*, 2005). Field plot 1 was cut once and sheep grazed 4 times yr^{-1} and therefore represented a high disturbance regime (H). Field plot 2 was sheep grazed once a year (low disturbance regime, L). Following extraction, each monolith was placed in a five-sided stainless-steel box with drain holes at the bottom. Twenty-four monoliths from each field plot (pre-experimental H and L) were placed at random in eight transparent (polyethylene film, $60\text{ }\mu\text{m}$) canopy enclosures ($1.5\text{ m} \times 0.5\text{ m} \times 0.75\text{ m}$). Each canopy enclosure therefore contained three monoliths from the same disturbance treatment.

The 16 canopy enclosures were exposed to natural light. Enclosures were part of an open-flow gas-exchange system (see Klumpp *et al.*, 2007a), where temperature and humidity of enclosure inlet air was adjusted to outdoors conditions by a computer con-

trolled cooling system and air humidifier, respectively. The experiment commenced in April 2003, whereupon the carbon dioxide (CO_2) concentration in the enclosures was maintained, from sunrise to sunset, comparable to the outdoor CO_2 concentration (mean difference of $13.2 \pm 9.5 \mu\text{mol mol}^{-1}$) by injection of CO_2 (Klump *et al.*, 2007a).

Depending on season, monoliths were watered between one and three times a week to target a soil volumetric water content of 33%, corresponding to a soil water potential of ca. -30 kPa . All enclosures received the same irrigation volumes.

Management and treatments

In September 2002, the canopy enclosures were closed and supplied with ambient air to adapt vegetation to enclosure conditions. At the start of the experiment, half of the enclosures per treatment (4, i.e. 12 monoliths) were switched to the opposite disturbance regime (HL and LH, crossed treatments), while half of the enclosures remained with the same disturbance level (HH and LL). Therefore, we applied a high disturbance (i.e. cut) to half the monoliths with pre-experimental low disturbance level (HL) and a low disturbance (uncut) to half the monoliths previously exposed to a high disturbance regime (LH). Hence, changes in disturbance level (HL and LH) can be compared with controls (HH, LL) in a fully factorial design, with four replicate enclosures per treatment.

On five occasions per year the monoliths of the experimental high disturbance regime HH and LH (24 and 4 controls) were cut simultaneously at 5 cm stubble height. Artificial urine (in average 5 g N m^{-2} per event, see Klump *et al.*, 2007a) was applied evenly following each cut, in order to simulate N returns at grazing. The remaining monoliths of the experimental low disturbance regime LL and HL (24) were neither cut nor fertilized. The harvested phytomass was separated into live and dead plant parts, oven dried for 48 h at 60°C and weighed. At the start and at the end of each growing season (in April and September) the standing aboveground biomass (including stubbles) was measured on half a monolith, in each enclosure.

C fluxes

The air flow and CO_2 concentration in each open-flow canopy enclosure was monitored continuously during the 2 years of the experiment. The above and soil CO_2 fluxes were calculated for each enclosure from these airflows and from changes in CO_2 concentrations (see Klump *et al.*, 2007a for full details). Gross primary

productivity (GPP) was the net photosynthesis (P_n) plus estimated daytime canopy dark respiration (R_d , see Casella & Soussana, 1997). The NEP was the net photosynthesis (P_n) minus aboveground (night-time, R_p) and soil (R_s) respiration. The annual total net carbon storage (NCS) in the monoliths was P_n minus total respiration ($R_s + R_p$) and minus annual C yield by cut (Chapin *et al.*, 2002). The aboveground net primary productivity (ANPP), of enclosures exposed to high disturbance levels (HH and LH), was the sum of the annual C yield by cut plus the C content of stubbles in September (date of peak biomass). For enclosures exposed to a low disturbance level (LL and HL), the ANPP was defined as the standing dead and live aboveground phytomass in September, plus stubbles, in order to integrate part of the biomass turnover during growing season (e.g. Scurlock *et al.*, 2002). Specific aboveground net primary productivity (SANPP), was the difference between log-transformed values of final and initial aboveground biomass divided by days of regrowth and growing season for cut and uncut monoliths. The BNCS was then calculated as the NEP minus the ANPP.

Soil organic C content

Soils were sampled once before the start of the experiment and then after 12 and 24 months. At each soil harvest, the 0–10 cm layer was air dried and the free organic matter fractions (OM) $> 200 \mu\text{m}$ (fast decomposing soil pool) were separated with water by wet sieving (Klump *et al.*, 2007a,b). Their C content was then analysed by an elemental analyzer (Carlo Erba Instruments, CNS NA 1500, ThermoFinnigan, Milan, Italy).

The top soil organic carbon (SOC) content $> 0.2 \text{ mm}$ was in average 10.7 (7.5), 6.2 (5.0), 5.0 (4.3), and 2.5 (3.3) mg C g^{-1} soil for LL, LH, HL and HH treatment in the first and second (parenthesis) year of the experiment. Year factor had no effect on SOC content, while SOC was significantly higher for monoliths of low pre-experimental and experimental disturbance, than for monoliths managed with a high disturbance regime (data not shown).

Plant community structure

Point quadrat measurements of plant species composition were carried out after monolith extraction in September 2002 and then five times during the experimental period (May and October 2003, April and July 2004, April 2005). During the experiment (September 2002 to April 2005) the mean observation area for plant species community was 0.5 m^2 . Presence/absence of species was recorded at 16 points per monolith (see Klump *et al.*, 2007a) by moving a pin down-

wards, which could hit more than one species. Shannon's diversity index (H') was calculated as

$$H' = \sum_i (p_i \log p_i), \quad (1)$$

where p_i is the presence of species i .

Leaf traits

Leaf traits, for plant species >5% presence, were measured on four occasions (June 2003, April and July 2004, April 2005), 1 week after the point quadrat measurements. Measured plant traits were vegetative height, leaf mass ratio, specific leaf area (SLA) and leaf dry-matter content (LDMC) (methodologies see Garnier *et al.*, 2001; Louault *et al.*, 2005).

For each enclosure and each sampling date, aggregated community scale trait values were calculated by weighing the individual trait value of species i (T_i) by its percentage presence (p_i) in the plant community (Garnier *et al.*, 2004). The community aggregated trait values (T_c) is

$$T_c = (1/s) \sum_{i=1}^s p_i T_i, \quad (2)$$

where s is the number of plant species in the community. The distribution (i.e. overlap) of trait values within the community, the functional divergence (FD), was calculated from species relative abundance and species leaf traits (SLA, LDMC, leaf mass ratio and mean height) using Rao's index, and was either used individually or averaged (FD_{vg}) across all traits (methodologies see Mason *et al.*, 2005; Lepš *et al.*, 2006)

$$FD = \sum_{i=1}^s \sum_{j=1}^s d_{ij} p_i p_j, \quad (3)$$

where d_{ij} is the distance between i -th and j -th species in a community, and p_i and p_j are the relative abundance of species i and j respectively, and s is the number of species in the community.

Root and rhizome traits

Belowground traits were measured from fresh soil samples of the September 2003 and April 2005 soil harvest. On both occasions, a vertical soil slice was sampled (50 cm × 6 cm) from the top soil layer (10 cm) of a monolith per enclosure. After washing on a 1 mm sieve, two subsamples were taken. A first subsample was sorted by hand into coarse roots (i.e. primary roots), fine roots and rhizomes. A second subsample was used to determine community root and rhizome traits. The following traits were measured: specific length (SL, m g^{-1} DM), tissue density (DENS,

g DM cm^{-3}), diameter (D , mm) and nitrogen content (NC, g N g^{-1} DM) (for methodology, see Roumet *et al.*, 2006).

Trait based response-and-effect framework

An ecosystem function (Y) applied to a plant community at time (t_1) can be broken down, according to the mass ratio hypothesis (Grime, 1998), in terms of the per-gram or per-capita effect (E_{jt1}) of a species j and its current abundance (n_{jt1}), where E is an effect trait. Using a trait-based prediction of the response to a changed environment (t_2), the abundance of species j in the new environment (n_{jt2}) is predicted to be $n_{jt1} R_j$, where R is a response trait. According to Suding *et al.* (2008), following a change in environment (from time 1 to time 2), the contribution of a given species j , to future ecosystem function Y_2 , can be assumed to be a function of the effect of a species on Y and its predicted abundance n_{jt2} . Hence as shown by Suding *et al.* (2008)

$$\begin{aligned} Y_2 &= \sum_{j=1} f(n_{jt2} E_j), \\ Y_2 &= \sum_{j=1} f(n_{jt1} R_j, E_j). \end{aligned} \quad (4)$$

In order to test this hypothesis, we now assume that f is a linear function with a constant slope a , and with an intercept (b_1 , b_2) which varies with environmental conditions from time 1 to time 2:

$$Y_2 = \sum_{j=1} a(n_{jt1} R_j, E_j) + b_2. \quad (5)$$

Let \bar{E} be, the mean of the effect trait weighted by species abundance at times 1 and 2:

$$\begin{aligned} \bar{E}_1 &= \sum_{j=1} (n_{jt1}, E_j), \\ \bar{E}_2 &= \sum_{j=1} (n_{jt1} R_j, E_j). \end{aligned}$$

Hence,

$$\begin{aligned} Y_1 &= a\bar{E}_1 + b_1, \\ Y_2 &= a\bar{E}_2 + b_2. \end{aligned} \quad (6)$$

According to Eqn (5), the change in ecosystem function will scale linearly with the change in mean effect trait (\bar{E}_1, \bar{E}_2) from time 1 to time 2:

$$Y_2 - Y_1 = a(\bar{E}_2 - \bar{E}_1) + (b_2 - b_1). \quad (7)$$

Whenever effect and response traits are related (see Suding *et al.*, 2008), $\bar{E}_2 = k\bar{E}_1$, where k is a constant

reflecting the change in response trait between the two time periods. Under these circumstances

$$Y_2 - Y_1 = (k - 1)a\bar{E}_1 + (b_2 - b_1). \quad (8)$$

Using this reasoning, the change in C flux with environmental conditions can be broken down into a direct effect of the environmental change ($b_2 - b_1$) and an indirect effect of response-and-effect trait change $[(k - 1)a\bar{E}_1]$. However, effect and response traits may completely overlap, we would then expect to see resilience or dampened changes in ecosystem function (Suding *et al.*, 2008).

Data analysis

As the experiment started in spring 2003 and finished in spring 2005, annual means of C fluxes and plant traits were calculated from the beginning of April to the end of April of the next year. Repeated measure analysis of variance (ANOVAS) and Fisher's LSD *post hoc* tests were performed to test effects of abiotic factors such as pre-experimental (H vs. L, disturbance) and experimental disturbance (H vs. L, disturbance level after the start of the experiment) and date. Effects of individual and combined factors on C fluxes (i.e. abiotic variables, functional diversity and community traits) were tested by Spearman's rank correlation and multiple regression models with a step-wise ascending procedure (see Diaz *et al.*, 2007b). In the multiple regression model, final combination of traits and trait distribution (FD) were retained according to *F*-value criteria. Community aggregated traits and FD's significantly affecting C fluxes, were fitted to Eqn (8) and slopes were compared using a comparison of regression-line procedure. When necessary, data were transformed by log or square root to satisfy tests of normality. Statistical analyses were performed with the STATISTICA 6 package (StatSoft Inc., Tulsa, OK, USA).

Results

Plant community dynamics and C fluxes

Effects of disturbance on plant community dynamics. Over 2 years of experiment, 41 plant species contributed to 99% of the total vegetation cover. Grasses had the highest ($P < 0.05$) presence (%) in the transient HL treatment (80%), followed by the HH, LH and LL treatments (70%, 61% and 44%, respectively, Table 1). Legumes had significantly ($P < 0.05$) lower contributions in monoliths of pre-experimental high disturbance (HL and HH), compared with monoliths adapted to low disturbance.

Applying frequent cuts to monoliths previously adapted to low disturbance (LH) resulted in a replacement of dominant species including legumes (*Vicia cracca*, *Dactylis glomerata*, *Holcus lanatus*, *Poa pratensis*, *Agrostis capillaris*) compared with low disturbance controls (LL) (data not shown). In contrast, shifting high disturbance controls (HH) to low disturbance (HL), changed the balance between codominant species (*Festuca arundinacea* and *A. capillaris*).

The Shannon diversity index (H') and the plant species richness (SNb) were higher in monoliths extracted from low as opposed to high disturbance field plots ($P < 0.001$, repeated measure ANOVA, data not shown). A shift to low experimental disturbance (HL) alleviated the decline ($P < 0.05$, data not shown) in SNb and H' , compared with high experimental disturbance controls (HH). FD of SLA, LDMC and total aboveground traits ranged from 0.23 to 0.58 (Table 1). A shift to high disturbance markedly ($P < 0.05$) increased FD_{SLA} and FD_{veg} (i.e. aboveground traits) of the HL-treatment.

Effects of disturbance and species number on C fluxes. In rank correlations, GPP correlates negatively ($P < 0.05$) with SNb, while a positive correlation is found for SANPP (supporting information, Appendix S1). Step-wise ascending regressions show a direct effect of plant species number on top soil SOC (> 0.2 mm) (Table 2).

Trait based response-and-effect framework

Response to disturbance of community scale traits (R). Community-aggregated leaf traits and root and rhizome traits are shown in Table 1. High compared with low pre-experimental disturbance (HH vs. LL), increased specific leaf area (SLA) and specific length of roots and rhizomes (SL), but reduced leaf dry-matter content (LDMC) and the density (DENS) and diameter (*D*) of roots and rhizomes. The N contents of leaves (LNC) and of roots and rhizomes (NC) did not differ significantly between the HH and LL treatments.

Under transient disturbance regimes (HL and LH), a reduced disturbance led to an increased dominance of plant species with high leaf dry-matter content (LDMC), low specific leaf area (SLA) and low leaf N content. Conversely, a shift from a low to a high experimental disturbance (HL vs. HH) reduced LDMC and increased SLA, LNC and SL.

The experimental disturbance significantly affected leaf traits (SLA, LDMC and LNC), while pre-experimental disturbance significantly affected root and rhizome traits (SL, *D* and DENS). All traits (except for LDMC) were affected by sampling date, indicating the successive change of traits during the experiment.

Table 1 Means (A, \pm SE) and statistical significance (B) of percentage presence of functional groups and community aggregated leaf and root and rhizome traits in a repeated measure analysis of variance (ANOVA) with pre-experimental (Pre-exp.), experimental disturbance (Exp.) and year factors and their interactions

| | Functional groups | | | Aggregated leaf traits | | | Root and rhizome traits | | | Functional divergence | | |
|------------------------------|----------------------|----------------------|----------------------|------------------------|--------------------------|------------------------|-------------------------|---------------------------|--------------------------|--------------------------|--------------------------|---------------------------|
| | Grasses | Forbes | Legumes | SLA | LDMC | LNC | SL | DENS | D | NC | FD _{SLA} | FD _{LDMC} |
| (A) Means (± SE) | | | | | | | | | | | | |
| LL | 51 ^a ± 4 | 24 ^a ± 10 | 25 ^a ± 7 | 213 ^a ± 29 | 0.27 ^a ± 0.03 | 2.8 ^a ± 0.4 | 11 ^a ± 4 | 0.36 ^a ± 0.04 | 0.80 ^a ± 0.15 | 1.40 ^a ± 0.17 | 0.23 ^a ± 0.03 | 0.47 ^{ab} ± 0.03 |
| LH | 65 ^a ± 5 | 25 ^a ± 8 | 10 ^{ab} ± 1 | 266 ^b ± 20 | 0.22 ^b ± 0.02 | 3.4 ^b ± 0.2 | 27 ^b ± 9 | 0.30 ^{a*} ± 0.03 | 0.69 ^b ± 0.20 | 1.24 ^a ± 0.13 | 0.34 ^b ± 0.02 | 0.52 ^b ± 0.02 |
| HL | 79 ^b ± 5 | 22 ^a ± 12 | 7 ^b ± 2 | 203 ^a ± 34 | 0.27 ^a ± 0.03 | 2.5 ^a ± 0.4 | 43 ^b ± 14 | 0.26 ^b ± 0.05 | 0.57 ^b ± 0.17 | 1.43 ^a ± 0.21 | 0.28 ^a ± 0.02 | 0.40 ^a ± 0.04 |
| HH | 61 ^a ± 14 | 42 ^a ± 10 | 3 ^b ± 1 | 258 ^b ± 17 | 0.21 ^b ± 0.02 | 3.2 ^b ± 0.4 | 52 ^b ± 28 | 0.24 ^b ± 0.05 | 0.59 ^b ± 0.14 | 1.27 ^a ± 0.09 | 0.28 ^a ± 0.04 | 0.42 ^a ± 0.03 |
| (B) Statistical significance | | | | | | | | | | | | |
| Pre-exp. disturbance | ** | ns | * | ns | ns | ns | ** | ** | ** | ns | ns | * |
| Exp. disturbance | ns | ns | * | *** | *** | *** | ns | ns | ns | ns | * | ns |
| Year | ** | ** | ns | * | ns | *** | ** | *** | *** | * | ns | ns |
| Pre-exp. × Exp. | ns | ns | ns | ns | ns | ns | ns | ns | ns | ns | * | ns |
| Pre-exp. × Year | *** | ns | ns | ** | ns | ns | ns | ns | ns | ns | ns | ns |
| Exp. × Year | ns | ns | * | ** | ns | ns | ns | ns | ns | ns | ns | ns |

Different letters in the same column indicate statistical differences ($P < 0.05$) between treatments.* $P < 0.05$, ** $P < 0.01$ and *** $P < 0.001$.

Trait values were log-transformed before ANOVA.

SLA, specific leaf area; LDMC, leaf dry-matter content; LNC, leaf nitrogen content; SL, specific length; DENS, tissue density; D and NC, diameter and nitrogen content of pooled root and rhizomes; FD_{SLA} and FD_{LDMC}, functional divergence of SLA and LDMC; ns, not significant.

Table 2 Summary of step-wise ascending regression procedure between carbon fluxes, abiotic factors (year and experimental disturbance, exD), species number (SNb), functional divergence of leaf traits (FD_{SLA}, FD_{LDMC}), and community scale specific leaf area, SLA; leaf dry matter content, LDMC and root and rhizome density, specific length, SL, diameter *D*

| Flux | Abiotic factors | | | Plant traits | | | <i>R</i> ² | <i>P</i> -model |
|-------|-----------------|--------------------------|-------|-----------------|----------------------|--------|-----------------------|-----------------|
| | Year | Experimental disturbance | SNb | Trait | Community scale mean | FD | | |
| NEP | - | - | - | LDMC | - | 0.011 | 19 | 0.011 |
| ANPP | <0.000 | 0.002 | - | SLA | - | 0.004 | 70 | <0.001 |
| | <0.000 | <0.000 | - | LDMC | 0.026 | ns | 65 | 0.046 |
| | 0.002 | 0.009 | ns | Log(SL) | 0.033 | - | 64 | 0.056 |
| | 0.042 | 0.009 | - | Log(<i>D</i>) | 0.062 | - | 64 | 0.043 |
| SANPP | - | <0.000 | - | SLA | ns | <0.001 | 92 | 0.008 |
| | - | <0.000 | ns | LDMC | - | 0.037 | 92 | 0.018 |
| NCS | ns | <0.000 | - | LDMC | ns | 0.021 | 60 | 0.016 |
| | - | <0.000 | ns | Log(SL) | ns | - | 59 | 0.021 |
| | - | <0.000 | - | DENS | 0.011 | - | 60 | 0.011 |
| | - | <0.000 | - | Log(<i>D</i>) | 0.034 | - | 57 | 0.034 |
| BNCS | 0.007 | 0.012 | - | LDMC | ns | 0.006 | 43 | 0.048 |
| | ns | - | - | Log(SL) | 0.013 | - | 32 | <0.001 |
| | ns | - | - | DENS | 0.002 | - | 34 | <0.001 |
| | ns | - | - | Log(<i>D</i>) | 0.016 | - | 30 | 0.001 |
| SOC | 0.020 | 0.002 | 0.023 | Log(SL) | 0.014 | - | 29 | 0.001 |
| | 0.006 | 0.013 | 0.009 | DENS | 0.002 | - | 40 | 0.001 |
| | 0.010 | <0.001 | 0.017 | Log(<i>D</i>) | 0.051 | ns | 35 | 0.006 |
| | | | | | | | | |

ns, not significant; -, variable not selected by the step-wise ascending regression. Data are means of 2003 and 2004 for four replicate enclosures per treatment (*n* = 32). When appropriate to satisfy Shapiro-Wilks test of normality, C fluxes and traits were transformed by log or square root before analysis. In bold, best model.

ANPP (g C m⁻² yr⁻¹), aboveground net primary productivity; SANPP (g C kg⁻¹ day⁻¹), specific aboveground net primary productivity; NCS (g C m⁻² yr⁻¹), total net carbon storage; BNCS (g C m⁻² yr⁻¹), belowground net carbon storage; SOC, top soil organic carbon content (>0.2 mm, g C m⁻²).

The fraction of rhizomes in the total belowground phytomass was significantly higher ($P < 0.001$, ANOVA, data not shown) at a pre-experimental low (LL, LH) compared with high (HH, HL) disturbance level, indicating a higher presence of the stoloniferous grass *Elytrigia repens* in those treatments. Treatment effects on the traits of fine roots, coarse roots and rhizomes are shown in supporting information (Appendix S2). The specific length of each of these belowground plant parts was significantly reduced at a low compared with a high pre-experimental disturbance, while the diameter of coarse roots and rhizomes was increased.

Effects of community scale traits on C fluxes (E). Rank correlation coefficients were used to analyse individual effects on C fluxes (supporting information, Appendix S1). A step-wise ascending regression procedure was applied to separate direct effects of disturbance (year) from indirect effects of plant species diversity, functional divergence and community-aggregated traits (Table 2).

Community scale leaf traits and C fluxes. Total C storage (NCS and SOC) and productivity (NEP, ANPP and SANPP) were affected by three single leaf traits (SLA, LNC and LDMC) and their distribution (FD) (Appendix S1). However, the step-wise procedure did not confirm a direct effect of community-aggregated leaf traits on C fluxes. Apart for the significant relationship between ANPP and community-aggregated LDMC, aboveground productivity and NCS were affected by disturbance and functional divergence of SLA and LDMC (see Table 2).

Community scale root and rhizome traits and C fluxes. Primary productivity (GPP, ANPP) and C storage (NCS, BNCS, SOC) were highly correlated to the specific length (SL), diameter (D) and density (DENS) of roots and rhizomes (Appendix S1). In step-wise procedure, community-scale specific length, diameter and density of roots and rhizomes had marked direct effects on ANPP and belowground C fluxes (NCS and BNCS) (Table 2). The distribution of traits was tested by using the functional divergence of shoot traits (FD_{vg}). FD_{vg} however, did not affect C fluxes.

Mathematical test of the trait based response-and-effect framework. In agreement with the framework proposed by Suding *et al.* (2008), our experiment demonstrated that root and rhizome traits (i.e. SL, D , DENS) responded to environmental change and significantly affected C fluxes (Table 2). Using Eqn (6), the framework was tested by comparison of linear regression between treatments. In each of the four disturbance treatments, slopes [a , Eqn (6)] and

intercepts of the regressions between C fluxes (Y) and effect traits (E) were significant (data not shown). Because effect slopes (a) did not differ ($P > 0.1$, data not shown) among treatments, equal slopes were assumed and Eqn (8) was applied (Table 3).

The corresponding linear regressions show that NCS, BNCS and SOC can be predicted ($P < 0.05$), according to Eqn (8), from roots and rhizome effect traits (SL, DENS and diameter) (Table 3). Treatment dependent intercepts for NCS and SOC indicate a direct effect of disturbance on these C fluxes (Table 3). Interestingly, intercepts do not differ among treatments for BNCS, showing that the change in C sequestration was mostly mediated by root trait changes. Applying the mathematical test to functional diversity of leaf traits (FD) shows that total C storage (NCS) and aboveground productivity (ANPP and SANPP) can be predicted ($P < 0.05$) according to Eqn (8) from FD_{SLA} and FD_{LDMC} (Table 3). The positive slopes indicate that aboveground productivity and total C-storage, increases with functional divergence of leaf traits. Significant differences in intercepts among treatments for NCS and SANPP underline that those C fluxes are also controlled by disturbance.

While important effect and response traits were related (see Suding *et al.*, 2008), changes in response traits between the two time periods were calculated as the constant k [see Eqn (8)] (Table 3). The measured change in C flux after a change in disturbance was thereby broken into two components [see Eqn (8)]: direct and indirect effects of disturbance and response-and-effect traits. Accordingly, community traits ($\log SL$, DENS, $\log D$) contributed between 6% and 61% to the change in C fluxes after disturbance shift (Table 3). These effects are displayed in Fig. 1, showing direct disturbance effects as vertical arrows and indirect response-and-effect as arrows parallel to the slope [a , Eqn (8)]. Similarly, up to 48% of changes in aboveground productivity (ANPP and SANPP) and total C-storage (NCS) were explained by response-and-effect changes of functional divergence of leaf traits (SLA and LDMC).

Discussion

Insight on the role of plant community structure for ecosystem functioning can best be gained in ecosystems that allow easy manipulation, such as perennial grassland communities (Loreau & Hector, 2001). Focusing on plant functional traits, rather than on species identities, allows generalizing complex community dynamics and predicting effects of ongoing environmental changes. The framework proposed by Suding *et al.* (2008) describes an approach to scale up from individuals to

Table 3 Linear regressions between carbon (C) fluxes and roots (and rhizome) community traits, FD_{SLA} and FD_{LDMC}

| Flux | Trait | Effect slope (<i>a</i>) | Model statistics (R^2) | Disturbance | Response <i>k</i> | % change | Treatment | Intercept (<i>b</i>) |
|-------|-----------------|-----------------------------------|----------------------------|------------------|-------------------|------------|-----------|------------------------|
| SANPP | FD_{SLA} | 42 ± 17 ($P = 0.016$) | 0.94 *** | L to H H to L | 1.47 1.01 | 8 −0.2 | LL | $-4.2^a \pm 4.5$ |
| | | | | | | | LH | $45.8^b \pm 3.7$ |
| | | | | | | | HL | $-7.7^a \pm 3.4$ |
| | | | | | | | HH | $36.4^b \pm 3.4$ |
| ANPP | FD_{SLA} | 951 ± 447 ($P = 0.043$) | 0.39 * | L to H H to L | 1.47 1.01 | 48 −0.9 | LL | $515^a \pm 160$ |
| | | | | | | | LH | $624^a \pm 116$ |
| | | | | | | | HL | $431^a \pm 103$ |
| | | | | | | | HH | $641^a \pm 97$ |
| NCS | FD_{LDMC} | 1304 ± 600 ($P = 0.039$) | 0.33 *** | L to H H to L | 1.11 0.97 | −10 −3 | LL | $987^a \pm 297$ |
| | | | | | | | LH | $266^b \pm 139$ |
| | | | | | | | HL | $735^a \pm 142$ |
| | | | | | | | HH | $225^b \pm 139$ |
| | DENS | 1039 ± 519 ($P = 0.056$) | 0.75 *** | L to H H to L | 0.83 1.13 | 8 7 | LL | $1369^a \pm 208$ |
| | | | | | | | LH | $626^b \pm 127$ |
| | | | | | | | HL | $941^a \pm 136$ |
| | | | | | | | HH | $518^b \pm 139$ |
| BNCS | Log(SL) | -150 ± 71 ($P = 0.045$) | 0.38 * | L to H H to L | 1.39 0.94 | 26 17 | LL | $1308^a \pm 194$ |
| | | | | | | | LH | $1134^a \pm 171$ |
| | | | | | | | HL | $1063^a \pm 184$ |
| | | | | | | | HH | $989^a \pm 185$ |
| | DENS | 1559 ± 680 ($P = 0.029$) | 0.39 ** | L to H H to L | 0.83 1.13 | 33 61 | LL | $404^a \pm 274$ |
| | | | | | | | LH | $204^a \pm 163$ |
| | | | | | | | HL | $120^a \pm 171$ |
| | | | | | | | HH | $90^a \pm 180$ |
| | Log(<i>D</i>) | 305 ± 125 ($P = 0.021$) | 0.41 *** | L to H H to L | 2.17 1.08 | 11 −6 | LL | $1048^a \pm 114$ |
| | | | | | | | LH | $836^a \pm 160$ |
| | | | | | | | HL | $745^a \pm 165$ |
| | | | | | | | HH | $651^a \pm 163$ |
| SOC | DENS | 7.3 ± 2.9 ($P = 0.016$) | 0.69 *** | L to H H to L | 0.83 1.13 | 21 27 | LL | $5.2^a \pm 1.1$ |
| | | | | | | | LH | $3.5^a \pm 0.7$ |
| | | | | | | | HL | $2.4^b \pm 0.7$ |
| | | | | | | | HH | $1.8^b \pm 0.8$ |

Response factor (*k*), effect slope (*a*, mean \pm SE) and direct effects of disturbance on C fluxes (intercept, *b*) [see Eqns (6) and (8)]. The % change in C flux resulting from changes in response-and-effect trait (and FD) were calculated from Eqn (8) (% change). Because slopes did not differ among disturbance treatments, a single slope was calculated. Different letters in the same column indicate statistical differences ($P < 0.05$) between treatments. Except for the relations BNCS–DENS and SANPP– FD_{SLA} all intercepts were significant ($P < 0.05$). For *P* values: * $P < 0.05$, ** $P < 0.01$ and *** $P < 0.001$.

ANPP ($\text{g C m}^{-2} \text{ yr}^{-1}$), aboveground net primary productivity; SANPP ($\text{g C kg}^{-1} \text{ day}^{-1}$), specific aboveground net primary productivity; BNCS ($\text{g C m}^{-2} \text{ yr}^{-1}$), belowground net C storage; SOC, top soil organic C content ($> 0.2 \text{ mm}$, g C m^{-2}); logSL, log-transformed specific length; DENS, tissue density; log*D*, log-transformed diameter of root and rhizomes; LL, constant low disturbance; LH, shift to high disturbance; HH, constant high disturbance; HL, shift to low disturbance; ns, not significant.

communities and ecosystems in the context of environmental change predictions. Thereby, plants are classified in terms of their response to environmental factors and their effects on ecosystem properties (Chapin *et al.*, 2000; Lavorel & Garnier, 2002). Although this framework deals with a highly relevant topic, empirical tests are rare.

In a mesocosm experiment, we were able to continuously measure C fluxes during 2 years in grassland

communities affected by contrasted disturbance regimes (Klump *et al.*, 2007a,b). In a fully factorial design, disturbance was either intensified or reduced. We have shown that changes in aboveground productivity and belowground C storage after a change in disturbance were neither directly related to plant species richness, nor to legume abundance, but were partly caused by response-and-effect traits and functional diversity.

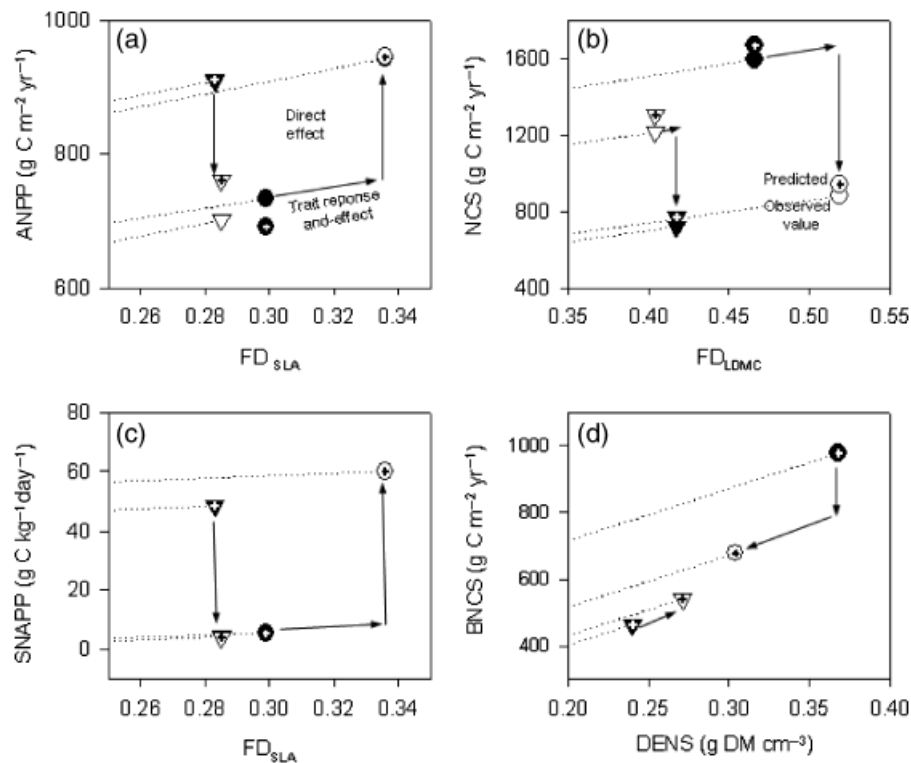


Fig. 1 Direct and response-and-effect components of carbon (C) flux changes [see Eqn (8)] after a change in disturbance. Direct effects are shown as vertical arrows. Response-and-effect changes mediated by tissue density of roots and rhizomes (DENS) (d) and functional divergence of specific leaf area (SLA) and leaf dry-matter content (LDMC) (a–c) are shown as arrows parallel to the slope (a) of the effect trait (dotted lines). Estimated C fluxes are for constant low disturbance (black circles), constant high disturbance (black triangles), shift to low disturbance (HL, open triangles) and shift to high disturbance (LH, open circles). Symbols with a cross are measured C fluxes. ANPP, aboveground net primary productivity; SANPP, specific aboveground net primary productivity; NCS, total net C storage and BNCS, belowground net C storage. When predicted values are invisible, they are masked by measured values (crossed symbols).

Response to disturbance of community scale traits (R)

The primary approach in identifying response traits is to evaluate how environmental conditions alter trait values. In our study, leaf and root (and rhizome) traits showed a common response to disturbance. In both steady-state (LL, HH) and transient disturbance regimes (HL and LH), a reduced disturbance led to plant growth strategies of resources conservation (i.e. high tissue density, low N content and low area per unit leaf mass) (e.g. Reich *et al.*, 1995; Wright & Westoby, 1999). Inversely, a growth strategy inducing resource exploitation (low tissue density, high N content and high area per unit mass leaves) was favoured by high disturbance (Louault *et al.*, 2005).

Roots and rhizome traits were also response traits to the disturbance regime. In contrast to leaf traits, root and rhizome traits were mostly controlled by the pre-experimental disturbance level (see supporting information, Appendix S2), indicating carry-over effects caused by the extended life span of roots compared

with leaves. A shift to high disturbance led to marked changes in root (and rhizome) morphology (increased specific length, reduced diameter and tissue density), presumably increasing root elongation (Eissenstat, 1992; Eissenstat *et al.*, 2000) and soil nutrient acquisition (Craine *et al.*, 2002) rates. As with leaf traits, these morphological changes correspond to an increased dominance of exploitative plant growth strategies (Roumet *et al.*, 2006). Conversely, a shift from high to low disturbance, led to small but nevertheless consistent changes (reduced SL, increased D and DENS) in the morphology of belowground plant parts. As with leaf traits, these changes indicate an increased dominance of conservative plant growth strategies (Roumet *et al.*, 2006).

Relating effects of community scale traits to C fluxes (E)

Functional effect traits are defined as those traits that have an impact on ecosystem functioning (Diaz &

Cabido, 2001; Lavorel & Garnier, 2002). In agreement with the response-and-effect framework (Suding *et al.*, 2008), our community traits were classified in terms of their response to environmental factors (*response traits*) and their effects on ecosystem services (C fluxes) (*effect traits*). As abiotic factors (i.e. disturbance, year) may explain a significant amount of variation, we applied a step-wise ascending regression procedure to separate abiotic effects from effects mediated by plant species number, functional divergence and (effect) traits. By including functional divergence effects, our procedure allowed a formal incorporation of functional diversity as a driving factor in the sensitivity to disturbance change.

In our experiment, the rank correlation between aboveground productivity (SANPP) and community leaf traits (SLA and LDMC) supported the mass ratio hypothesis in agreement with the report by Garnier *et al.* (2004). However, in a step-wise regression procedure the functional divergence (FD_{SLA}), rather than the mean value of these traits, explained aboveground net primary productivity (ANPP and SANPP). The mass ratio hypothesis has been theoretically linked to a selection mechanism (i.e. selection of dominant species), whereas the functional divergence underlines a reduced overlap of trait values in a community (Mason *et al.*, 2005). Effects of functional trait distributions, can thus be related to a niche complementarity effect (Petchey & Gaston, 2006), suggesting functional diversity as the driving factor of productivity (Diaz *et al.*, 2007b).

ANPP and total belowground C storage (NCS, BNCS, SOC) were highly correlated to the specific length (SL), diameter (D) and density (DENS) of roots and rhizomes. Irrespective of current disturbance regime and of year, the step-wise ascending procedure confirms a direct role of mean root and rhizome trait values on both above- and belowground C fluxes. However, this effect is most consistent with BNCS, which is significantly affected by SL, D and DENS. High tissue density and low specific length of roots has been shown to reduce the decomposition rate of belowground litter (Personeni & Loiseau, 2005), which in turn explains the increase in mean residence time of C in coarse particulate soil organic matter (Klumpp *et al.*, 2007b) and, hence, belowground C sequestration (BNCS). Root and rhizome traits would therefore primarily control soil processes such as belowground C sequestration, but would also have an indirect effect on other C fluxes through N-cycling (Bardgett *et al.*, 2005; De Deyn *et al.*, 2008). In contrast our findings show, the functional diversity of leaf traits would have a direct effect on ANPP, but no significant effect on other C fluxes.

Mathematical test of response-and-effect hypothesis. Can we incorporate functional traits and diversity to successfully predict effects of environmental change in a conceptual way? The work presented here, provides a mathematical test of the response and effect framework by Suding *et al.* (2008). Our approach was tested by (i) assuming (for simplification) a linear effect of the response-and-effect traits on C fluxes [see Eqns (4–8)], allowing us to quantitatively determine the role of disturbance and (ii) by integrating other mechanisms apart from the mass ratio hypothesis (i.e. FD).

Highly significant regressions were found with root and rhizome traits and functional divergence of SLA and LDMC. Noticeably, the response-and-effect changes in C flux account for between -3% and 48% of the total C flux changes (ANPP and NCS), indicating that some fluxes are more susceptible to changes in trait values and their distribution than others (SANPP). Interestingly, the positive slope of the response of ANPP, SANPP and NCS to functional divergence of SLA and LDMC indicates that the distribution of trait values conditions aboveground NPP. This demonstrates that the prediction of ecosystem services requires data on functional diversity factors, which is in agreement with results of Diaz *et al.* (2007b). Nevertheless, differences between intercepts among treatments for NCS and SANPP highlight the fact that C fluxes are also controlled by disturbance. Moreover, our study shows that FD does not always explain changes in C fluxes (i.e. rejection of FD_{vg} for BNCS) and the community trait values can be sufficient for practical reasons. However, we did not measure the functional diversity of root traits, which might yield better prediction of belowground C fluxes (BNCS) than community trait values. To overcome this limitation, new methods allowing the determination of belowground species traits are required.

As with ANPP, transient effects of disturbance are asymmetric for soil C sequestration (NCS, BNCS and SOC) because increasing disturbance (LH vs. LL) has stronger effects (response factor k) than reducing disturbance (HL vs. HH). The asymmetry results from a stronger response of community traits to an increase as opposed to a decline in disturbance. For example, with regard to SL, k increased by $+40\%$ in response to disturbance increase, whereas it was reduced by 6% in response to disturbance decline. Our data confirms that organic C is lost more rapidly from soil than it is gained (Körner, 2003; Soussana *et al.*, 2004). Nevertheless, we found a common effect slope (a) for both increase and decline in disturbance, suggesting that asymmetry is mostly mediated by trait responses and not by trait effects.

Because the disturbance induced trade-off between C sequestration, and herbage production (Klumpp *et al.*,

2007a) is mediated by community-scale response-and-effect traits and functional diversity, plant diversity is presumably a major driver of C cycle responses to disturbance in grasslands. In contrast to species-rich grasslands, sown grass mixtures are likely to have a small functional divergence of traits following changes in disturbance change, which may limit their C sequestration potential.

In conclusion, we were able to show how environmental changes affect community traits and their distribution and how they relate to C fluxes in grasslands. Moreover, we identified a set of community scale traits that predict changes in C sequestration in species-rich grasslands. In contrast, for herbage production, functional diversity of leaf traits has been shown to be the best predictor. Applying a set of statistical procedures reduced uncertainties in predicting ecosystem processes, allowing, for the first time: (i) to test mathematically the *response-and-effect* approach, (ii) to scale up from individuals to communities and ecosystems in the context of environmental change (iii) to test hypothesis other than the mass ratio and to (iv) to determine quantitative effects of disturbances, community traits and functional diversity on C fluxes. Further tests will be required with other environmental factors (e.g. elevated CO₂, warming, etc.) in order to predict future changes in ecosystem services under global change.

Acknowledgements

We thank Robert Falcimagne, Patrick Pichon and the trainees Julie Blanchon and Rémi Pilon for their scientific and technical contributions. We also thank Dr Catherine Picon-Cochard for her help with the techniques concerning root traits and Drs Sébastien Fontaine, Juliette Bloor and Nicolas Gross for helpful comments on previous versions of this manuscript. This work was supported by a Marie Curie Individual Fellowship (EVK2-CT2002-50026) to Katja Klumpp, by the European FP5 'GREENGRASS' project (EVK2-CT2001-00105) and was part of the French ECCO PNBC 'Geotraits' and GDR 'UTILITERRES'.

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Supporting Information

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

Appendix S1. Spearman's rank correlation coefficients between annual means of carbon fluxes ($\text{g C m}^{-2} \text{yr}^{-1}$), community aggregated traits and indices of plant community structure, with species number (SNb), percentage legumes (%Leg), functional divergence of the vegetation (FD_{vg}), specific leaf area (SLA), leaf dry matter content (LDMC), leaf nitrogen content (LNC) and specific length (SL), tissue density (Dens), diameter (D) and nitrogen content (NC) of pooled root and rhizomes. Functional divergence of SLA, LDMC (FD_{SLA} , FD_{LDMC}) and above-ground traits (FD_{vg}), gross primary productivity (GPP), net ecosystem productivity (NEP), total net carbon storage (NCS), belowground carbon storage (BNCS), aboveground net primary productivity (ANPP), specific annual net primary productivity (SANPP) and of total C in soil OM >0.2 mm (SOC). Correlations marked in bold are significant at $P < 0.05$. Data correspond to means in 2003 and 2004 of four replicate enclosures per treatment ($n = 32$).

Appendix S2. Annual means (A) of coarse root, fine root and rhizome aggregated trait values and statistical significance of pre-experimental and experimental disturbance factors and year in an ANOVA (B). Constant low disturbance (LL), shift to high disturbance (LH), constant high disturbance (HH) and shift to low disturbance (HL). Specific length (SL), tissue density (DENS), diameter (D) and nitrogen content (NC) of pooled root and rhizomes. For P values, not significant (–), $P < 0.05$ (*), $P < 0.01$ (**) and $P < 0.001$ (***).

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