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Addition of nitrogen fertiliser increases net ecosystem carbon dioxide uptake and the loss of soil organic carbon in grassland growing in mesocosms

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ABSTRACT

Maintaining and increasing soil organic carbon stocks in grasslands is essential for sustainable productivity and to offset anthropogenic carbon emissions. Direct measurements of net ecosystem carbon dioxide exchange, F_N, can be used to detect whether an ecosystem is a net sink or a source of carbon. However partitioning heterotrophic respiration, R_H, from ecosystem respiration, R_E, is needed to determine the impacts of land-use and global change on soil organic carbon stocks. We extracted intact soil cores from an intensively grazed dairy farm to establish mesocosms growing in controlled conditions and we subjected them to low and high nitrogen treatments (100 and 400 kgN ha^1 y^{-1}, respectively). After concurrent clipping and addition of nitrogen, we measured the timing for the recovery of F_N and its components, photosynthesis, A, and ecosystem respiration, R_E, by measuring them daily. Subsequently, we measured R_H from the same mesocosms seven days after the treatments were applied, using a non-disruptive, natural abundance carbon isotope technique. To test the significance of the presence of living roots when measuring R_H, we compared the results obtained from the isotopic approach to those obtained from a root exclusion technique, which involved removing the roots from the mesocosms. As the plants grew after clipping, F_N decreased (increasing net CO_2 uptake) exponentially to mean (±standard error) steady-state values of 1.11 ± 0.26 μmol m^{-2} s^{-1} (net source) and −0.19 ± 0.33 μmol m^{-2} s^{-1} (near neutral) for the low and high nitrogen treatments, respectively. When measured using the isotopic approach, R_H increased by 60%, from 1.26 ± 0.29 μmol m^{-2} s^{-1} in the low, to 2.06 ± 0.55 μmol m^{-2} s^{-1} in the high nitrogen treatment. Thus, addition of the high nitrogen resulted in an increase in soil organic carbon loss concurrently with an increase in net uptake of carbon by the ecosystem in the high nitrogen treatment compared with the low nitrogen treatment. When measured in the absence of living roots using the root exclusion technique, R_H was overall much higher than the value obtained with the isotopic technique (4.34 ± 0.13 μmol m^{-2} s^{-1}), indicating an apparent negative rhizosphere priming effect. Furthermore, when using the root exclusion technique, no difference was found between the nitrogen treatments, suggesting that the presence of roots mediated the response of heterotrophic respiration to addition of nitrogen. These results highlight the need to include measurements of changes in R_H alongside measurements of F_N in non-disturbed ecosystems to interpret the processes regulating the effects of management practices on long-term changes in soil organic carbon stocks.

1. Introduction

The terrestrial biosphere absorbs approximately one third of anthropogenic carbon emissions (Schimel et al., 2001) and is crucial for mitigating the increase in atmospheric carbon dioxide (CO_2) partial pressure and the impacts of climate change (Pachauri et al., 2014). Grazed grasslands cover 26% of the earth's ice free land surface (Steinfeld et al., 2006) and represent 70% of agriculture land (FAOSTAT, 2011). Grasslands have high inherent soil organic matter content (Miller and Donahue, 1990), which is comprised of more than 55% of carbon (Stockmann et al., 2013), and is a key factor in soil productive capacity (Jenny, 1941; Miller and Donahue, 1990). Maintaining soil organic matter in grasslands is therefore critical, for both the Earth's carbon balance and sustainable land productivity (Conant et al., 2001).

In New Zealand, conversion of dryland grazed grasslands to dairy farming is a major land-use change (MacLeod and Moller, 2006).

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Grassland ecosystems represent 30% of the total land surface area and are an important component of the national carbon budget (Trotter et al., 2004). Grazed grasslands used for dairy farming typically are intensively managed, including high stock numbers, high inputs of nitrogen fertiliser and irrigation at sites with low rainfall. Such practices are known to result in changes in the rates of soil organic matter decomposition and soil carbon stocks (Paul et al., 1996; Conant et al., 2001) but there are few studies to quantify these impacts. Using sequential measurements of soil carbon from cores across 31 sites converted to dairy farming over two to three decades, Schipper et al. (2010) observed a mean (± standard error) net loss in soil carbon of 730 ± 160 kg C ha^{-1} y^{-1}. In contrast, in a short-term two-year study to measure net ecosystem CO_2 exchange using eddy covariance, Mudge et al. (2011) estimated an increase in net carbon uptake for a dairy grassland. Eleven years after conversion of a dryland site to dairy farming using irrigation, Kelliher et al. (2014) found a 28% increase in soil carbon in the upper 0.3 m of soil compared with the change in an adjacent non-irrigated site. However, the difference in carbon content at a depth of 0.8 m was not significant. These apparently contradictory findings highlight the need for further studies to interpret the spatial and temporal complexities and environmental and management drivers of soil carbon dynamics.

The ecosystem carbon balance depends on the net ecosystem CO_2 exchange, F_N, comprising the input of carbon from photosynthesis, A, and losses from ecosystem respiration, R_E. Ecosystem respiration consists of respiration from the above-ground component of plants, R_P, and soil respiration, R_S. Soil respiration is comprised of autotrophic respiration, R_A, originating from roots and their associated mycorrhizal fungi and rhizosphere microbes, and heterotrophic respiration, R_H, from microbial decomposition of soil organic matter, such that (Amundson, 2001; Paterson et al., 2009).

$$F_N = R_E - A = R_P + R_S - A = R_P + R_A + R_H - A. \quad (1)$$

For long-term analysis of carbon balance, leaching and exported biomass can also be significant components (Soussana et al., 2007).

Estimates of F_N using eddy covariance are used widely to determine if an ecosystem is a sink or a source of carbon to the atmosphere (Baldocchi, 2008) but further detail is required to reveal the mechanisms driving changes in soil organic carbon (Kuzyakov, 2006). Soil respiration, R_S, is a major component and can account for 60–90% of R_E (Kuzyakov, 2006). The autotrophic component, R_A, represents the rapid turnover of a recently assimilated carbon pool that has only a small effect on long-term changes in soil organic carbon whereas R_H represents the slow turnover (up to millennia) of much larger carbon pools (Trumbore, 2000; Stockmann et al., 2013).

Determining differences in the drivers regulating changes in R_H and R_A is important, especially to predict changes in soil carbon stocks with changes in climate and management practices (Kuzyakov, 2006). However, partitioning R_S into its heterotrophic and autotrophic components is problematic. One difficulty is that the presence of roots can influence R_H, the so-called 'rhizosphere priming effect' (Kuzyakov, 2002). Many approaches for partitioning R_H from R_S, such as the root exclusion techniques, are based on manipulations to remove R_A, for example, using trenches to exclude roots (Buchmann, 2000; Lee et al., 2003; Jiang et al., 2005) or shading and clipping leaves (Craine et al., 1999). In a comprehensive meta-analytical review of studies designed to partition R_S into its components, Subke et al. (2006) documented a wide range in the ratio of R_H:R_S and suggested that this could be due partially to the different techniques employed. The review also highlighted potentially overlooked interactions between soil respiration components as a consequence of disrupting the ecosystem. Dungait et al. (2012) showed that the losses of soil organic carbon is regulated by microbial accessibility to soil organic matter and this is related closely to soil physical structure (Six et al., 2002). This structure can be modified by soil physical disturbance such as sieving (Zakharova et al., 2014, 2015). This suggests that attempts to partition R_H from R_S needs to be done in intact, undisturbed systems. One approach to achieve this is the use of stable carbon isotopes (Hanson et al., 2000). These methods are based on measurable differences in the ^{13}C isotopic signatures ($\delta^{13}C$) of the CO_2 emitted from R_H and R_A ($\delta^{13}CR_A$ and $\delta^{13}CR_H$, respectively). Most studies to date have used C_3/C_4 plant isotopic shifts to increase the difference between $\delta^{13}CR_A$ and $\delta^{13}CR_H$. However, such an approach is restricted to ecosystems where C_3 or C_4 plants have invaded naturally (Millard et al., 2008) or have been introduced (Uchida et al., 2010) into C_3 or C_4 systems. In pure C_3 systems, the isotopic signature of respiration from soil organic matter (SOM) turnover, $\delta^{13}CR_H$, is typically 2–4‰ enriched compared with that from the roots and associated microbes, $\delta^{13}CR_A$ (Bowling et al., 2008). Midwood et al. (2008) demonstrated that this difference can be estimated in an undisturbed C_3 system and used to partition R_S. This 'natural abundance $\delta^{13}C$' approach has been used successfully by Millard et al. (2010) and Graham et al. (2012).

Photosynthesis and R_E decrease immediately after grazing, then increase over a number of weeks as the new leaves expand (Parsons and Penning, 1988). In artificial ryegrass swards, Kuzyakov et al. (2002) showed that 80% of the carbon respired by the rhizosphere originated from recently assimilated carbon by photosynthesis. Defoliation of plants by removing photosynthetic material reduces carbon allocation below-ground (Craine et al., 1999; Kuzyakov, 2006) and thus has a strong impact on R_A (Bremer et al., 1998; Cheng and Kuzyakov, 2001). It is well known that the addition of nitrogen fertiliser to grassland increases photosynthesis and light use efficiency (Evans, 1989; Sinclair and Horie, 1989; Muchow and Sinclair, 1994) and leaf respiration (Reich et al., 2008). The effect of high nitrogen addition on R_H is more difficult to predict because no studies are available on undisturbed grasslands. However, priming effects as a consequence of added nitrogen have been observed mostly to be positive (Hart et al., 1986; Raun et al., 1998; Sembiring et al., 1998).

Our objectives in this study were to investigate the effects of clipping and application of nitrogen fertiliser on the components of the net ecosystem CO_2 exchange for an intensively grazed grassland. We extracted intact cores with soil and plants from a grassland site and grew the grass in well-watered mesocosms in controlled conditions for six months. We measured F_N, R_E, A and R_S daily after clipping and addition of nitrogen fertiliser to determine the time constants for recovery of ecosystems growing with high and low additions of nitrogen fertiliser. At the end of these measurements, we measured the effects of addition of nitrogen on R_H using natural abundance $\delta^{13}C$. Comparing the results obtained from the isotopic technique to those from a root exclusion technique, we also investigated the magnitude and direction of rhizosphere priming, at high and low nitrogen supply.

2. Materials and methods

2.1. Preparation of the mesocosms

Material for the study was collected from Beacon Farm, a commercial dairy farm on the Canterbury plains, New Zealand (lat. 43.58° S, long. 171.92° E, elevation 203 m above sea level). The site was formally a dry-land sheep farm, with low application of nitrogen fertiliser, and conversion took place four years prior to the start of our study. The site is dominated by perennial ryegrass (*Lolium perenne* L.), with minor presence of dandelion (*Taraxacum officianle* F. H. Wigg) and white clover (*Trifolium repens* L.). The soil is a shallow (0.20–0.45 m depth) stony silt loam (typic dystrustept) and well drained. The soil characteristics (mean ± standard error, n = 4) were bulk density 1.16 ± 0.03 g L^{-1}, bulk soil ^{13}C isotopic signature ($\delta^{13}C$) 27.4 ± 0.07 ‰, volumetric percentage of stones 7.8 ± 1.4%, and carbon and nitrogen concentrations 46.8 ± 0.02 and 3.4 ± 0.01 g kg^{-1}, respectively.

In May 2013, 28 intact soil cores (200 mm diameter, 300 mm depth) were sampled and placed in cylinders of PVC. The bases of

the cylinders were sealed with 1 mm mesh netting, allowing excess of water to drain. The mesocosms were placed in two growth cabinets (Model HGC 1514, Weiss Gallenkamp, UK) with constant conditions 14 °C, photoperiod 15 h and relative humidity 85%. Gaps between the soil and sides of the cylinders were filled with petroleum wax to prevent pathways for drainage of water. To prepare for the root exclusion technique, twelve mesocosms were chosen randomly for a 'bare soil' treatment, from which all plant material was eliminated by clipping covering the mesocosms with dark plastic sheets for four months. The other 16 mesocosms comprised the 'planted soil' treatment. A collar for measurements of soil respiration rates (100 mm diameter, 70 mm height) was placed to a depth of 30 mm in the centre of each mesocosm. The grass inside the collars was removed using black plastic sheets for four months, whilst allowing roots from the surrounding plants to colonise underneath the rings. Water was applied to the soil surface daily to retain soil water content near field capacity. To reproduce rotational grazing by dairy cows in field conditions, the plants in the planted mesocosms were clipped to a constant height every two weeks and the biomass was collected, dried at 65 °C for three days and weighed. The leaves were analysed for carbon and nitrogen concentrations using a Dumas elemental analyser (Europa Scientific ANCA-SL, Crewe, UK).

When the dark plastic sheets were removed, after four months, from the bare soil mesocosms, and from the central collars of the planted soil mesocosms, two nitrogen treatments were applied to both the planted and bare mesocosms, following each fortnightly clipping. The high nitrogen treatment, N_1, was supplied using 144 mL of a nutrient solution with 41 g N L^{-1} (ammonium nitrate). The low nitrogen treatment, N_0, was 144 ml of the same nutrient solution but with a lower concentration of 10 g N L^{-1} (ammonium nitrate), equivalent to approximately 400 kg N ha^{-1} y^{-1} and 100 kg N ha^{1} y^{-1}, for N_1 and N_0 treatments, respectively. The nitrogen treatments were maintained in the controlled conditions for two months prior to the start of the measurements.

Preparation of the material resulted in the establishment of 12 bare soil mesocosms, 6 for each nitrogen treatment, and 16 planted mesocosms, 8 for each nitrogen treatment.

2.2. Measurements of net ecosystem carbon dioxide exchange and soil respiration

Net ecosystem CO_2 exchange was measured on each mesocosm using a purpose-built cylindrical chamber (200 mm diameter and 210 mm height) made from polycarbonate. The bottom edge was covered with a ring of high density foam to form a seal. Measurements of CO_2 exchange in the chamber were made over a period of 70 s, using a portable photosynthesis system (LI-6400XT, LI-COR Inc., Lincoln, NE, USA) designed to work as a closed system on top of the mesocosms. The chamber had an open vent to the atmosphere with a 9 mm diameter polyethylene tube (Xu et al., 2006) to avoid pressure fluctuations (Hutchinson and Livingston, 2001; Rochette and Hutchinson, 2005) and a small fan moving the air at 50 L min^{-1} (V249L, 6V, Micronel®) was mounted inside the chamber in order to maintain well-mixed conditions. Incident irradiance (Q, 400–700 nm) was measured with a quantum sensor (Q40205, LI-COR Inc., Lincoln, NE, USA) placed at the top inside the chamber. Air temperature was measured using a thermocouple (Type E, Omega Engineering Ltd., Stamford, CT, USA) shaded from incident irradiance.

On each measurement day, F_N was measured for each mesocosm in the planted soil treatment under full irradiance (650–700 μmol m^{-2} s^{-1}). Subsequent measurements of F_N were taken at four levels of shade to establish light response curves, achieved by draping sheets of shade cloth over the chamber, and finally a dark cloth excluding all light to give a measurement of R_E. A linear equation was used to estimate light use efficiency, α, from the light response curve described by Luo et al. (2000) as

$$A_G = \alpha Q + R_E \quad (2)$$

where A_G is the rate of gross photosynthesis.

Immediately following the measurements of F_N, three replicate measurements of soil respiration rate, R_S, were made using a closed dynamic system (LI-8100, LI-COR Inc., Lincoln, NE, USA) on the central collar.

2.3. Partitioning soil respiration

Both the natural abundance $\delta^{13}C$ and the root exclusion techniques were used to partition R_H from R_S. For the root exclusion technique, R_H was assumed to be equal to the value of R_S measured from the bare soils (Craine et al., 1999). The proportion of respiration derived from heterotrophic respiration, fR_H, was estimated by comparison of mean values of respiration fluxes between planted soils and bare soils. The natural abundance $\delta^{13}C$ technique requires the measurement of ^{13}C isotopic signatures of the CO_2 respired from the undisturbed soil (soil efflux), $\delta^{13}CR_S$, from roots and associated microorganisms, $\delta^{13}CR_A$, and from soil organic matter decomposition, $\delta^{13}CR_H$. The rate of heterotrophic respiration, R_H, and fR_H are calculated using a mass balance approach (Lin et al., 1999; Millard et al., 2010) where

$$fR_H = 1 - \left(\left(\delta^{13}CR_S - \delta^{13}CR_H\right) / \left(\delta^{13}CR_A - \delta^{13}CR_H\right)\right) \quad (3)$$

and

$$R_H = fR_H \times R_S. \quad (4)$$

Simultaneous measurements of $\delta^{13}CR_S$ from six mesocosms were made by collecting air respired from the soil surface using a partially automated open chambers system described by Midwood et al. (2008). The chambers were placed on the rings in each mesocosm and approximately 500 ml of respired air was collected after approximately 90 min of equilibration (Midwood et al., 2008; Millard et al., 2010) into bags (Tedlar® Keika Ventures, Chapel Hill, NC, USA) that were flushed twice with CO_2 free air and evacuated prior to use.

Measurements of the isotopic signatures of the end members for root-free soil, $\delta^{13}CR_H$, and roots, $\delta^{13}CR_A$, were made following Millard et al. (2010). After the soil surface CO_2 efflux sample had been collected, samples of roots and soils were collected from each mesocosm. A steel tube identical in diameter of the collars was hammered into the soil to a depth of 200 mm. The presence of large stones in some of the mesocosms prevented sampling deeper than 170–180 mm and this was taken into account in the statistical analysis. The soil from the core was broken up loosely and roots were removed by hand. Samples of root-free soil and roots were placed in separate Tedlar® bags which were sealed and flushed three times with CO_2 free air, then filled with approximately 500 ml of CO_2 free air. An aliquot of gas was removed and the CO_2 partial pressure checked to make sure it fell within the range of 300–700 μmol mol^{-1} needed to ensure optimum precision for the isotope analysis. The concentration was then adjusted if needed, by either adding CO_2 free air or extending the period of incubation. Incubations were kept to the minimum time possible (typically 5 to 7 min for the root-free soils, and 20 min for the roots) to minimise shifts in $\delta^{13}C$ values caused by a switch to different carbon substrates due to root death or physical disturbance of the soil (Millard et al., 2008; Zakharova et al., 2014). All gas samples were analysed for $\delta^{13}C$ values using a cavity ringdown spectrometer (G2121-I, Picarro Inc., Santa Clara, CA, USA).

2.4. Experimental design

The experimental period consisted of two phases. For the first phase we selected randomly ten planted mesocosms, five for each nitrogen treatment. To estimate the effect of clipping and addition of nitrogen on the recovery of ecosystem CO_2 exchange components, we made daily measurements of F_N, R_S and α during 19 days after concurrent clipping and nitrogen additions. To disentangle the effects of clipping and adding nitrogen, the grass was subsequently clipped without nitrogen addition and F_N, R_S and α were measured daily for 9 days. On the tenth day after clipping, nitrogen was applied, without clipping and F_N, R_S and α were measured daily for 12 days.

The second phase consisted of measuring heterotrophic soil respiration, R_H, using the natural abundance $\delta^{13}C$ and the root exclusion technique. All 28 mesocosms were used. Just after the first phase was completed, the treatments applied were: concurrent clipping and nitrogen addition for the planted mesocosms, and nitrogen addition to the bare mesocosms. Measurements to determine R_H were made on day 7 after treatment application. For the root exclusion technique, measurements were contained within 1 h. For the natural abundance technique, measuring R_H from 16 mesocosms required 8 h of the daylight period, during which potential variations of $\delta^{13}CR_S$ were checked using two mesocosms selected randomly for repeated sampling. In addition, four root samples and four root-free soil samples were selected randomly for longer incubation times, up to 2.5 h, in order to estimate the effect of length of incubation on $\delta^{13}CR_H$ and $\delta^{13}CR_A$.

For each mesocosm, soil water content, Θ_S, (Model SM300, Delta-T Devices Ltd., Cambridge, UK) and soil temperature, T_S, (Model HH 603A, Omega Engineering Ltd., Stamford, CT, USA) were measured at a depth of 50 mm daily. After all the measurements were completed, soil and roots samples were collected, dried, ground to a powder in a ball mill and analysed for carbon and nitrogen concentrations using a Dumas elemental analyser (Europa Scientific ANCA-SL, Crewe, UK).

2.5. Statistical analyses

Changes in F_N, R_S, R_E and α after concurrent clipping and adding nitrogen fertiliser, clipping alone and adding nitrogen fertiliser alone, were tested using non-linear mixed-effect models conducted in the 'nlme' package of R version 3.2.1 (Pinheiro et al., 2014). Each measurement of F_N, R_S, R_E and each calculated value of α was treated as a sample. To account for non-independence of repeated measurements, replicate number was included as a random effect in each model. F_N and α were modelled as common asymptotic exponential functions of number of days after treatment (n) (Crawley, 2007) as

$$F_N = a + b\exp.(-cn) \quad (5)$$

where a is the steady-state value of F_N, b is the difference between a and the value of F_N at day 0 and c characterises the shape of the curve, and

$$\alpha = p(1-\exp.(-qn)) \quad (6)$$

where p is related to the initial value and to the steady-state value of α and q characterises the shape of the curve.

To characterise the time constants for the recovery of α and F_N, the number of days to reach 95% of the changes (n_{95}) was calculated from p and c respectively as

$$n_{95} = \ln(1/0.05)/X \quad (7)$$

where X represents p and c for the functions for α and F_N, respectively.

R_E was observed to decrease after grazing for a period of three days before starting to increase. To capture this initial decrease, a 3rd degree polynomial function was tested to model R_E. R_S was modelled as a linear function of n. Models with different coefficients for the high nitrogen treatment, N_1, and the control treatment, N_0, were compared with models fixing the same coefficients for N_1 and N_0. A top-down stepwise regression approach was used to model fR_H and R_H. For the natural abundance technique, fR_H was modelled as a function of root and soil carbon and nitrogen concentrations, R_S, nitrogen treatment and core sampling depth. R_H was modelled similarly without the inclusion of R_S. For the root exclusion technique, R_H was modelled as a function of soil carbon and nitrogen concentrations and nitrogen treatment. Soil temperature, T_S and soil water content, Θ_S, at a depth of 50 mm were also included. Model comparisons were based on Akaike's Information Criterion (AIC), the model with the lowest AIC being the most strongly supported, following Burnham and Anderson (2002). As a rule of thumb, models with ΔAIC < 2 were also considered to be strongly supported (Burnham and Anderson, 2002). Treatment values of R_H obtained with the two techniques were compared with a Student t-test. The effects of addition of nitrogen on root and soil carbon and nitrogen concentrations were assessed using analysis of variance (ANOVA). Analyses of specific leaf area and leaf nitrogen concentration over time included replicate number as a random effect and was assessed with linear modelling. Differences in T_S and Θ_S between nitrogen treatments and measurement phases were assessed using analysis of variance. Analyses of residuals were undertaken to check on model assumptions, including independence from T_S and Θ.

3. Results

3.1. Soil temperature and soil water content

Air temperature in the controlled environment cabinets remained constant to within 1 °C of the set point, but soil temperature, T_S, increased during the day due to radiation loading from the lamps (21.6 ± 0.07 °C, range 19.4–25.8 °C). There was no significant difference in T_S for the mesocosms in the two nitrogen treatments ($P = 0.22$). Mean volumetric soil water content, Θ_S, was 43.3 ± 0.2% and ranged from 33.4 to 53.4%. For the N_1 treatment, mean Θ_S (42.0 ± 0.3%) was significantly lower than the value for the N_0 treatment (44.6 ± 0.3%) ($P < 0.001$). T_S and Θ_S were not significantly different between the measurement phases.

3.2. Soil, root and leaf properties

For both the planted and bare soil treatments, there were no differences in soil carbon and nitrogen concentrations between the N_0 and N_1 treatments (Table 1). Root nitrogen concentration in the N_1 treatment was higher than that of the N_0 treatment (Table 1) but the difference was not significant ($P = 0.09$). No differences were found in root carbon concentrations ($P = 0.7$). Accordingly, no differences were found in the C:N ratios for soil and roots between the treatments (Table 1). A linear model best described cumulative leaf dry mass with time (Fig. 1) with different slopes for the N_0 and N_1 treatments (0.118 ± 0.003 and 0.169 ± 0.005 g day^{-1}, respectively), and with the same initial values not significantly different from 0. This resulted in higher cumulative leaf dry mass for the N_1 treatment (27.61 ± 1.68 g) compared with the value for the N_0 treatment (19.60 ± 2.05 g) at the end of the

Table 1
Soil and root carbon and nitrogen concentrations and C:N ratios at the end of the experiment for the low, N_0, and high nitrogen, N_1, treatments. All values shown are mean ± standard error.

	Planted soils		Bare soils	
	N_0	N_1	N_0	N_1
Root nitrogen (g kg^{-1})	7.8 ± 0.3	8.7 ± 0.4	–	–
Root carbon (g kg^{-1})	238.3 ± 14	228.1 ± 22	–	–
Root C:N	30.7 ± 2.3	26.3 ± 2.9	–	–
Soil nitrogen (g kg^{-1})	3.2 ± 0.1	3.3 ± 0.1	3.2 ± 0.1	3.2 ± 0.1
Soil carbon (g kg^{-1})	37.8 ± 0.7	38.0 ± 0.8	38.1 ± 0.8	37.6 ± 0.5
Soil C:N	11.6 ± 0.1	11.7 ± 0.3	11.9 ± 0.1	11.7 ± 0.1

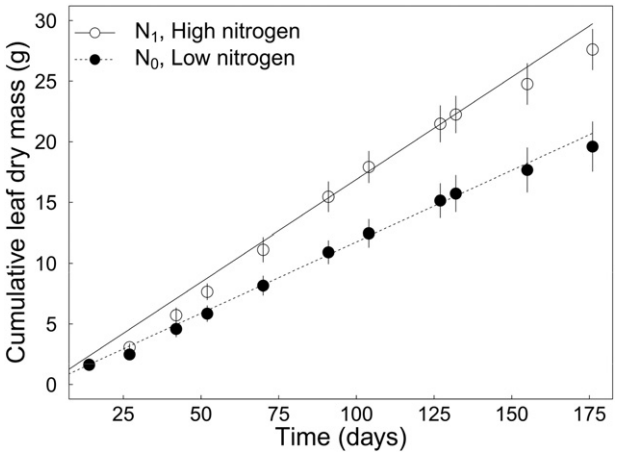

Fig. 1. Cumulative leaf dry mass with time throughout the experiment for the low, N_0, and high, N_1, nitrogen treatments. The vertical bars show standard errors of the mean.

experiment ($P = 0.02$). No significant changes were measured in specific leaf area throughout the experiment (Table 2) for the N_0 and N_1 treatments ($P = 0.3$ and $P = 0.9$, for nitrogen treatment and date of the clipping event, respectively). Leaf nitrogen concentration (Table 2) showed significant variability with sampling date and nitrogen treatment ($P < 0.001$ and $P = 0.02$, respectively). Mean nitrogen concentration was higher for leaves in the N_1 treatment compared with the value for leaves from the N_0 treatment (24.2 ± 0.4 and 21.2 ± 0.6 g kg^{-1} respectively). The overall leaf nitrogen concentration on the last clipping event was significantly lower (by 2.3 ± 0.5 g kg^{-1}) than that for all values during the measurement period.

3.3. Net ecosystem carbon dioxide exchange and light use efficiency

The estimated parameters describing changes in F_N (a, b and c) and α (p and q) as functions of number of days after treatment, n, are shown in Table 3. The best model describing the change in F_N after concurrent clipping and nitrogen addition was an exponential decrease (Eq. (5)) to a steady-state value becoming a small net sink of carbon, near neutral ($a = -0.19 \pm 0.33$ μmol m^{-2} s^{-1}) for the N_1 treatment, while remaining a net source of carbon for the N_0 treatment (Fig. 2). Changes in F_N were also greater in magnitude for the N_1 treatment than for the N_0 treatment (e.g., the estimated value of b was higher for the N_1 treatment). The shapes of the curves were not statistically different and 95% of the changes occurred within the first seven days for both the N_1 and N_0 treatments ($n_{95} = 7.1$ days). The best model describing the response of α after clipping and addition of nitrogen was an increasing exponential function (Eq. (6)) with the steady-state value for the N_1 treatment being higher than the value for the N_0 treatment. The shapes of the curves were not significantly different between nitrogen treatments and 95% of the change, n_{95}, occurred in 9.1 days. Asymptotic exponential functions best described changes of F_N and α after clipping alone and addition of nitrogen fertiliser alone. There were no significant differences in the response of N_1 and N_0 treatments for measurements made after clipping alone, neither for F_N nor for α. Significant differences appeared after adding nitrogen alone, where the value for c for the N_0 treatment was very close to 0, suggesting there was almost no further decrease in F_N after addition of the N_0 treatment. For the N_1 treatment the system became a small net carbon sink within three days after addition of nitrogen alone ($n_{95} = 2.6$ days), reaching a similar steady-state value to that for the measurements made after concurrent clipping and nitrogen addition. The estimated steady-state value of α following addition of nitrogen only was higher for the N_1 treatment than for the N_0 treatment, but the shapes of the curves were not statistically different, with $n_{95} = 1.7$ days.

3.4. Ecosystem respiration and soil respiration

Changes in R_E with time were best described by a 3rd degree polynomial function. Values of R_E following concurrent clipping and nitrogen addition were higher for the N_1 treatment than the N_0 treatment except for the initial value, for which the difference was not statistically significant (Fig. 3). There was a small decrease in R_E on the first two days after clipping alone, and this was not significantly different for the N_1 and N_0 treatments. R_E was nearly constant after addition of nitrogen alone, except for the N_1 treatment which slightly increased from $n = 5$ days.

Changes in R_S after concurrent clipping and nitrogen addition were linear (Fig. 3) with the same initial value (6.38 ± 0.30 μmol m^{-2} s^{-1}) but differences in slopes for the nitrogen treatments. This was also the case for measurements made after clipping alone and addition of nitrogen alone, with initial values of 5.45 ± 0.23 and 5.67 ± 0.28 μmol m^{-2} s^{-1}, respectively. However, absolute values of the estimated slopes were all below, or very close to, the instrument detection limits of 0.02 μmol m^{-2} s^{-1} day^{-1}, with the maximum slope value of −0.04 ± 0.02 (μmol m^{-2} s^{-1}) day^{-1} for the N_1 treatment after only clipping.

For the measurements made after concurrent clipping and nitrogen addition, a trend was observed in the residuals as a function of T_S. A new model was thus fitted including T_S and this resulted in an improvement (lower AIC and independence of the residuals). There was a positive linear effect of T_S on R_S with a slope of 0.30 ± 0.03 (μmol m^{-2} s^{-1}) $°C^{-1}$.

3.5. Partitioning soil respiration using the natural abundance $\delta^{13}C$ technique

During the 8 h of measurements, there were no changes in the values of $\delta^{13}CR_S$ from the two test mesocosms ($P = 0.29$). $\delta^{13}CR_A$ from four mesocosms did not vary over a 2.5 h incubation period ($P = 0.7$). Changes in $\delta^{13}CR_H$ over the incubation time followed an exponential decay function (Eq. (4)) with values for $a = -28.89 \pm 0.24$‰, $b = 0.037 \pm 0.012$‰ and $c = 4.05 \pm 0.40$ day $‰^{-1}$. For one mesocosm from the 16 measured, $\delta^{13}CR_A$ was close to, but slightly enriched compared with $\delta^{13}CR_S$, suggesting that variability associated with measurements resulted in no difference between $\delta^{13}CR_S$ and $\delta^{13}CR_A$, so the value of fR_H for that replicate was constrained to 0.

Values of $\delta^{13}CR_S$, $\delta^{13}CR_A$ and $\delta^{13}CR_H$ were not significantly different between nitrogen treatments. Overall, mean values of $\delta^{13}CR_S$ were 3.6 ± 0.3‰ more depleted than the values for $\delta^{13}CR_H$ and 1.8 ± 0.4‰ more enriched than the values for $\delta^{13}CR_A$ (Table 4).

Table 2
Leaf nitrogen concentration and specific leaf area at different times throughout the measurement period when the grass was clipped. The mention 'NA' indicates non available data. All values shown are mean ± standards error.

Days since start of the experiment	Days since clipping	Leaf nitrogen (g kg^{-1})		Specific leaf area (m^2 kg^{-1})	
		N_0	N_1	N_0	N_1
14	14	22.4 ± 0.9	23.7 ± 0.8	292.0 ± 55.9	228.6 ± 64.9
27	14	22.6 ± 0.7	25.2 ± 0.8	NA	NA
104	13	21.7 ± 0.8	24.9 ± 0.5	269.3 ± 7.3	251.9 ± 12.2
127	23	18.3 ± 1.2	22.7 ± 0.8	209.0 ± 10.6	251.9 ± 15.4

Table 3
Estimated values of the parameters of modelled net ecosystem CO_2 exchange, F_N (a, b and c), and light use efficiency, α (p and q) for the low, N_0, and high nitrogen, N_1, treatments, after concurrent addition of nitrogen and clipping, clipping alone and addition of nitrogen alone. Values of parameters centred are not significantly different between the nitrogen treatments. All values shown are mean ± standards error.

Model	Parameter		Clipping + addition of nitrogen		Clipping alone		Addition of nitrogen alone	
			N_0	N_1	N_0	N_1	N_0	N_1
F_N	a	(μmol m^{-2} s^{-1})	1.11 ± 0.26	−0.19 ± 0.33	2.05 ± 0.25		−1.36 ± 0.53	−0.22 ± 0.49
	b	(μmol m^{-2} s^{-1})	4.87 ± 0.63	7.42 ± 0.87	3.63 ± 0.31		2.85 ± 0.44	
	c	((m^2 s μmol^{-1}) day^{-1})	0.42 ± 0.04		0.55 ± 0.09		0.03 ± 0.02	0.66 ± 0.17
α	p	(μmolC μmol $quanta^{-1}$)	0.018 ± 0.001	0.023 ± 0.001	0.011 ± 0.001		0.013 ± 0.001	0.017 ± 0.001
	q	((μmol quanta $\mu molC^{-1}$) day^{-1})	0.33 ± 0.05		0.24 ± 0.04		1.75 ± 0.3	

The best model describing fR_H and R_H included nitrogen treatment, soil core depth and T_S. The estimated effect of T_S was small (0.04 ± 0.02 $°C^{-1}$ and 0.31 ± 0.16 μmol m^{-2} s^{-1} $°C^{-1}$ for fR_H and R_H, respectively). Values of fR_H were higher when the presence of stones prevented the core from being sampled to a depth of 200 mm. This resulted in an increase in R_H of 1.69 ± 0.37 μmol m^{-2} s^{-1}. This occurred in 3 and 4 mesocosms for the N_1 and N_0 treatments, respectively. Thus, there was no co-variation between soil core depth and nitrogen treatment. The value of fR_H was significantly higher for the N_1 treatment (Table 4). This resulted in a higher value of R_H for the N_1 treatment (2.06 ± 0.55 μmol m^{-2} s^{-1}) compared with the value for the N_0 treatment (1.26 ± 0.29 μmol m^{-2} s^{-1}) (Fig. 4).

3.6. Root exclusion technique and method comparison

From the measurements using the bare soil mesocosms, R_H increased linearly with the soil C:N ratio and T_S (Fig. 3), but there were no significant differences between the nitrogen treatments (Fig. 4). R_H was 4.34 ± 0.13 μmol m^{-2} s^{-1}, which was significantly higher than the mean value of R_H measured with the natural abundance isotope technique ($P < 0.001$) (Fig. 3). The overall mean value for fR_H from the root exclusion technique (0.86) was also higher than the overall mean value estimated using the natural abundance $\delta^{13}C$ approach ($fR_H = 0.33$).

4. Discussion

This study contributed new insights to carbon cycling in managed grasslands by integrating measurements of net ecosystem CO_2 exchange and its components with measurements to partition R_H from R_S in undisturbed mesocosms. We showed that the increase in net ecosystem CO_2 uptake (decrease in F_N) with the addition of high concentration of nitrogen fertiliser masked a smaller, concomitant increase in soil organic matter decomposition (increase in R_H). The additional carbon input to the system was at least partly allocated to above-ground biomass, as shown by the greater cumulative biomass in the N_1 treatment compared with N_0 treatment.

4.1. Net ecosystem CO_2 exchange

Our findings highlight a strong increase in ecosystem CO_2 uptake (decrease in F_N) to a steady-state value with increasing time after clipping that was associated strongly with increasing light use efficiency, α. The addition of high nitrogen resulted in a smaller steady-state value of F_N and higher steady-state value of α, but time constants for

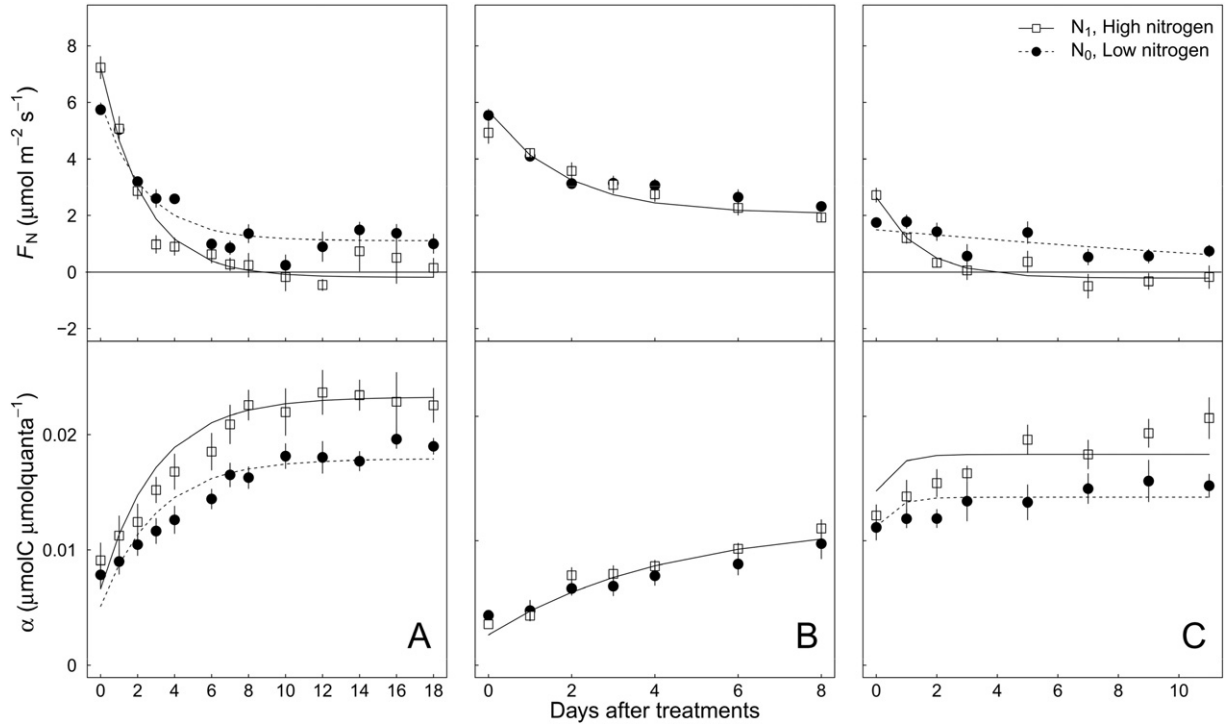

Fig. 2. Net ecosystem CO_2 exchange, F_N, and light use efficiency, α, modelled as a function of number of days after (A) clipping and addition of nitrogen, (B) clipping only and (C) addition of nitrogen only for the low, N_0, and high, N_1, nitrogen treatments. The vertical bars show standard errors of the mean. A single solid line is used when the model does not differ significantly between the nitrogen treatments.

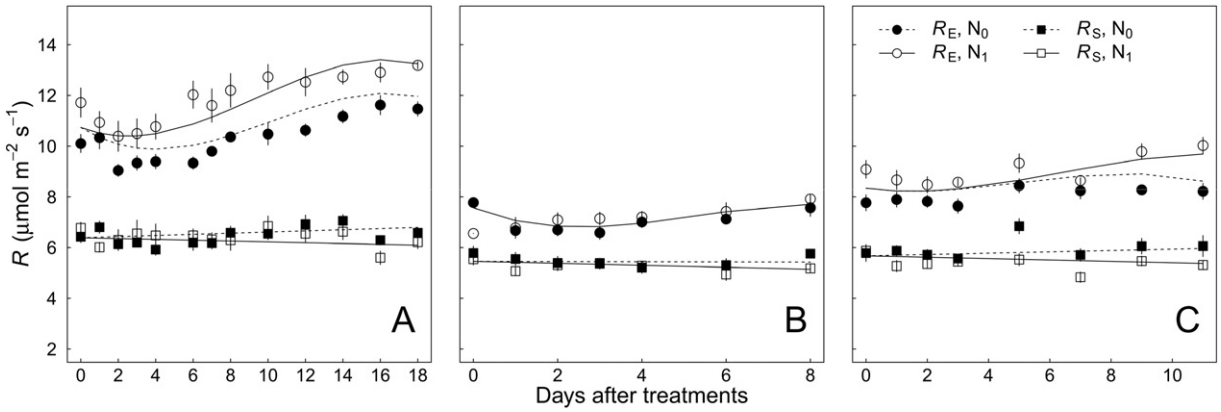

Fig. 3. Soil respiration, R_S, and ecosystem respiration, R_E, modelled as a function of number of days after (A) concurrent clipping and addition of nitrogen, (B) clipping alone and (C) addition of nitrogen alone, for the low, N_0, and high nitrogen, N_1, treatments. The vertical bars show standard errors of the mean. A single solid line is used when the model does not differ significantly between nitrogen treatments.

the responses were similar for both nitrogen treatments. This demonstrates that addition of high nitrogen resulted in an increase in α, leading to more efficient conversion of intercepted irradiance into photosynthesis that exceeded the increase in ecosystem respiratory losses, R_E. This resulted in a greater net ecosystem carbon uptake for the high nitrogen treatment, N_1, compared with the control treatment, N_0. The additional cumulative biomass in response to added nitrogen was attributable mainly to increased canopy photosynthesis. The resulting similar specific leaf area but higher leaf nitrogen concentration in the N_1 treatment compared with values for the N_0 treatment also suggests that photosynthesis per unit leaf area was enhanced by increasing Rubisco activity associated with leaf nitrogen concentration (Friend, 1991). Consistent with other studies, the effects of adding high nitrogen increased rates of photosynthesis more than leaf respiration (Field and Mooney, 1986). The lack of a difference in soil and root nitrogen concentration between the nitrogen treatments suggests that most of the added nitrogen was utilised by the plants for above-ground biomass growth.

In our study, as the changes in soil respiration, R_S, with time and between the treatments were very small, differences in R_E were dominated by changes in leaf respiration rates. Ourry et al. (1988) showed that regrowth of perennial ryegrass after clipping can be described by two physiological phases. During the first six days, nitrogen supply to leaves is derived from remobilisation from reserves in roots and stubble, then nitrogen is supplied by root uptake from the soil. We interpret the limited response of *A* and biomass growth in the treatment where leaves were clipped without adding nitrogen to the exhaustion of nitrogen root reserves during the few days following the treatment. When nitrogen was added without clipping during the second phase of development, there was a rapid stimulation in *A* that was larger than the proportional increase in leaf respiration rate, resulting in enhanced biomass production. Moreover, high nitrogen supply has also been shown to reduce the initial rate of nitrogen remobilisation and uptake by roots (Millard et al., 1990). Atkinson (1986) demonstrated an increase in leaf respiration rate within 20 h after defoliation in sheep fescue (*Festuca ovina* L.) and a similar observation was made in tobacco leaves (*Nicotiana tabacum* L.) in the few hours following defoliation (Macnicol, 1976), attributed to a wounding response. Increases in respiration per unit leaf area following defoliation could explain the initial higher values of R_E in the first two days after clipping in our mesocosms.

4.2. Components of soil respiration

The exponential increase in R_S resulting from increasing T_S is well documented (Lloyd and Taylor, 1994; Davidson et al., 2000; Brown et al., 2009) and maximum values of R_S are associated with values of Θ_S near field capacity (Davidson et al., 2000). Brown et al. (2009) observed mean rates of R_S in a ryegrass-dominated grassland in New Zealand of around 3 μmol m^{-2} s^{-1} in field conditions, roughly half of the measured values from our controlled environment conditions. This suggests that, in our study, T_S and Θ_S were not limiting for R_S. Furthermore, the small variations in T_S and Θ_S did not affect R_S, R_A and R_H significantly.

Several studies have demonstrated a strong decrease in R_S following clipping. Bremer et al. (1998) showed a decrease of 20 to 50% in R_S in the first two days after clipping in a tallgrass prairie and Cheng and Kuzyakov (2001) observed a decrease of 50% in R_S from wheat mesocosms after a shading treatment was applied. In our study, the

Table 4
Carbon isotopic signatures for air collected from soil respiration, $\delta^{13}CR_S$, incubation of root-free soil, $\delta^{13}CR_H$, and incubation of roots, $\delta^{13}CR_A$, and calculated values of the proportion of soil respiration resulting from heterotrophic respiration, fR_H, for the low, N_0, and high nitrogen, N_1, treatments. The asterisk indicates a significant difference in values between nitrogen treatments. All values shown are mean ± standard error.

		N_0	N_1
$\delta^{13}CR_S$	(‰)	−29.37 ± 0.23	−29.34 ± 0.26
$\delta^{13}CR_A$	(‰)	−30.83 ± 0.48	−31.45 ± 0.49
$\delta^{13}CR_H$	(‰)	−25.41 ± 0.18	−26.27 ± 0.45
fR_H		0.26 ± 0.06*	0.39 ± 0.10*

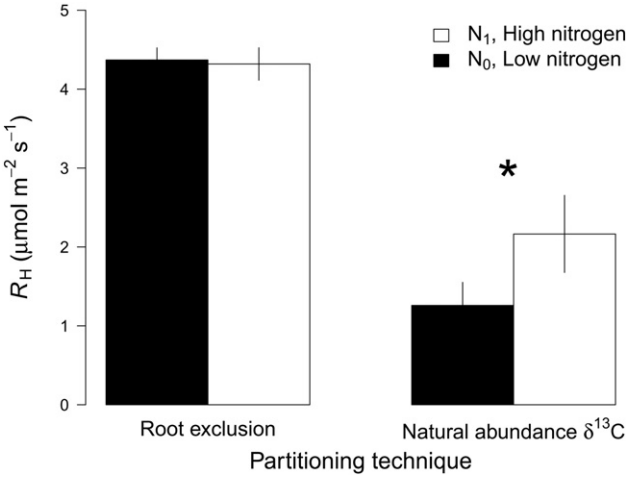

Fig. 4. Rates of soil heterotrophic respiration, R_H, on day 7 after concurrent clipping and addition of nitrogen using two partitioning techniques, for the low, N_0, and high, N_1, nitrogen treatments. The vertical bars represent standard errors of the mean. The asterisk indicates a significant difference in values between nitrogen treatments.

response of R_S was insignificant in comparison. Kuzyakov (2002) cites numerous studies that have shown negative rhizosphere priming effects where the presence of plant roots decreases decomposition rates of soil organic matter by 10 to 30%. One mechanism proposed to explain negative rhizosphere priming effects is competition for nutrients between living roots and soil microorganisms (Jingguo and Bakken, 1997; Bottner et al., 1999). The review by Wang and Fang (2009) highlights that the short-term effects of clipping on R_S are attributable to the physiological response of plants. It is likely that clipping reduced carbon allocation below ground (Craine et al., 1999; Kuzyakov, 2006) resulting in reduced root and rhizosphere activity. A decrease in root activity as a consequence of clipping in our mesocosms would have led to reduced competitiveness of the rhizosphere and therefore to a proportional increase in R_H, with no net effect on R_S.

Using the isotope natural abundance technique, our data suggest that high nitrogen supply resulted in increased fR_H and R_H. This result is supported by several studies showing increases in soil organic matter decomposition rates with the addition of nitrogen (Hart et al., 1986; Raun et al., 1998; Sembiring et al., 1998). A competition mechanism involved in the rhizosphere priming would also explain this result. Millard et al. (1990) found that addition of high nitrogen reduced ryegrass root biomass. Other studies in grasslands observed a decrease in R_S due to reduced carbon allocation below-ground as a result of nitrogen addition (Jong et al., 1974; Ammann et al., 2007). Although we were not able to measure root biomass directly, a reduction in biomass in response to high nitrogen supply seems like a reasonable assumption. By reducing root activity in the planted soils, addition of nitrogen would have enhanced competitiveness of the soil microorganisms, therefore increasing R_H.

The higher estimates of R_H using the root exclusion technique compared with the isotope approach in our study could be attributable partly to the presence of remnant decaying roots. Nakane et al. (1996) and Craine et al. (1999) showed that the presence of decaying roots could be responsible for increases in R_S by up to 20% in a forest ecosystem. While this could account for some of the difference in estimates of R_H between the two techniques we used, this would not amount to the 50% difference that we observed. The most plausible explanation is that the difference is attributable to the existence of a negative rhizosphere priming effect. This finding supports our use of the natural abundance $\delta^{13}C$ technique to estimate R_H in undisturbed systems where plants are growing in soil and indicates that the root exclusion technique introduces bias in estimates of R_H. In a similar study to ours, Chen et al. (1996) showed that ryegrass roots alone (separated from the rhizosphere) accounted for between 49 and 58% of R_S, which is closer to the result we obtained from the natural abundance $\delta^{13}C$ technique (68% for root and the rhizosphere). This supports the validity of our use of the natural abundance $\delta^{13}C$ technique.

5. Conclusions

The larger net ecosystem CO_2 uptake (decrease in F_N) associated with addition of high nitrogen in our study was concurrent with an increase in soil organic matter decomposition, R_H. Our data strongly support the existence of a negative rhizosphere priming effect on soil organic matter decomposition. Based on these observations, we conclude that (i) measuring F_N and its components ecosystem respiration, R_E, and photosynthesis, A, alone can be misleading when trying to predict long-term changes in soil organic carbon stocks, and (ii) when making measurements to partition the components of soil respiration in response to treatments, it is important to use non disturbed systems. This can be achieved using the natural abundance $\delta^{13}C$ technique.

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