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## ► To cite this version:

Florence Baptist, Iker Aranjuelo, Nicolas Legay, Luis Lopez-Sangil, Gemma Molero, et al.. Rhizodeposition of organic carbon by plants with contrasting traits for resource acquisition: responses to different fertility regimes. *Plant and Soil*, 2015, 10.1007/s11104-015-2531-4 . hal-02508998

**HAL Id: hal-02508998**

**<https://hal.science/hal-02508998>**

Submitted on 18 Mar 2020

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Rhizodeposition of organic carbon by plants with contrasting traits for resource acquisition: responses to  
different fertility regimes

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**Abstract (206 words)**

**Background and Aims:** Rhizodeposition plays an important role in mediating soil nutrient availability in ecosystems. However, owing to methodological difficulties (i.e. narrow zone of soil around roots, rapid assimilation by soil microbes) fertility-induced changes in rhizodeposition remain mostly unknown.

**Methods:** We developed a novel long-term continuous  $^{13}\text{C}$  labelling method to address the effects of two levels of nitrogen (N) fertilization on rhizodeposited carbon (C) by species with different nutrient acquisition strategies.

**Results:** Fertility-induced changes in rhizodeposition were modulated by root responses to N availability rather than by changes in soil microbial biomass. Differences among species were mostly related to plant biomass: species with higher total leaf and root biomass also had higher total rhizodeposited C, whereas species with lower root biomass had higher specific rhizodeposited C (per gram root mass). Experimental controls demonstrated that most of the biases commonly associated with this type of experiment (i.e., long-term steady-state labelling) were avoided using our methodological approach.

**Conclusions:** These results suggest that the amount of rhizodeposited C from plants grown under different levels of N were driven mainly by plant biomass and root morphology rather than microbial biomass. They also underline the importance of plant characteristics (i.e. biomass allocation) as opposed to traits associated with plant resource acquisition strategies in predicting total C rhizodeposition.

**Key words:** carbon, exudation, leaf traits, root traits, nitrogen, plant nutrient use strategies.

## Introduction

The availability and management of nitrogen (N) is a major constraint on plant productivity in many agro-ecosystems worldwide (Passioura 2002; Goll et al. 2012). In most natural ecosystems and croplands that are not amended with inorganic fertilizers, nutrient availability to plants is largely determined by the activity of saprotrophic soil microbes that decompose soil organic matter (SOM). The resulting products, carbon dioxide (CO<sub>2</sub>) and inorganic nutrients, are in turn readily taken up by plants and associated mycorrhizas (Molina and Smith 1998; Valé et al. 2005). In parallel, nutrient cycling in the rhizosphere can also be regulated by the provision of plant carbon (C) to saprotrophic microbiota (Paterson 2003; Bahn et al. 2013). Rhizodeposition, inputs of labile organic solutes and sloughed-off cell tissues from plant roots, can represent around 11% of net fixed C and up to 30% of the C allocated to roots (Jones et al. 2009). This process is believed to constitute a strategy whereby growing plants improve soil nutrient availability by supplying additional energy-rich C (i.e., carbohydrates) to soil microbes capable of decomposing SOM (Bardgett et al. 1998; 2008). Soluble compounds exuded by roots - mainly sugars, carboxylic acids and amino acids - may account for 1 to 10% of rhizodeposition (Paterson 2003; Jones et al. 2004) and together with other rhizodeposits, they provide energy to soil microbiota for the mineralization of organic N and other nutrients from SOM (Paterson 2003; Jones et al., 2004; Kuzyakov et Xu 2013). Although mineralized N is initially incorporated into the microbial biomass, the rapid turnover of microbial cells relative to roots, ultimately makes it available to plants (Schmidt et al. 2007). Rhizodeposition may thus play an important role in accelerating nutrient cycling in the rhizosphere, which facilitates plant growth (Lynch 1990; Bardgett et al. 1998). Among other factors, the amount of C allocation by plants into the soil can be affected by nutrient availability (Merckx et al. 1987).

The role of rhizodeposition in soil nutrient availability has previously been addressed in studies comparing plants grown under different fertility conditions (Paterson and Sim 1999; 2000; Denef et al. 2009). Nevertheless, the results of these studies are highly variable, which leads to substantial discrepancies in the subsequent conclusions. For instance, studies with plants grown in artificial substrates (such as hydroponics and axenic sand culture systems) demonstrated that the loss of C per unit root mass increased under low N supply compared to high N inputs (Paterson and Sim 1999; 2000). In contrast, the results of studies in which plants are grown in soil are inconclusive, with both positive and negative effects of low N supply on rhizodeposition (Jones et al. 2004; Denef et al. 2009). These contradictory findings may be partly due to methodological constraints. Rhizodeposited compounds are rapidly assimilated by soil microbes, which limits accurate estimates of total plant rhizodeposit-C (Paterson et al. 2003; 2005; Dilkes et al. 2004). Furthermore, most studies investigating

rhizosphere effects on SOM cycling have used pulse labelling or natural tracer techniques, using  $^{13}\text{C}$  or  $^{14}\text{C}$  isotopes to differentiate plant-derived  $\text{CO}_2\text{-C}$  from SOM-derived  $\text{CO}_2\text{-C}$  fluxes (Meharg 1994; Andrews et al. 1999; Nguyen et al. 1999). On the one hand, continuous  $^{14}\text{C}$  labelling is hazardous and requires special facilities that are not readily available to many researchers. On the other hand, pulse-labelling precludes the separation of total plant-derived  $\text{CO}_2\text{-C}$  from SOM derived  $\text{CO}_2\text{-C}$  because it does not label all plant C pools uniformly (Kuzakov and Cheng 2004) and consequently, the estimated release of C from roots is biased towards those pools receiving the greatest contribution from recent photoassimilates (Meharg 1994; Paterson et al. 2009).

A further possible source of the discrepancies among studies of rhizodeposition could be the use of plant species with distinct nutrient acquisition strategies. Two of these strategies have been described in detail and are strongly linked to soil nutrient availability (Grime 1979; Tilman 1980; Wright et al. 2004). Nutrient-poor ecosystems are dominated by species with low relative growth rates (RGR), coupled with low nutrient concentrations in tissues, such as root nitrogen concentration (RNC) and leaf nitrogen concentration (LNC) (Lavorel and Garnier 2002; Roumet et al. 2006). Conversely, nutrient-rich ecosystems are dominated by species with high RGR and high LNC and RNC (Craine et al. 2001). These contrasting strategies may result in highly variable fertility-induced changes in root exudation among different plant species (Rovira 1969; Dakora and Phillips 2002). Plant species in nutrient-rich ecosystems are most likely to have high C rhizodeposition (Van der Krift et al. 2001) because (i) rapid plant growth is linked to large quantities of photoassimilates, which can be allocated to roots and (ii) root morphologies with high biomass (Craine et al. 2001) and low levels of structural C allow passive diffusion of exudates through root tissue (Valé et al. 2005). In contrast, plant species in nutrient-poor ecosystems have low relative growth rates, which may result in lower rates of C rhizodeposition in soil. Although these species may increase exudation to stimulate soil N mineralization (Van der Krift et al. 2001), its subsequent uptake by plants could be limited by competition with active microbes, which can reduce N-acquisition by plants in the short term (Jones et al. 2004; Kuzakov and Xu 2013).

In this study, we aimed to elucidate the effects of two levels of N availability on rhizodeposited C and the subsequent impact on soil microbiota. To distinguish the effects of N availability from plant nutrient acquisition strategies, we used four grassland plant species with contrasting strategies: exploitative species were represented by *Dactylis glomerata* L. and *Lolium perenne* L.; conservative species were represented by *Anthoxanthum odoratum* L. and *Briza media* L. (Quétier et al. 2007; Harrison and Bardgett, 2010). We hypothesised that (i) plants with exploitative strategies would have higher rhizodeposition rates, which would in turn stimulate microbial activity; and that (ii) the changes in rhizodeposition rates of plant species in response to

106 N fertilization would be dependent on plant nutrient acquisition strategies. To achieve this, plants were subjected  
107 to continuous  $^{13}\text{C}$  labelling in growth chambers for almost three months. Additional soil and plant analyses  
108 ( $\text{NH}_4^+$  and  $\text{NO}_3^-$  analysis, soil pH,  $^{13}\text{CO}_2$  respired by the roots) were performed in order to identify underlying  
109 processes of rhizodeposition patterns among species and fertilization treatments

## Material and methods

### Plant cultures

Two exploitative species (*Dactylis glomerata*, DG and *Lolium perenne*, LP), and two conservative species (*Anthoxanthum odoratum*, AO and *Briza media*, BM) were selected for this study, all of which belong to the *Poaceae* family. Ramets of *Dactylis glomerata* and *Briza media* were collected from adult tussocks in an alpine grassland (French Alps, 2000 m a.s.l., 45° 4' N - 6°34' E) and ramets of *Lolium perenne* and *Anthoxanthum odoratum* were collected from adult tussocks in an English grassland (54°18' N - 2°5' W, Yorkshire Dales, United Kingdom) during July 2009.

For each species, Roots and leaves were cut 5-cm from the base of each ramet and planted individually in the pot. Each pot was filled with 220 g d.w. grassland topsoil (0–30-cm) collected at the Helmholtz Zentrum experimental station in Scheyern (Germany, 479 m a.s.l., 48°30' N, 11°28' E). The soil was sandy (7.6% clay, 10.3% of silt and 82.1% sand) with total soil organic C and N concentration of 0.19g kg<sup>-1</sup> and 0.01g kg<sup>-1</sup> respectively and a soil pH<sub>H2O</sub> of 6.5.

A total of 22 pots per species were prepared and an additional 18 pots without plants ('bare soil pots') were included as controls. In order to inhibit algal growth on the soil surface, the soil in each pot was covered by a circle of dark paper with a hole cut in the center. All pots were distributed in two environment-controlled growth chambers (Conviron E15, Controlled Environments Ltd., Winnipeg, Manitoba, Canada). In both growth chambers, the photoperiod was 10 h day with a photosynthetic photon flux density (PPFD) of *c.* 400 ± 30 μmol m<sup>-2</sup> s<sup>-1</sup>, mean air temperatures were 22°C during the day and 18°C at night, and relative air humidity was maintained constant at 46%. The soil moisture content in the pots was maintained constant at 70% soil water holding capacity (WHC) by counterbalancing evapotranspiration losses by adding the corresponding amount of water to the pots each every day.

### Fertilization treatment

The fertilization treatment aimed to simulate the fertility of the grassland soils where the plant species were collected. The fertilizer was dissolved in irrigating solution containing macronutrients (0.7 mM K, 3.18 mM Ca, 0.55 mM P) and micronutrients (6 μM Cl, 14 μM B, 3 μM Zn, 0.7 μM Cu, 0.7 μM Mo, 0.1 μM Co and 200 μM Fe as EDTA) to avoid limitation by nutrients other than N. Half of the replicate pots of each plant species and half of the bare soil controls were fertilized with ammonium nitrate to provide 100 kg N ha<sup>-1</sup>, whereas the other half received the same irrigating solution without nitrogen. Irrigating solution (with and without nitrogen) was applied twice during the experiment, on the 30/10/09 and on the 06/11/09; for N-fertilized plants, the first and

second applications corresponded to a supply of 40 kg N ha<sup>-1</sup> and 60 kg N ha<sup>-1</sup>, respectively.

#### **Labelling procedure**

In order to estimate rhizodeposited C, we conducted steady state labelling in a <sup>13</sup>CO<sub>2</sub> enriched atmosphere. The labelling procedure lasted 81 days in total, from the 02/11/09 until the 21/01/10. Sixteen replicates per species ( $n = 8$  per fertilization level) and twelve bare soil pots ( $n = 6$  per fertilization level) were placed in a growth chamber with a <sup>13</sup>C-labelled atmosphere (76 pots in total). The remaining six replicates per species ( $n = 3$  per fertilization level) and six bare soil pots ( $n = 3$  per fertility level) were kept as controls in the second growth chamber with an unlabelled atmosphere (30 pots total). The design of the experiment is described in detail in S1 and S2.

In the <sup>13</sup>C-labelled growth chamber, a 50 ml syringe (SGE, Ringwood, Australia) and needle (model microlance 3, BD, Plymouth, UK) were filled with 99.9% <sup>13</sup>C-CO<sub>2</sub> each day and placed on a syringe pump, which delivered 6 ml h<sup>-1</sup>. This system allowed homogeneous labelling throughout the day. Calculations based on the CO<sub>2</sub> injected by the syringe pump and the volume of the growth chamber gave a total concentration of 410 ppm CO<sub>2</sub> in the growth chamber without taking plant gas fluxes into account. IRGA measurements showed that CO<sub>2</sub> concentration varied from 300 to 500 ppm during the day depending on plant gas exchange activity (see S3). Diurnal isotopic composition of atmospheric CO<sub>2</sub> in the growth chamber reached 200 to 300 ‰ (see S3). No <sup>13</sup>C-CO<sub>2</sub> was injected at night.

#### **Plant and soil sampling**

After 71 days, two harvests of pots in the <sup>13</sup>CO<sub>2</sub>-enriched atmosphere were performed 7 days apart to estimate <sup>13</sup>C flows and mass-balance <sup>13</sup>C of the soil-plant systems ( $t_i$  and  $t_f$ , see S2). In the growth chamber with the <sup>13</sup>C-labelled atmosphere, 8 replicates per species ( $n = 4$  per fertilization level) and 6 ‘bare soil’ pots ( $n = 3$  per fertilization level) were harvested on 11/01/10 ( $t_i$ ), and the remaining pots were harvested on 18/01/10 ( $t_f$ ). Thus, plants harvested at  $t_f$  were grown in the enriched atmosphere for an additional seven days.

In the growth chamber with the unlabelled atmosphere, all pots (with and without plants) were sampled in a single day on 21/01/10 (see S2).

All pots and treatments were processed following the same procedure: when plants were present, the whole plant was removed from the soil and was shaken by hand to recover soil adhering to the roots; roots and leaves were then separated. Fresh soil from the pots was homogenized by sieving (2-mm mesh) and any remaining root fragments were removed and pooled with the respective root samples. Soil CO<sub>2</sub> efflux and its isotopic signature were analysed as described below. Leaf samples were dried at 60°C for 48h, weighed and ground. Root samples



were washed, gently dried with tissue paper and immediately used for measuring root CO<sub>2</sub> efflux and its isotopic signature ( $\delta^{13}\text{C}$ ; see subsequent section), after which, the samples were dried at 60°C for 48h and weighed. The analyses of root CO<sub>2</sub> efflux were used for the interpretation of processes underlying rhizodeposition patterns among species and fertilization treatments but did not allow estimates of  $^{13}\text{C}$  rhizodeposition (see section on 'Isotopic calculations and mass-balance  $^{13}\text{C}$  budget calculations').

#### **CO<sub>2</sub> gas exchange and isotopic composition measurements**

On both sampling dates ( $t_i$  and  $t_f$ ), empty 0.4L Perspex™ dark chambers were previously flushed for 30 min with CO<sub>2</sub>-free air passing through the soda-lime column (CO<sub>2</sub> absorbent) of an infra-red gas analyser (Li-6200, LI-COR, Inc., Lincoln, NE, USA). Each root and soil sample was then enclosed separately in one of the chambers. Root and SOM derived CO<sub>2</sub>-C effluxes were estimated every minute for 10–15 min. Air temperature (*c.* 20°C) and relative air humidity were recorded during all measurements. The dark chambers were then closed hermetically and CO<sub>2</sub> concentrations were allowed to rise between 600-1000 ppm, after which air samples were collected using the same 50 ml syringe and needle described above (Nogués et al. 2004). The gas samples were passed through a magnesium perchlorate column (water vapour trap) and then immediately injected into a 10 ml vacutainer (BD vacutainer, Plymouth, UK). To avoid contamination, the vacutainers were overpressurized with N<sub>2</sub> and the syringe and needle were also flushed with N<sub>2</sub> before taking each sample.

The isotopic composition of root and SOM derived CO<sub>2</sub>-C efflux was analysed by gas chromatography-combustion-isotope ratio mass spectrometry (GC-C-IRMS) as described by Nogués et al. (2008). After gas sampling, isotope composition was determined in roots using an Elemental Analyser Flash 1112 (Carlo Erba, Milan) coupled to isotope ratio mass spectrometry IRMS Delta C through a Conflo III Interface (Thermo-Finnigan, Germany).

#### **Soil analysis**

Sieved, root-free fresh soil samples were used for all subsequent analyses; soil samples of 18 g dry-weight (DW) equivalent were used to estimate the amount and isotopic signature ( $\delta^{13}\text{C}$ ) of SOM-derived CO<sub>2</sub>-C efflux, following the same procedure described for roots above. Additional subsamples were used to determine gravimetric soil water content by drying at 105 °C for 5 h and soil pH in water (1:5 ratio of soil to deionised water). Soil microbial and extractable C and N fractions were determined by the fumigation-extraction method (Vance et al. 1987) using 8 g soil (DW equivalent). In brief, control (unfumigated) subsamples were immediately extracted in 20 ml 0.3 M K<sub>2</sub>SO<sub>4</sub>, then shaken for 60 minutes, centrifuged and filtered. Fumigated subsamples were exposed to ethanol-free CHCl<sub>3</sub> inside a dark vacuum chamber for 24 h prior to K<sub>2</sub>SO<sub>4</sub>-extraction. The

content and the isotopic composition of the soil C in both the fumigated and unfumigated soil extracts were estimated by injecting 1 ml of extract into a Surveyor MS HPLC Pump plus (Thermo-Finnigan) coupled to an IRMS (Delta V Advantage, Thermo-Finnigan, Germany) via an LC Isolink interface (Thermo-Finnigan). The mobile phase used was a degassed MilliQ water constantly set to 400 µl/min. The quantitative chemical oxidation of compounds was performed in the LC Isolink interface with sodium peroxodisulfate (Na<sub>2</sub>S<sub>2</sub>O<sub>8</sub>) solution (100 g/l) and orthophosphoric acid (1.5M H<sub>3</sub>PO<sub>4</sub>) within an oxidation reactor kept at 99.9°C. Soil ammonium (NH<sub>4</sub><sup>+</sup>) and nitrate (NO<sub>3</sub><sup>-</sup>) concentrations were analysed by spectrophotometry using 1 to 3 ml of extract, following the protocols described in Baethgen and Alley (1989) and Cataldo et al. (1975) respectively. Soil microbial C and N fractions were subsequently calculated as the difference between fumigated and non-fumigated extractions without correction for extraction efficiency (Vance et al. 1987).

#### Isotopic calculations and mass-balance <sup>13</sup>C budget calculations

To estimate <sup>13</sup>C enrichment in leaf, root and soil samples, the Atom <sup>13</sup>C % excess for each type of sample was calculated as difference in the Atom %<sup>13</sup>C between labelled and unlabelled samples (see S1 and S2):

$$\begin{aligned} Atom\%^{13}C_{excess} &= Atom\%^{13}C_{labelled} - Atom\%^{13}C_{unlabelled} \\ &= \left[ 100 \cdot \left( \frac{R_{labelled}}{R_{labelled} + 1} \right) \right]_{labelled} - \left[ 100 \cdot \left( \frac{R_{unlabelled}}{R_{unlabelled} + 1} \right) \right]_{unlabelled} \end{aligned} \quad (1)$$

Where *R* is the absolute isotope <sup>13</sup>C/<sup>12</sup>C ratio for labelled and unlabelled samples.

The labelling-derived <sup>13</sup>C content (γ<sup>13</sup>C, in µg <sup>13</sup>C) in leaf, root and soil samples was then calculated as:

$$\gamma^{13}C = \frac{Atom\%^{13}C_{excess}}{100} \cdot massC_{sample} \quad (2)$$

where massC<sub>sample</sub> represents the C content of the sample (in µg).

Root- and SOM-derived <sup>13</sup>CO<sub>2</sub>-C (γ<sup>13</sup>R<sub>C</sub>, in µg <sup>13</sup>C g<sup>-1</sup> DW h<sup>-1</sup>) were calculated as:

$$\gamma^{13}Resp = \frac{Atom\%^{13}C_{excess}}{100} \cdot Resp_{sample} \cdot mass_{sample} \quad (3)$$

where mass<sub>sample</sub> is the mass of root or soil sample (g DW) and Resp<sub>sample</sub> is the CO<sub>2</sub>-C efflux from root or microbial biomass (µg C g<sup>-1</sup> DW h<sup>-1</sup>).

Cumulative rhizodeposited <sup>13</sup>C was calculated as the sum of (1) the cumulative labelling-derived <sup>13</sup>C content in extractable soil organic C between t<sub>i</sub> and t<sub>f</sub>, (2) the cumulative labelling-derived <sup>13</sup>C content immobilized in microbial biomass between t<sub>i</sub> and t<sub>f</sub>, and (3) the cumulative labelling-derived <sup>13</sup>C respired by microbial biomass between t<sub>i</sub> and t<sub>f</sub>. As the air temperature during soil CO<sub>2</sub> efflux measurements was similar to temperature inside

the growth chamber, cumulative labelling-derived  $^{13}\text{C}$  respired by microbial biomass was estimated by integrating soil  $\text{CO}_2$  efflux measurements over seven days.

Hence, cumulative rhizodeposited  $^{13}\text{C}$  ( $\mu\text{g}^{13}\text{C}$ ) over seven days was thus equal to:

$$\int_{t_i}^{t_f} {}^{13}C_{\text{rhizodeposited}} = \int_{t_i}^{t_f} \gamma^{13}C_{\text{labile soil}} + \int_{t_i}^{t_f} \gamma^{13}C_{\text{microbial biomass}} + \int_{t_i}^{t_f} \gamma^{13}\text{Resp}_{\text{microbial respired}} \quad (4)$$

Rhizodeposited  $^{13}\text{C}$  ( $\mu\text{g}^{13}\text{C d}^{-1}$ ) was calculated as cumulative rhizodeposited  $^{13}\text{C}$  divided by the number of days between  $t_i$  and  $t_f$  (i.e. seven days). Finally, specific rhizodeposited  $^{13}\text{C}$  ( $\mu\text{g}^{13}\text{C g}^{-1}$  root DW  $\text{d}^{-1}$ ) was calculated as rhizodeposited  $^{13}\text{C}$  divided by root dry weight.

### Statistical analyses

Differences between parameters at times  $t_i$  and  $t_f$  were calculated as the mean value at  $t_f$  subtracted from the replicate  $i$  value. Then the mean of the  $i$  replicates was determined. For all data, a three-way ANOVA was performed in order to test for the effects of N fertility, species and time ( $t_i$  and  $t_f$ ) effects. Regression analyses were used to explore the relationships between rhizodeposition and plant parameters (plant biomass, root biomass and leaf and root nitrogen concentration). Where main treatment effects were significant, *a posteriori* differences among species were determined by Tukey's HSD (Honestly Significant Differences) test. All analyses were performed with Jump software (V7.0 for window, SAS Institute Inc., Cary, NC, USA).

## Results

### *Plant characteristics*

Total plant biomass, leaf and root biomass differed significantly among species and the observed ranking, DG>LP>AO=BM, mainly reflected variations in root biomass (Tables 1 and 2). LP exhibited a significantly higher root:shoot ratio than the others species (1.53 vs. 0.88 on average for the other three species). There were also slight but significant variations in leaf- and root N concentrations among species, where BM had the highest values (Table 1 and 2).

Fertilization with N led to a 22% increase in total biomass and a 12% increase in leaf biomass across all species but had no consistent effect on leaf and root N concentration (Table 1 and 2). Root biomass was strongly inversely related to root N concentration (S4,  $R^2=0.62$ ,  $F=99.7$ ,  $P<0.0001$ ) but there was no relationship between leaf biomass and leaf N concentration (S4).

The species  $\times$  fertility interaction was significant for all biomass parameters ( $P<0.001$ ; Table 2). Leaf and root biomass increased in response to N fertilization in DG and AO, but decreased in BM and LP displayed an increase in leaf biomass but a decrease in root biomass in response to N fertilization. However, root:shoot ratios did not change in response to fertilization (data not shown) but differed among species as DG and LP had higher root:shoot ratios than AO and BM (data not shown,  $F=9.23$ ,  $P<0.001$ ).

### *Steady-state $^{13}\text{C}$ labelling*

The isotopic composition of C in leaves differed significantly between  $t_i$  and  $t_f$  but the isotopic composition of root C was similar between the two sampling dates (S6). The isotopic composition of root C was lower in AO (mean 238‰) compared to the others species (mean 276‰; S5 and S6) but this pattern was not apparent in isotopic composition of leaf C (overall means for LP, BM and DG: 322‰ compared to 324‰ for AO; S5 and S6).

The isotopic composition of extractable soil C and microbial biomass in bare soil pots was similar in growth chambers with labelled and unlabelled atmosphere (S7). The  $^{13}\text{CO}_2$  respired by the microorganisms was strongly correlated with  $^{13}\text{C}$  microbial immobilization ( $R^2=0.56$ ,  $F=36.28$ ,  $P<0.0001$ ; S8).

### *Estimation of total rhizodeposited $^{13}\text{C}$*

When all species and fertility treatments were considered, cumulative labelling-derived  $^{13}\text{C}$  content in extractable soil C was very low ( $-0.0068 \pm 0.0012 \mu\text{g } ^{13}\text{C}$ ). In contrast, the mean total labelling-derived  $^{13}\text{C}$  immobilized in the microbial biomass was high ( $48.1 \pm 8.0 \mu\text{g } ^{13}\text{C}$ ). The mean  $\text{CO}_2$  respired by microbial biomass was  $0.58 \pm 3.31 \text{ ng } ^{13}\text{C.g}^{-1} \text{ soil DW min}^{-1}$  over the 7 days; the mean of the  $^{13}\text{CO}_2$  respired by microbial biomass efflux was

0.014 ± 0.001 ng <sup>13</sup>C.g<sup>-1</sup> soil DW min<sup>-1</sup>. Mean cumulative labelling-derived <sup>13</sup>C respired by microbial biomass over the 7 day-period was 30.0 ± 3.7 μg <sup>13</sup>C. Total rhizodeposited <sup>13</sup>C varied between 5 and 18 μg <sup>13</sup>C d<sup>-1</sup> depending on species and fertilization treatment (Fig. 1a).

#### ***Effect of species and fertility on rhizodeposited <sup>13</sup>C***

More than 65% of the rhizodeposited <sup>13</sup>C was immobilized in the microbial biomass and the rest was respired (Fig. 2a and 2b). Accordingly, the immobilization of <sup>13</sup>C in the K<sub>2</sub>SO<sub>4</sub>-extractable soil organic C fraction represented less than 0.001% of total rhizodeposited <sup>13</sup>C (data not shown).

When both fertilization treatments were considered together, there was a threefold difference in total rhizodeposited <sup>13</sup>C and specific rhizodeposited <sup>13</sup>C among species (Table 3, Fig. 1a and 1b). Total rhizodeposited <sup>13</sup>C was significantly higher in DG and significantly lower in BM compared to all other species but there was no difference between AO and LP mainly due to the strong effect of fertilization (Fig. 1a). In contrast, specific rhizodeposited <sup>13</sup>C was highest in AO, followed by BM, DG and LP and all species differences were significant (Fig. 1b).

Fertilization significantly increased total rhizodeposited <sup>13</sup>C by 13% across all species (Fig. 1a; Table 3), which was mainly explained by increases in <sup>13</sup>C microbial immobilization and <sup>13</sup>C respired by microbial biomass in DG and AO pots (Fig. 2a and 2b). In contrast, there was no consistent effect of fertilization on specific rhizodeposited <sup>13</sup>C (Fig. 1b; Table 3).

The species × fertility interaction was significant for both total rhizodeposited <sup>13</sup>C and specific rhizodeposited <sup>13</sup>C (Table 3). Whereas total <sup>13</sup>C rhizodeposition in DG and AO was higher with fertilization, the opposite was observed for LP and BM. Specific <sup>13</sup>C rhizodeposition was lower with fertilization in DG, LP and BM but increased in AO.

Total rhizodeposited <sup>13</sup>C was significantly correlated to leaf biomass and leaf N concentration (Fig. 3a, b) and to root biomass and root N concentration (Fig. 3c, d). A significant negative relationship was observed between specific rhizodeposited <sup>13</sup>C and root biomass (Fig. 4c) but there was no relationship between specific rhizodeposited <sup>13</sup>C and leaf biomass (Fig. 4a), leaf N concentration (Fig. 4b) or root N concentration (Fig. 4d).

#### ***Impact of species and fertility on soil properties***

Soil nitrate concentrations were extremely low in all treatments (*i.e.* below detection limit; data not presented). Concentrations of soil NH<sub>4</sub><sup>+</sup> and microbial biomass N were influenced by plant species, fertilization and sampling date (Table 4 and 5). Soil NH<sub>4</sub><sup>+</sup> concentrations were significantly higher for LP than for AO, DG and BM, whereas microbial biomass N was lower in LP compared to the other species. Soil NH<sub>4</sub><sup>+</sup> content was

299 significantly lower in fertilized pots for all species except BM, whereas microbial biomass N was significantly  
300 higher (Table 4 and 5). Soil  $\text{NH}_4^+$  concentrations and microbial biomass N were consistently higher at  $t_i$   
301 compared to  $t_f$ . Total microbial biomass C was not affected by plant species or fertilization (Table 4 and 5) but  
302 microbial biomass C was higher at  $t_f$  than at  $t_i$  across all treatments.

303 Soil pH was significantly affected by species and fertilization (Table 5): soil pH in bare soil pots was 0.30 to  
304 0.50 units lower than in pots with plants. Furthermore, soil pH was lowest in BM pots, followed by AO, LP and  
305 DG and the difference between the lowest and the highest values was 0.12 units. Finally, fertilization  
306 significantly decreased soil pH by a mean of 0.06 units.

## Discussion

The aim of this study was to elucidate the impact of two levels of fertilization on the rhizodeposition of grassland species with different nutrient acquisition strategies. Our experimental design avoided several methodological biases reported in the literature (Paterson et al. 2005; 2009), which limit the comparison of rhizodeposited C between species or under different treatments such as fertility levels. Previous techniques based on pulse-labelling were rarely sufficient to uniformly label plant inputs into soil and consequently, only the contribution of recently labelled assimilates could be estimated (Paterson et al. 2005). In our study, the isotopic composition of roots remained constant from  $t_i$  to  $t_f$  for each species, which indicates that a steady-state was reached and plant inputs to the soil were uniformly labelled. Additionally, a further bias in  $^{13}\text{C}$  labelling studies can be introduced by direct fixation of  $^{13}\text{C}$  in the microbial biomass by phosphoenolpyruvate carboxylation. In our experiment, the isotopic composition of extractable soil C and microbial biomass in bare soil pots were highly similar in growth chambers with labelled and unlabelled atmosphere and hence direct  $^{13}\text{C}$  carboxylation by soil microbes was negligible. Collectively, these results demonstrate that the common biases related to the nature of experiment (i.e. steady-state long-term labelling) were avoided. Although we did not measure soil and root respiration *in situ*, our estimates of microbial- and root  $\text{CO}_2$  effluxes were similar to those reported in the literature (Tjoelker et al. 2005; Baptist et al. 2009). Hence, although our study precludes estimates of rhizodeposited  $^{13}\text{C}$  fluxes in natural conditions, our experimental design provides accurate comparative data to assess differences among species and fertilization treatments.

We observed large differences in the total amount of rhizodeposited  $^{13}\text{C}$  as well as specific  $^{13}\text{C}$  rhizodeposition, among the four species. Contrary to our expectations, the conservative species displayed higher rhizodeposition fluxes per unit dry weight of root than the two exploitative species. Fertilization consistently affected total rhizodeposited  $^{13}\text{C}$  but not specific rhizodeposited  $^{13}\text{C}$ . The rate of total rhizodeposited  $^{13}\text{C}$  was higher in DG and AO with fertilization whereas the opposite was observed in LP and BM. Hence, the two exploitative species studied, as well as the two conservative species, showed respectively an opposite response to fertilization. These findings suggest that plant rhizodeposition responses to the fertilization were not directly related to nutrient acquisition strategies as we had anticipated, but were species dependent. Therefore, rhizodeposition patterns could not be associated with the leaf economic spectrum (Freschet et al. 2010). Various underlying processes can explain these results including differences in sink strength applied by rhizospheric microbial biomass changes (Jones et al., 2009; Bahn et al., 2013), differences in plant biomass allocation and root morphology (Van der Krift et al. 2001; Warembourg et al. 2003; Darwent et al. 2003) or in shoot architecture which might induce

changes in light interception and therefore in whole plant carbon partitioning (Hodge et al. 1997, Paterson et al. 1999).

Although we observed changes in rhizodeposition patterns among plant species and fertility treatments, we found no differences in soil C microbial biomass; this suggests that, in contrast to other studies, rhizospheric microbial biomass was not the main driver of rhizodeposition process (Jones et al. 2009; Bahn et al. 2013). Indeed, in accordance with the study of Henry et al. (2005), no differences in soil C microbial biomass were found between plant species and fertility treatment whereas changes in rhizodeposition patterns were observed. Furthermore, there was no correlation between total or specific rhizodeposited  $^{13}\text{C}$  and soil microbial biomass variations between  $t_i$  and  $t_f$  (data not shown). The stability of microbial biomass C between sampling dates could be explained by the fact that soil microorganisms were not limited by C availability in any of the treatments (Paterson et al. 2009) but might have suffered from limitation by another resource, such as nitrate. Indeed, nitrate concentrations in the soil in all treatments were below detection limits. It is conceivable that N availability to the soil microbial community may have been limited by competition with plants (Legay et al. 2013) or by exuded plant compounds inhibiting nitrification (Subbarao et al. 2009), resulting in a reduction in microbial growth. Although microbial biomass did not change between sampling dates, we cannot exclude a change in community composition in response to fertilization. Although analyses of microbial community structure was beyond the scope of this work, fertilization with N can favour the development of bacteria at the expense of the fungal community (Denef et al. 2009), which would modify the strength of the C sink in rhizospheric microbial biomass (Jones et al., 2009; Bahn et al., 2013) and affect plant rhizodeposition rates.

Microbial biomass did not change in response to different rhizodeposition patterns of plant species, which lends further support the hypothesis that microbial biomass was not a driving force for rhizodeposition rate in our study. In contrast, we observed strong relationships between total rhizodeposited  $^{13}\text{C}$  and leaf or root biomass, which supports previous assumptions that exudation patterns are affected by species identity in general and root biomass in particular (Van der Krift et al. 2001; Blagodatskaya et al. 2014). Collectively, these results suggest that changes in rhizodeposition rates following fertilization were not driven by the soil microbial community but rather indicate a substantial influence of plant characteristics on rhizodeposition processes (Farrar et al., 2003; Warembourg et al., 2003; Kuzyakov and Cheng, 2004; Badri and Vivanco, 2009), as demonstrated by previous studies on grassland (Van der Krift et al. 2001; Pausch et al. 2013) and forest species (Bowden et al. 2004). Interestingly, total and specific rhizodeposited  $^{13}\text{C}$  were both strongly related to total root biomass. Total rhizodeposited  $^{13}\text{C}$  was positively correlated to root biomass and leaf biomass, which indicates that



rhizodeposition increases with plant growth. Conversely, specific rhizodeposited  $^{13}\text{C}$  (i.e.,  $^{13}\text{C}$  per g root dry weight) decreased with increasing root biomass, indicating a loss in rhizodeposition efficiency when belowground biomass increases (Henry et al. 2005) but there was no relationship between specific rhizodeposited  $^{13}\text{C}$  and leaf biomass, or leaf- and root N concentrations. This loss of rhizodeposition efficiency has previously been observed in two grasses with contrasting nutrient acquisition strategies, *L. perenne* (exploitative) and *Festuca rubra* (conservative), which both displayed a decrease in rhizodeposition rate in response to fertilisation, despite larger root biomass (Paterson and Sim, 1999, 2000). This was attributed to greater specific root length (SRL) at low N, whereby longer but finer roots would increase soil exploration and exudation efficiency, allowing greater nutrient acquisition and stimulation of microbial mineralization processes (Paterson and Sim, 1999, 2000). Moreover, high SRL is associated with a greater number of root tips (Arredondo and Johnson 1999), which have the highest rhizodeposition rate compared to other parts of the root system (Darwent et al. 2003). Taken together, these findings suggest that changes in the amount of rhizodeposited C per unit dry weight root were mainly driven by belowground biomass and root morphology rather than traits associated to plant resource acquisition strategies such as leaf and root N concentration.

## Conclusions

The new continuous labelling method developed in this study permitted the separation of plant-derived  $\text{CO}_2\text{-C}$  from microbial-derived  $\text{CO}_2\text{-C}$  and avoided most of the biases related to this type of experiment (i.e. steady state long term labelling). It provides a useful approach in order to assess mechanisms involved in patterns of plant rhizodeposition under different soil fertility levels. We suggest that the observed changes in the amount of rhizodeposited C under different levels of N availability were driven mainly by plant biomass rather than soil microbes. Finally, our results underline the potential importance of plant characteristics (i.e; root biomass) as opposed to traits associated with plant resource acquisition strategies in predicting total C rhizodeposition. However, further studies with more species will be necessary in order to confirm these findings.

## **Acknowledgments**

The authors wish to thank Pilar Teixidor from the Scientific Technical Services of the University of Barcelona which performed the GC-C-IRMS and EA-IRMS analyses and Sonia Ba Forne for her technical help. Sandra Lavorel, Jean-Christophe Clement and Emma J. Sayer are also thanked for their helpful comments on an earlier version of the manuscript. This study was financed by the BIODIVERSA-VITAL (Ministère de l'Ecologie, du Développement Durable et de l'Energie, France) and the OPTIMA (European Community) projects.

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**Table 1.** Responses of plant traits to fertilization treatments without (-N) or with nitrogen addition (+N); as there was no effect of sampling date (see Table 2), data from all pots per treatment and date were pooled to give means and standard errors for  $n=8$  per treatment.

| Species                     | Abbr. | Total biomass (g DW) |             | Leaf biomass (g DW) |             | Root biomass (g DW) |             | Leaf N concentration (% DW) |             | Root N concentration (% DW) |             |
|-----------------------------|-------|----------------------|-------------|---------------------|-------------|---------------------|-------------|-----------------------------|-------------|-----------------------------|-------------|
| Fertility level             |       | -N                   | +N          | -N                  | +N          | -N                  | +N          | -N                          | +N          | -N                          | +N          |
| <i>Dactylis glomerata</i>   | DG    | 0.92 (0.07)          | 1.16 (0.07) | 0.56 (0.05)         | 0.82 (0.04) | 0.47 (0.06)         | 0.89 (0.09) | 1.63 (0.14)                 | 1.51 (0.04) | 0.76 (0.03)                 | 0.62 (0.03) |
| <i>Lolium perenne</i>       | LP    | 0.60 (0.03)          | 0.84 (0.03) | 0.29 (0.02)         | 0.41 (0.04) | 0.56 (0.04)         | 0.47 (0.07) | 1.70 (0.05)                 | 2.00 (0.10) | 0.65 (0.03)                 | 0.78 (0.03) |
| <i>Anthoxantum odoratum</i> | AO    | 0.54 (0.04)          | 0.83 (0.10) | 0.15 (0.02)         | 0.40 (0.03) | 0.16 (0.02)         | 0.31 (0.03) | 1.71 (0.17)                 | 1.51 (0.20) | 0.86 (0.04)                 | 0.80 (0.03) |
| <i>Briza media</i>          | BM    | 0.54 (0.05)          | 0.49 (0.12) | 0.42 (0.04)         | 0.33 (0.08) | 0.27 (0.05)         | 0.19 (0.05) | 1.71 (0.12)                 | 2.35 (0.25) | 0.92 (0.09)                 | 1.21 (0.18) |

**Table 2.** Results of analysis of variance (ANOVA) investigating the effects of plant species, fertilization, and time (sampling date) on plant biomass and plant functional traits; F statistics and degrees of freedom (*df*) are given, and significant results at  $P < 0.05$  are show in bold.

| Variable                                    | Effect                            | <i>F</i> ( <i>df</i> ) | <i>P</i>          |
|---|-----------------------------------|------------------------|-------------------|
| <b>Total biomass<br/>(g DW)</b>             | <i>Species</i>                    | 37.47 (3,47)           | <b>&lt;0.0001</b> |
|   | <i>Time</i>                       | 2.00 (1,47)            | 0.16              |
|   | <i>Fertility</i>                  | 16.16 (1,47)           | <b>0.0002</b>     |
|   | <i>Species</i> × <i>Time</i>      | 1.64 (3,47)            | 0.19              |
|   | <i>Species</i> × <i>Fertility</i> | 5.88 (3,47)            | <b>0.001</b>      |
|   | <i>Time</i> × <i>Fertility</i>    | 0.10 (1,47)            | 0.77              |
| <b>Leaf biomass<br/>(g DW)</b>              | <i>Species</i>                    | 34.15 (3,47)           | <b>&lt;0.0001</b> |
|   | <i>Time</i>                       | 1.01 (1,47)            | 0.32              |
|   | <i>Fertility</i>                  | 17.38 (1,47)           | <b>&lt;0.0001</b> |
|   | <i>Species</i> × <i>Time</i>      | 0.97 (3,47)            | 0.41              |
|   | <i>Species</i> × <i>Fertility</i> | 6.28 (3,47)            | <b>0.001</b>      |
|   | <i>Time</i> × <i>Fertility</i>    | 0.002 (1,47)           | 0.96              |
| <b>Root biomass<br/>(g DW)</b>              | <i>Species</i>                    | 32.82 (3,47)           | <b>&lt;0.0001</b> |
|   | <i>Time</i>                       | 2.60 (1,47)            | 0.11              |
|   | <i>Fertility</i>                  | 7.04 (1,47)            | <b>0.01</b>       |
|   | <i>Species</i> × <i>Time</i>      | 2.16 (3,47)            | 0.10              |
|   | <i>Species</i> × <i>Fertility</i> | 9.77 (3,47)            | <b>&lt;0.0001</b> |
|   | <i>Time</i> × <i>Fertility</i>    | 0.20 (1,47)            | 0.65              |
| <b>Leaf N<br/>concentration<br/>(% DW )</b> | <i>Species</i>                    | 3.32 (3,44)            | <b>0.03</b>       |
|   | <i>Time</i>                       | 0.56 (1,44)            | 0.45              |
|   | <i>Fertility</i>                  | 1.96 (1,44)            | 0.16              |
|   | <i>Species</i> × <i>Time</i>      | 0.18 (3,44)            | 0.90              |
|   | <i>Species</i> × <i>Fertility</i> | 2.66 (3,44)            | 0.06              |
|   | <i>Time</i> × <i>Fertility</i>    | 0.12 (1,44)            | 0.73              |
| <b>Root N<br/>concentration<br/>(% DW)</b>  | <i>Species</i>                    | 16.93 (3,59)           | <b>&lt;0.0001</b> |
|   | <i>Time</i>                       | 1.07 (1,59)            | 0.30              |
|   | <i>Fertility</i>                  | 3.65 (1,59)            | 0.06              |
|   | <i>Species</i> × <i>Time</i>      | 2.11 (3,59)            | 0.11              |
|   | <i>Species</i> × <i>Fertility</i> | 6.94 (3,59)            | <b>0.0006</b>     |
|   | <i>Time</i> × <i>Fertility</i>    | 1.54 (1,59)            | 0.22              |



**Table 3.** Results of analysis of variance (ANOVA) investigating the effects of species and fertilization on total rhizodeposited  $^{13}\text{C}$ , specific rhizodeposited  $^{13}\text{C}$ , microbially immobilized  $^{13}\text{C}$ , microbially respired  $^{13}\text{CO}_2$  and  $\text{K}_2\text{SO}_4$ -extractable soil organic  $^{13}\text{C}$ ;  $F$  statistics and degrees of freedom ( $df$ ) are given, and significant results at  $P < 0.05$  are show in bold.

| Variable   | Effect                                   | $F$ ( $df$ )  | $P$               |
|--|--|---------------|-------------------|
| <b>Total rhizodeposited <math>^{13}\text{C}</math></b><br>( $\mu\text{g } ^{13}\text{C d}^{-1}$ )                            | <i>Species</i>                           | 66.4 (3,22)   | <b>&lt;0.0001</b> |
|  | <i>Fertility</i>                         | 10.9 (1,22)   | <b>0.0032</b>     |
|  | <i>Species</i> $\times$ <i>Fertility</i> | 83.2 ( 3,22)  | <b>&lt;0.0001</b> |
| <b>Specific rhizodeposited <math>^{13}\text{C}</math></b><br>( $\mu\text{g } ^{13}\text{C g}^{-1}$ root DW $\text{d}^{-1}$ ) | <i>Species</i>                           | 55.3 (3,22)   | <b>&lt;0.0001</b> |
|  | <i>Fertility</i>                         | 0.14 (1,22)   | 0.70              |
|  | <i>Species</i> $\times$ <i>Fertility</i> | 27.1 ( 3,22)  | <b>&lt;0.0001</b> |
| <b><math>^{13}\text{C}</math> microbially immobilized</b><br>( $\mu\text{g } ^{13}\text{C d}^{-1}$ )                         | <i>Species</i>                           | 66.0 (3,22)   | <b>&lt;0.0001</b> |
|  | <i>Fertility</i>                         | 15.9 (1,22)   | <b>0.006</b>      |
|  | <i>Species</i> $\times$ <i>Fertility</i> | 150.1 ( 3,22) | <b>&lt;0.0001</b> |
| <b><math>^{13}\text{CO}_2</math> respired by microbial biomass</b><br>( $\mu\text{g } ^{13}\text{C d}^{-1}$ )                | <i>Species</i>                           | 12.7 (3,22)   | <b>&lt;0.0001</b> |
|  | <i>Fertility</i>                         | 1.38 ( 1,22)  | 0.25              |
|  | <i>Species</i> $\times$ <i>Fertility</i> | 17.1 ( 3,22)  | <b>&lt;0.0001</b> |
| <b>Extractable soil <math>^{13}\text{C}</math></b><br>( $\mu\text{g } ^{13}\text{C}$ )                                       | <i>Species</i>                           | 2.55 (3,22)   | 0.08              |
|  | <i>Fertility</i>                         | 5.72 ( 1,22)  | <b>0.03</b>       |
|  | <i>Species</i> $\times$ <i>Fertility</i> | 1.58 ( 3,22)  | 0.22              |

**Table 4.** Soil properties without (-N) or with nitrogen addition (+N); soil nitrate concentrations are not given as they were below analytical detection limits; as there was no effect of sampling date (see Table 5), data from all pots per treatment and date were pooled to give means and standard errors for  $n=4$  per plant species and.  $n=3$  for bare soil pots; NA = not measured.

| Species / Bare soil plots   | Time  | Soil $\text{NH}_4^+$<br>( $\mu\text{g N-NH}_4^+ \text{ kg}^{-1}$ soil DW) |              | Immobilized $\text{NH}_4^+$ N<br>microbial biomass<br>( $\mu\text{g N kg}^{-1}$ soil DW) |              | C microbial biomass<br>( $\mu\text{g C g}^{-1}$ soil DW) |               | pH          |             |
|-----------------------------|-------|---|--------------|--|--------------|--|---------------|-------------|-------------|
|                             |       | -N  | +N           | -N   | +N           | -N   | +N            | -N          | +N          |
| <i>Dactylis glomerata</i>   | $t_i$ | 0.56 (0.08)   | 0.46 (0.001) | 3.05 ( 0.06)   | 3.44 (0.12)  | 312.6 (125.5)  | 115.0 (6.6)   | 6.87 (0.04) | 6.83 (0.04) |
|                             | $t_f$ | 0.38 (0.02)   | 0.40 (0.03)  | 2.44 (0.21)  | 3.05 (0.27 ) | 399.9 (72.2 )  | 539.7 (65.5)  | 6.88 (0.02) | 6.80 (0.02) |
| <i>Lolium perenne</i>       | $t_i$ | 0.73 (0.04)   | 0.67 (0.03)  | 2.68 (0.09)  | 2.87 (0.11)  | 330.9 (156.5)  | 141.0 (67.8)  | 6.82 (0.01) | 6.79 (0.06) |
|                             | $t_f$ | 0.75 (0.04)   | 0.63 (0.04)  | 2.46 (0.12 )   | 2.52 (0.02 ) | 462.0 (119.7)  | 382.7 (142.7) | 6.85 (0.02) | 6.77 (0.03) |
| <i>Anthoxantum odoratum</i> | $t_i$ | 0.63 (0.001)  | 0.63 (0.03)  | 2.95 (0.09)  | 3.67 (0.16)  | 158.0 (37.1)   | 58.4 (25.4)   | 6.78 (0.02) | 6.75 (0.03) |
|                             | $t_f$ | 0.63 (0.07)   | 0.34 (0.06)  | 2.85 (0.13 )   | 2.71 (0.10)  | 329.6 (30.0)   | 520.8 (47.2 ) | 6.80 (0.01) | 6.75 (0.01) |
| <i>Briza media</i>          | $t_i$ | 0.46 (0.05)   | 1.92 (0.80)  | 2.94 (0.06)  | 3.17 (0.05)  | 73.5 (40.1)  | 68.9 (20.4)   | 6.76 (0.04) | 6.69 (0.03) |
|                             | $t_f$ | 0.30 (0.05)   | 10.21 (5.70) | 2.84 (0.13)  | 2.84 (0.11)  | 434.0 (84.1 )  | 479.4 (46.6 ) | 6.76 (0.02) | 6.69 (0.03) |
| Bare soil                   | -     | 0.46 (0.02)   | 0.42 (0.001) | 1.80 (0.09)  | 1.98 (0.35)  | NA   | NA            | 6.54 (0.04) | 6.29 (0.01) |

**Table 5.** Results of analysis of variance (ANOVA) investigating the effects of species, fertilization and time on soil properties and microbial biomass; F statistics and degrees of freedom (df) are given, and significant results at  $P < 0.05$  are show in bold.

| Variable   | Effect                     | F (df)      | P                 |
|--|----------------------------|-------------|-------------------|
| <b>Soil NH<sub>4</sub><sup>+</sup><br/>(µg N-NH<sub>4</sub><sup>+</sup> kg<sup>-1</sup> soil<br/>DW)</b> | <i>Species</i>             | 41.4 (3,46) | <b>&lt;0.0001</b> |
|  | <i>Time</i>                | 22.8 (1,46) | <b>&lt;0.0001</b> |
|  | <i>Fertility</i>           | 12.7 (1,46) | <b>0.0009</b>     |
|  | <i>Species × Time</i>      | 2.16 (3,46) | 0.10              |
|  | <i>Species × Fertility</i> | 0.96 (3,46) | 0.41              |
|  | <i>Time × Fertility</i>    | 1.50 (1,46) | 0.23              |
| <b>N microbial<br/>biomass<br/>(µg N kg<sup>-1</sup> soil<br/>DW)</b>                                    | <i>Species</i>             | 8.37 (3,46) | <b>0.0002</b>     |
|  | <i>Time</i>                | 34.6 (1,46) | <b>&lt;0.0001</b> |
|  | <i>Fertility</i>           | 15.5 (1,46) | <b>0.0003</b>     |
|  | <i>Species × Time</i>      | 1.44 (3,46) | 0.24              |
|  | <i>Species × Fertility</i> | 1.91 (3,46) | 0.14              |
|  | <i>Time × Fertility</i>    | 3.65 (1,46) | 0.06              |
| <b>C microbial biomass<br/>(µg C g<sup>-1</sup> soil DW)</b>   | <i>Species</i>             | 0.98 (3,45) | 0.41              |
|  | <i>Time</i>                | 48.3 (1,45) | <b>&lt;0.0001</b> |
|  | <i>Fertility</i>           | 0.34 (1,45) | 0.56              |
|  | <i>Species × Time</i>      | 1.05 (3,45) | 0.38              |
|  | <i>Species × Fertility</i> | 0.98 (3,45) | 0.41              |
|  | <i>Time × Fertility</i>    | 5.73 (1,45) | <b>0.02</b>       |
| <b>pH</b>  | <i>Species</i>             | 11.8 (3,47) | <b>&lt;0.0001</b> |
|  | <i>Time</i>                | 0.03 (1,47) | 0.86              |
|  | <i>Fertility</i>           | 14.9 (1,47) | <b>0.0004</b>     |
|  | <i>Species × Time</i>      | 0.07 (3,47) | 0.97              |
|  | <i>Species × Fertility</i> | 0.24 (3,47) | 0.86              |
|  | <i>Time × Fertility</i>    | 1.03 (1,47) | 0.31              |

## Figure legends

**Figure 1.** Total rhizodeposited  $^{13}\text{C}$  (a) and specific rhizodeposited  $^{13}\text{C}$  (b) measured in a stable isotope labelling experiment with (+N) or without (-N) nitrogen addition in pots with planted with *Dactylis glomerata* (DG), *Lolium perenne* (LP), *Anthoxantum odoratum* (AO), or *Briza media* (BM); significant differences among species at  $P < 0.05$  (for both fertilization treatments pooled) are indicated by different letters above bars; standard error bars are shown for  $n = 4$ .

**Figure 2.** Microbially immobilized  $^{13}\text{C}$  (a) and  $^{13}\text{CO}_2$  respired by microbial biomass measured in a stable isotope labelling experiment with (+N) or without (-N) nitrogen addition in pots with planted with *Dactylis glomerata* (DG), *Lolium perenne* (LP), *Anthoxantum odoratum* (AO), *Briza media* (BM); significant differences among species at  $P < 0.05$  (for both fertilization treatments pooled) are indicated by different letters above bars; standard error bars are shown for  $n = 4$ .

**Figure 3.** Relationships between total rhizodeposited  $^{13}\text{C}$  and (a) leaf biomass, (b) leaf N concentration, (c) root biomass and (d) root N concentration in a stable isotope labelling experiment with (+N) or without (-N) nitrogen addition in pots with planted with *Dactylis glomerata* (DG), *Lolium perenne* (LP), *Anthoxantum odoratum* (AO), *Briza media* (BM).

**Figure 4.** Relationships between specific rhizodeposited  $^{13}\text{C}$  and (a) leaf biomass, (b) leaf N concentration, (c) root biomass and (d) root N concentration in a stable isotope labelling experiment with (+N) or without (-N) nitrogen addition in pots with planted with *Dactylis glomerata* (DG), *Lolium perenne* (LP), *Anthoxantum odoratum* (AO), *Briza media* (BM).

Figure 1

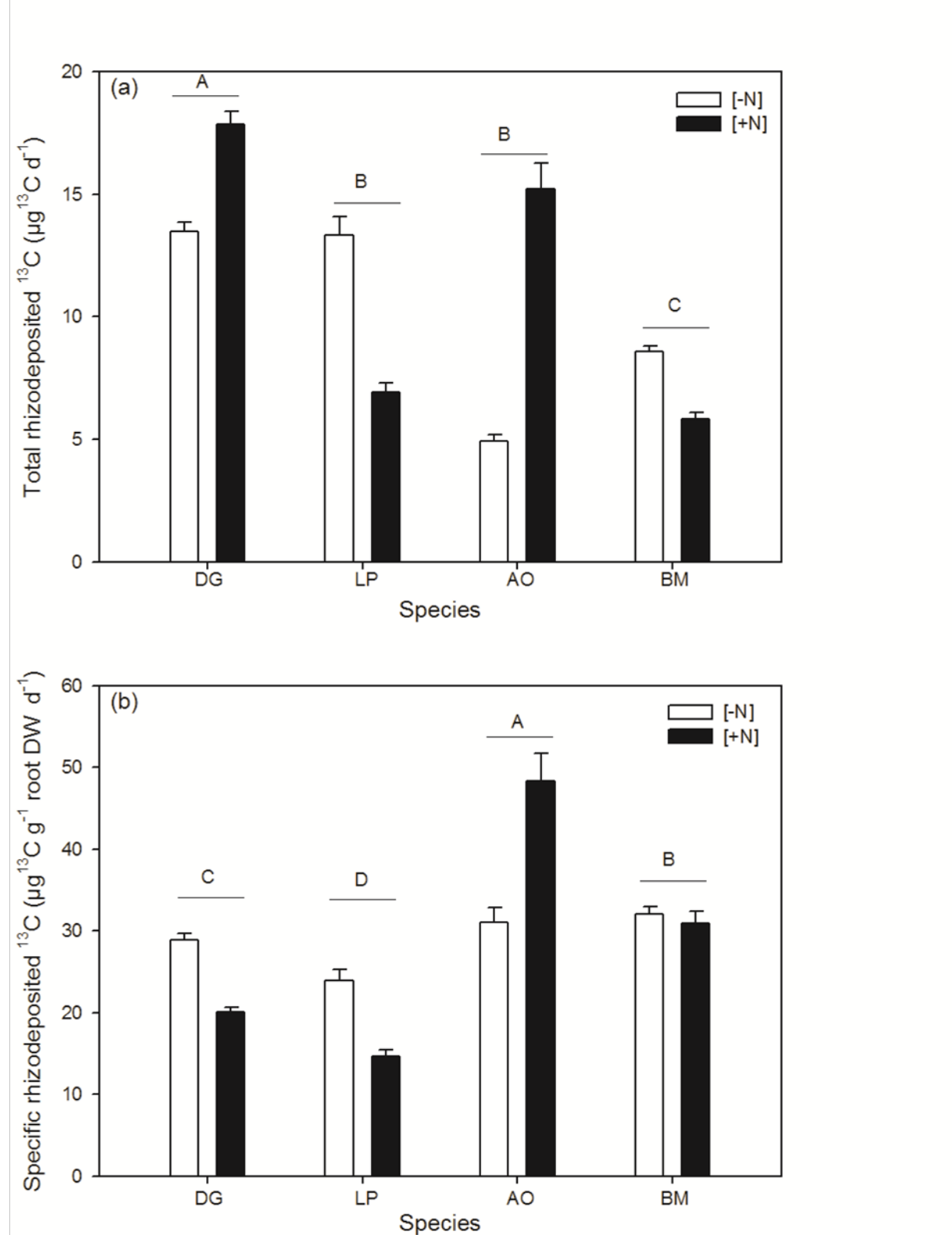


Figure 2

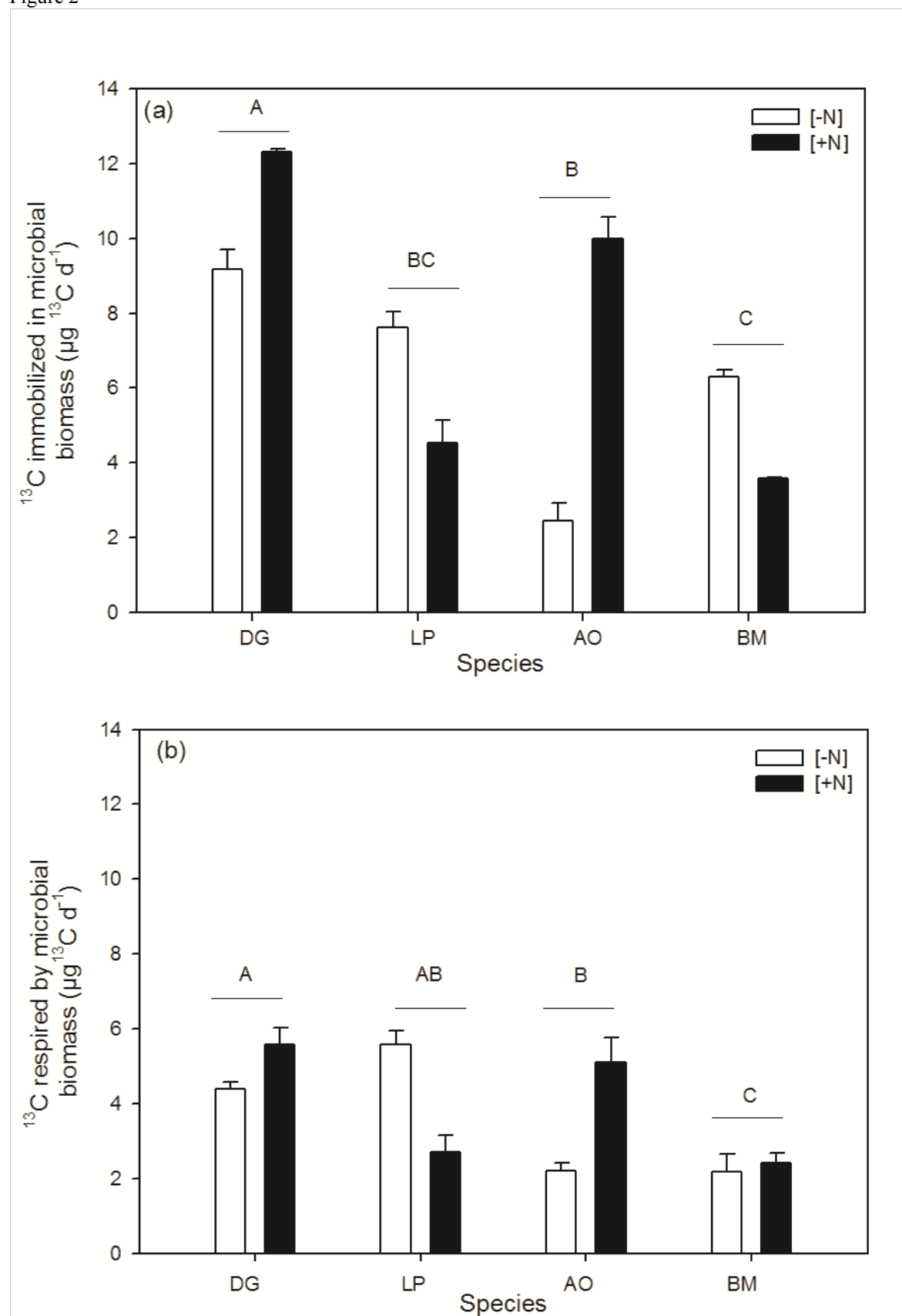


Figure 3

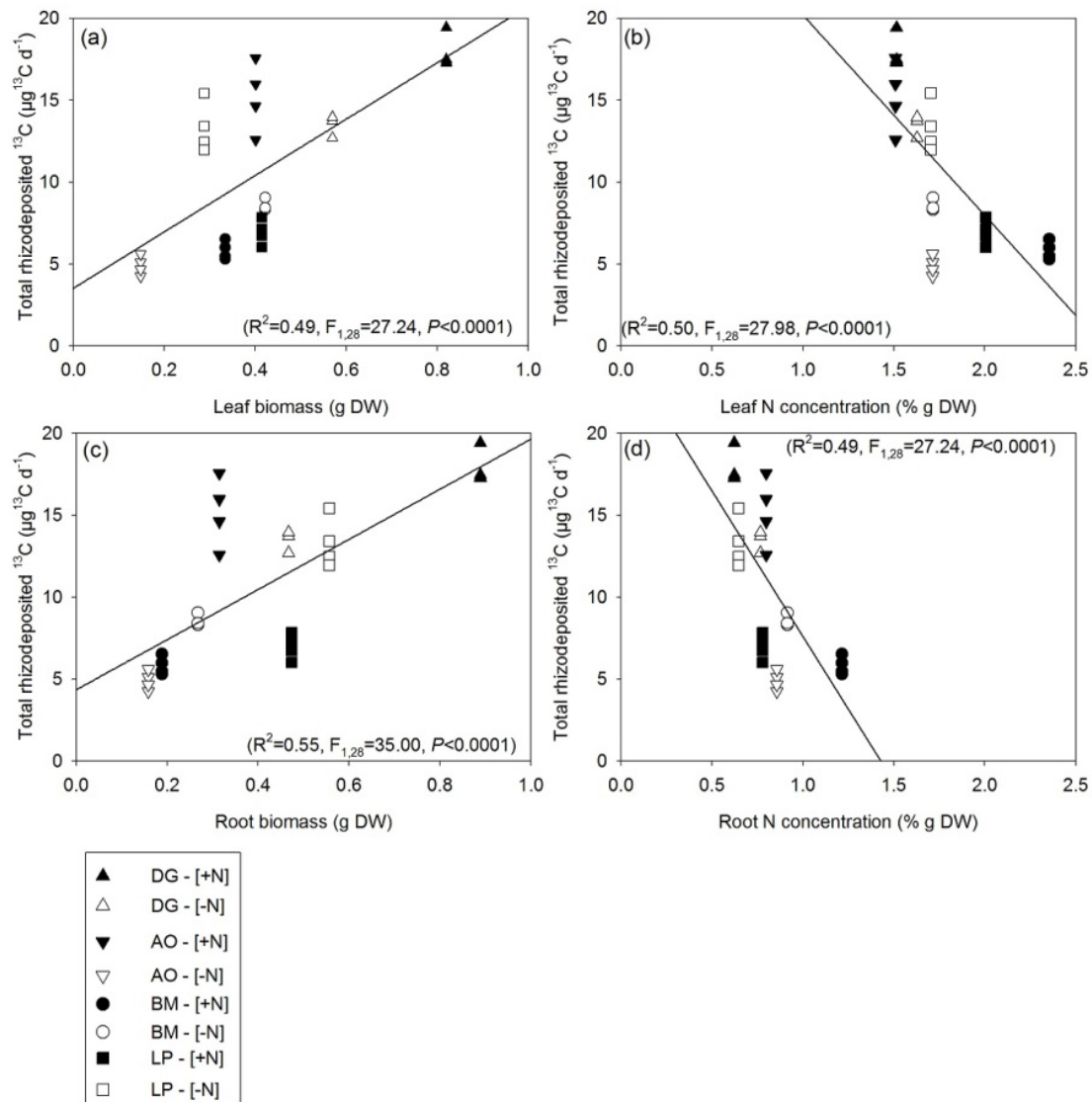


Figure 4

