Plant and Soil (2005) 268: 233–242
DOI 10.1007/s11104-004-0277-5
© Springer 2005

Sucrose application, soil microbial respiration and evolved carbon dioxide isotope enrichment under contrasting land uses

F.M. Kelliher[1,2], M.M. Barbour[1] & J.E. Hunt[1]
[1]*Manaaki Whenua – Landcare Research, P.O. Box 69, Lincoln, New Zealand.* [2]*Corresponding author**

Received 1 October 2003. Accepted in revised form 10 May 2004

Key words: carbon isotope, forest, grazed pasture, soil respiration, sucrose

Abstract

Heterotrophic decomposition of organic matter dictates that substrate supply rate, including energy and nutrients, can limit soil microbial activity. In New Zealand, soils are naturally deficient in nitrogen and phosphorus. Fertiliser application is a part of pastoral agriculture, the country's most widespread land use. We postulated that organic soils under grazed pasture and pristine forest would be at the extremes of substrate quality and supply rate, and thus potential microbial response to food opportunities. Soil microbial responses to the addition of fresh energy (sucrose) were determined by laboratory experiments with root-free samples and intact cores including roots. Responses were quantified by respiration and respired carbon (C) isotope (δ^{13}C) enrichment measurements. A supra-trace sucrose dose (0.002 mol kg^{-1} (soil)) caused the forest soil's microbial respiration rate to nearly double within 2 h. The peak response took 20 h, and saturation occurred beyond a sucrose dose of 0.05 mol kg^{-1} (soil). Intact soil cores from the forest had similar respiration rates and responses. For root-free soil samples from the grazed pasture, respiration response to sucrose was nearly immediate, dose dependent, and there was up to a 9-fold increase in the rate. Intact cores from the pasture had much higher respiration rates, but a similar response to sucrose. For both soils, the similarity of sucrose application effects on respiration and relative δ^{13}C enrichment of the respired carbon was striking.

Introduction

The poor quality of carbon generally limits the amount of energy available to soil microbes (Fontaine et al., 2003; Vance et al., 2001). These microbes are, however, poised in a state of '*metabolic alertness*' to seize every food opportunity (De Nobili et al., 2001). Addition of organic matter to soils has long been used to examine the substrate limitation to soil microbial activity (e.g., Bingeman et al., 1953; Löhnis, 1926), and more recently the fate of added carbon has been traced using isotope techniques (e.g., Hobbie et al., 2003; Högberg and Ekblad, 1996). Tracer experiments sometimes suggest an increase in the soil organic matter decomposition rate after addition of fresh organic matter such as simple sugars, green manure or straw. The mechanisms of this so-called priming effect are poorly understood, but recent work (Fontaine et al., 2003) suggests that competition between microbes specialised in decomposition of fresh organic matter and those feeding on polymerised soil organic matter may explain the wide variability of responses reported. Fontaine et al. (2003) highlight the importance of nutrient availability in determining microbial response to added carbon, a point raised by DeNobili et al. (2001) and investigated by Ekblad and Nordgren (2002).

Energy and nutrient availabilities to soil microbes can vary widely with land use and management. Ecosystem productivity and sustainability are at stake, and although increasingly rare, pristine sites are valuable for comparison (Price et al., 2003; Vance and Chapin, 2001). In New Zealand, soils are naturally deficient in nitrogen and phosphorus (McLaren and Cameron, 1996). For the country's second most widespread land use, native forest over 6 M ha, soils are mostly pristine with a high carbon content near the

*FAX No: 64-3-325-3607.
E-mail: KelliherF@LandcareResearch.Co.NZ

surface and a significant, stable humus fraction (Tate et al., 1993). For New Zealand's primary land use of pastoral agriculture (over 13 M ha), carbon content is also high near the surface. These soils have often been amended with phosphorous and nitrogen fertilisers, although legumes in the pasture are also used for nitrogen fixation. Moreover, for these soils, up to around 85 million grazing animals ingest, excrete and thus regularly cycle vast quantities of carbon and nitrogen.

For this paper, we compare the microbial response to food opportunities of two soils representing extremes of substrate quality and supply rate. Microbes in soil beneath a grazed pasture, with high nutrient availability and substrate quality, were expected to be quickly and highly responsive to the addition of a simple carbohydrate. Slower and lesser responses to such addition was expected of microbes in soil beneath a pristine forest, with naturally low fertility and poorer quality substrate. To test this hypothesis, we sampled organic soils at two sites that were formed beneath swamp forest vegetation. The natural forest has remained pristine for about 12,000 years at one site, while the other was drained and developed for grazed pasture about 140 years ago. Forest litter and pasture herbage samples were collected at the two sites and analysed to assess substrate quality. Laboratory experiments were conducted to determine responses of the two soil microbial communities to sucrose application. The responses were quantified by measurements of respiration and evolved carbon dioxide isotope enrichment.

Materials and methods

Description of sites

Samples of litter, pasture herbage and soil were collected at native forest and grazed pasture sites located about 200 km apart. The two sites were at a similar latitude, but near the west and east coasts of New Zealand's South Island. Historically, both of the site's organic soils were developed under swamp forest vegetation. To the west, Okarito Forest was established around 12,000 years ago (Vandergoes and Fitzsimons, 2003). It has been pristine since then, and is dominated by rimu (*Dacrydium cupressinum* Lamb.) trees between 100 to 400 year-old. The forest site was located about 7 km east of the Tasman Sea near the hamlet of Okarito (43.2° S, 170.3° E, 50 m above sea level). Annual rainfall, average air temperature and air temperature range are about 3.4 m, 11 °C, and 9 °C, respectively. High rainfall and poor drainage means the site's soil, known as Okarito peaty silt loam, is often very wet. The peat is highly decomposed, and the soil's USDA classification great group level is Haplosaprist.

The grazed pasture site, at Wainui Farm in an area known as Ladbrooks, is 11 km west of Governor's Bay and the Pacific Ocean (43.6° S, 172.6° E, about 10 m above sea level). At nearby Lincoln, annual rainfall, average air temperature and air temperature range average about 0.7 m, 11 °C, and 21 °C, respectively. The farm is a remnant of the former swamp forest, being mostly low lying, springs from an artesian system supplied water to the slightly higher ground. This forest was probably established around 10,000 years ago (McGlone et al., 1993), but dominated by kahikatea (*Dacrycarpus dacrydioides* (A. Rich.) de Laub) trees (Colin Meurk, personal communication). The forest was undisturbed until burnt by Polynesian settlers around 700 years ago (McGlone et al., 1994), and buried charcoal is common at the farm. The vegetation naturally regenerated to raupo, a rush plant (*Typha orientalis* C. Presl; Cox, 1978). After European settlement during the 1860s, the raupo was cleared and open drains dug although soils still became very wet in winter. The vegetation at Wainui Farm before the 1860s meant that the peat formed was naturally more fertile than that at Okarito Forest. Further, drainage would have enhanced the carbon and nutrient mineralisation rates. Perennial ryegrass (*Lolium perenne* L.) and other grass species were sown after the 1860s for dairy pasture, and vegetables have also been grown near Wainui Farm. However, Wainui is a stud farm that produces cattle for the dairy industry, and the site's land use for the past 25 years has been grazed pasture (Murray Stone, personal communication). The pasture was grazed on 50 days each year at a stocking of 31 cattle per hectare (Murray Stone, personal communication). The soil is known as Waimairi peaty loam, and is classified as a Haplosaprist.

Soil sampling and biochemical analyses

Soil and litter or pasture herbage were sampled from both sites for assessment of substrate quality and soil properties. Methods for the determination of pH in water, total C and N, extractable C, microbial C and N concentrations were described by Ross et al. (1999). To calculate microbial C from the (0.5 M K_2SO_4) extractable-C flush value in the fumigation-extraction

method, a k_{EC}-factor of 0.41 was applied, based on the value in Sparling et al. (1990) and adjusted for determination of extracted C using a Shimadzu TOC-5000 total C analyser rather than dichromate oxidation. Cellulose and lignin contents of forest litter and pasture were determined by isolation using an acid-detergent fibre pre-extraction and cellulose hydrolysis with sulphuric acid (Rowland and Roberts, 1994).

Response of soil microbial respiration to sucrose application

Microbial response to applied sucrose concentration was assessed by applying a range of sucrose doses (0 to 1.75 mol (sucrose) kg^{-1} (soil)) to root-free soil placed in glass jars and measuring the microbial respiration rate using a portable chamber system (PP Systems, Ltd., Model SRC-1 chamber and EGM-3 gas analyser, Hitchin, UK) (Law et al., 2001). Soil for this experiment was collected from over a depth of 0–100 mm on 5 December 2001 at Okarito Forest and 19 February 2002 at Wainui farm. From each site, samples were collected at a single location, returned to the laboratory, roots were gently removed by hand, and the root-free soil was stored at 4 °C for up to 3 days.

Soils were packed into jars in a field-moist state and left for 24 h to pre-incubate in the laboratory (20 °C). At sampling, a well at the forest site indicated that the water table was 80 mm below the soil surface. This wet condition and that of the so-called field capacity water content (upon the completion of drainage following thorough wetting by rainfall; see below) were characteristic of soil at this site. Each of the fifteen jars included 24 g of root-free soil and 76 g of water. Sucrose solution was applied at rates of 0, 0.002, 0.01, 0.05 and 0.16 mol kg^{-1} (soil) to 3 replicate jars after pre-treatment respiration rates had been measured. Soil from the grazed pasture site had also been thoroughly wetted by rainfall prior to sampling, and each of twenty jars included 52 g of root-free soil and 48 g of water. Sucrose solution was applied at rates of 0, 0.01, 0.02, 0.10 and 0.14 mol kg^{-1} (soil) to 4 replicate jars after pre-treatment respiration rates had been measured. Post-treatment respiration rates were measured 2 to 2.5, 20 to 26 and 45 to 50 h after sucrose application.

Tracing the fate of applied sucrose using stable isotope ratios of respired CO_2

Isotopes have long been recognised as valuable tracers in a wide range of experimental systems. In this experiment we exploit natural variation in the stable isotope ratio of carbon in organic matter to trace the fate of applied sucrose. Soil samples for this experiment were collected from a single location at the Okarito forest on 12 February 2002. On this occasion, the water table was 160 mm below the surface indicating the soil had reached its field capacity. Intact soil cores, of 0.18 m^3 volume (including roots), were removed from the forest and grazed pasture soils collected in February and placed in jars in the laboratory. From the forest, each of three jars contained 24 g of soil and 19 g of water. From the grazed pasture, each of three jars contained 55 g of soil and 30 g of water. Sucrose (5 mL of 0.2 M aqueous solution) was applied at rates of 0.04 and 0.02 mol kg^{-1} (soil) to the forest and grazed pasture soil cores, respectively.

To distinquish the catabolism of isotopically distinct carbon substrates in soil samples, we measured respiration rate, as before, and collected respired carbon dioxide before and 2 and 20 h after sucrose application. This was done using a closed-loop chamber, pump and infrared gas analyser (LICOR, model 6262, Lincoln, Nebraska). Part of the loop contained soda lime, and we began by reducing CO_2 concentration in the chamber to zero. After switching off the soda lime CO_2 trap, soil respiration returned the chamber CO_2 concentration to the ambient level in about 2 minutes. A chamber gas sample was then collected in a 30 mL evacuated flask, after passing the gas line through a 160 mL $Mg(ClO_4)_2$ water trap for at least 2 min. The chamber lid was sealed with silicone grease and the chamber placed into a water bath so that the bottom edge of the lid sat below the water level, providing both a good seal between the chamber and the lid and a constant temperature inside the chamber. The system was leak tested with an empty chamber and a steep CO_2 concentration gradient (at least 500 ppm CO_2 difference between outside and inside the chamber). No significant change in CO_2 concentration of the air within the system was observed over 15 min.

The samples were analysed for $\delta^{13}C$ at the National Institute of Water and Atmospheric Research (NIWA) using a Finnigan MAT 252 mass spectrometer (Finnigan Mat, Brenan, Germany) as described by Ferretti et al. (2000). The $\delta^{13}C$ of CO_2 is expressed

relative to the Pee Dee belemnite (PDB) standard;

$$\delta^{13}C = (R_s/R_r) - 1, \quad (1)$$

where R is the $^{13}C/^{12}C$ ratio and subscripts s and r are for sample and reference, respectively.

The stable carbon isotope ratio of three replicate samples of sucrose, forest leaf litter and pasture herbage were analysed at the Research School of Biological Sciences, Australian National University (ANU) using a Carlo-Erba elemental analyser linked to a stable isotope mass spectrometer (Micromass Isochrom; VG Isotech, Middlewich, UK). Isotope ratios are also expressed relative to the PDB standard.

Statistical analysis

Respiration rate and isotope measurements are reported as means of the three replicates ± standard errors of the mean. These were computed by conventional procedures for normally distributed data. Response to sucrose application was determined for individual replicates in two ways; namely, by calculation of the difference between measurements made before and after sucrose application and by the ratio of these two measurements. The latter is known as a relative response. Responses are also reported as means of the three replicates ± standard errors of the mean. The response error calculations recognised that measurements made before and after sucrose application were not independent. This meant the conventional procedure was used for the standard error of the mean response determined by difference. The standard error of the mean response determined by ratio was calculated differently. The required variance is the square of the standard error of the mean response multiplied by $\sqrt{n-1}$ where n is the number of replicates. This variance (V), designating b and a to represent respiration rate or $\delta^{13}C$ before and after sucrose application, was determined following Stuart and Ord (1987) as:

$$V(a/b) = [V(a)/\bar{b}^2] + [(\bar{a}^2 V(b))/\bar{b}^4] - [(2\bar{a}C V(a,b))/\bar{b}^3], \quad (2)$$

where an overbar designates the arithmetic mean and CV denotes the covariance of a and b.

Results

Forest litter, pasture herbage and soil biochemical properties

The pristine forest litter and grazed pasture herbage had similar cellulose contents (Table 1). However, the forest litter's lignin content was much higher, and it's total nitrogen (N) and phosphorus contents lower. The forest litter's total-N to phosphorus ratio of 26 was near the maximum measured in New Zealand (Roger Parfitt, personal communication). The forest has not been fertilised. The farm is regularly fertilised. The pasture herbage's low lignin content and relative high N and phosphorus contents reflect its use as feed for cattle.

The forest soil was acidic. The pasture soil had a much higher pH with about 70 times more hydroxyl ions. The pasture soil's total C, extractable C, microbial C and total N contents also averaged 1.6 to 3.8 times higher. In the forest soil, the percentages of microbial C in total C and microbial N in total N were 0.9 and 4.4%, respectively, and the microbial-C to microbial-N ratio was 4.3. In the pasture soil, the corresponding percentages were lower at 1.2 and 2.7%, and the ratio higher at 6.7.

Sucrose application and respiration response of root-free soil samples

Prior to sucrose application, the root-free forest soil had a microbial respiration rate of 0.53 ± 0.06 μmol CO_2 kg^{-1} s^{-1} at 20 °C. During 2 to 2.5 h after an application of 0.002 mol kg^{-1}, this soil's respiration rate nearly doubled (Figure 1a). After 20 to 26 h, the relative response was halved (Figure 1b) and respiration had returned to the pre-application rate after 45 to 50 h (data not show, but see Figure 2a).

Over 2 h, sucrose doses ≥ 0.012 mol kg^{-1} corresponded with a 2.6-fold increase in the forest soil's microbial respiration rate and a saturating response. Twenty hours after sucrose application, respiration had increased 8.3-fold relative to the pre-application rate, and it was saturated for doses ≥ 0.012 mol kg^{-1}.

Prior to sucrose application, the root-free pasture soil had a microbial respiration rate of 0.06 ± 0.01 μmol CO_2 kg^{-1} s^{-1}. After 2–2.5 h, this soil's respiration had increased up to 9-fold relative to the pre-application rate, and the response was dose dependent without saturation (Figure 1c). After 20–26 and 45–50 h, the responses had not changed significantly (Figures 1d and 2b).

Table 1. Properties of litter, herbage and soil (0–0.1 m depth) samples from pristine forest and grazed pasture sites. The data are mean ± standard error of the mean ($n = 3$). No error is given when it was negligible

Forest litter and farm pasture herbage						
Site	Cellulose (g kg^{-1})	Lignin (g kg^{-1})	Total nitrogen (g kg^{-1})	Phosphorus (g kg^{-1})		
Pristine forest	255	464 ± 8	5.1 ± 0.1	0.2		
Grazed pasture	204 ± 8	26 ± 4	41.1 ± 3.3	5.6 ± 0.2		
Soil						
Site	pH	Total carbon (g kg^{-1})	Extractable carbon (mg kg^{-1})	Microbial carbon (mg kg^{-1})	Total nitrogen (g kg^{-1})	Microbial nitrogen (mg kg^{-1})
Pristine forest	3.8	188 ± 4	171 ± 6	1709 ± 104	9.0 ± 0.2	400 ± 84
Grazed pasture	5.6	295	643 ± 34	3570 ± 291	19.5	532 ± 5

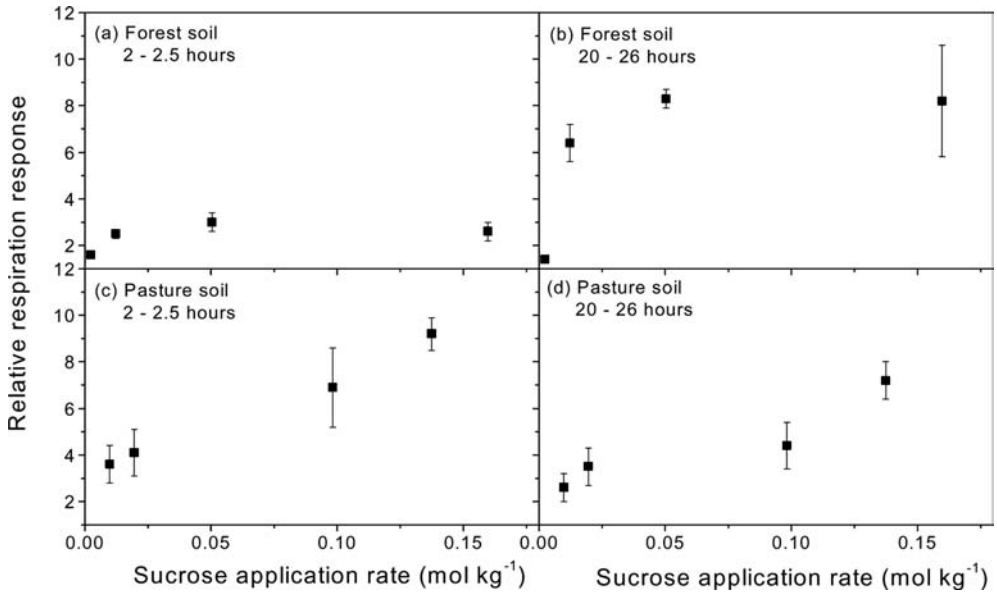

Figure 1. The relative responses of microbial respiration rate following four rates of sucrose application (mol (sucrose) kg^{-1} (soil)) to root-free soil samples from pristine forest (a, b) and grazed pasture (c, d) sites. Responses are shown 2–2.5 (a, c) and 20–26 (b, d) hours after application. Pre-application respiration rates averaged 0.53 ± 0.06 and 0.06 ± 0.01 μmol CO_2 kg^{-1} s^{-1} for the forest ($n = 12$) and pasture ($n = 16$) soils, respectively.

Sucrose application, respiration response of intact soil, including roots, and carbon isotope enrichment

Prior to sucrose application, the forest and pasture soils, including roots, had respiration rates of 0.55 ± 0.06 and 0.40 ± 0.05 μmol CO_2 kg^{-1} s^{-1}, respectively, at 20 °C (Table 2). The forest soil's root density was 10.5 ± 0.4 kg (biomass) m^{-3} (soil), all roots were woody, most had mycorrhizal nodules (Russell et al., 2002), and 91 ± 2% had a diameter greater than 0.5 mm. The pasture soil's root density was 8.9 ± 0.9 kg (biomass) m^{-3} (soil) with 74 ± 8% of roots having a diameter greater than 0.5 mm.

The δ^{13}C of the applied sucrose was −11.9‰, typical of a C_4 plant. For the forest litter and pasture herbage, δ^{13}C was −30.7 and −31.2‰, respectively.

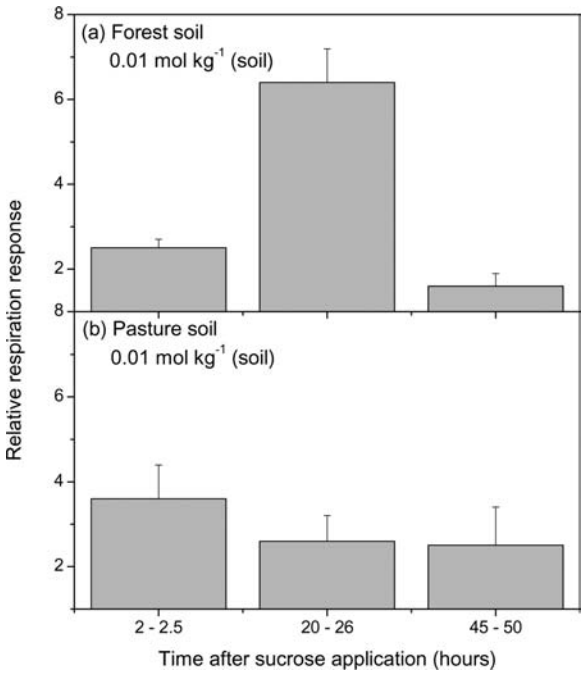

Figure 2. The relative responses of microbial respiration rate following sucrose application to root-free soil samples (0.01 mol (sucrose) kg^{-1} (soil)) from pristine forest (a) and grazed pasture (c) sites. Responses are shown for three periods after application. Pre-application respiration rates averaged 0.54 ± 0.19 and 0.09 ± 0.02 μmol CO_2 kg^{-1} s^{-1} for the forest ($n = 3$) and pasture ($n = 4$) soils, respectively.

For the forest soil, 2 and 20 h after sucrose application, the respiration rate had increased 0.67 ± 0.29 and 1.35 ± 0.01 μmol CO_2 kg^{-1} s^{-1}, respectively (Table 2). The time-dependent relative increases were thus 2.2 ± 0.5 and 3.5 ± 0.3. After sucrose application, $\delta^{13}C$ of the respired carbon was relatively enriched because the microbes catabolised the sucrose. Thus, 2 and 20 h after sucrose application, enrichment was 3.2 ± 0.2 and 7.1 ± 0.2‰.

For the pasture soil, 2 and 20 h after sucrose application, the respiration had increased 0.52 ± 0.15 and 0.66 ± 0.26 μmol CO_2 kg^{-1} s^{-1}, respectively, or relatively by 2.4 ± 0.5 and 2.7 ± 0.7. The response was thus relatively constant. At 2 h after sucrose application, $\delta^{13}C$ of the respired carbon was relatively enriched by 6.7 ± 0.9‰. The relative enrichment was virtually identical 20 h after application (6.5 ± 1.8‰). Like the respiration response, relative enrichment, as a measure of the applied sucrose's fate, was thus also completed in 2 h.

Table 2. Respiration rates of peaty soil, including roots, cores collected (0–0.1 m depth) from pristine forest and grazed pasture sites. Measurements were made at 20 °C, before and 2 and 20 h after sucrose applications of 0.04 and 0.02 mol kg^{-1} to the forest and pasture soil cores, respectively. The data are mean ± standard error of the mean ($n = 3$). Also shown are isotope ratios of carbon ($\delta^{13}C$) in the respired carbon dioxide (CO_2)

Respiration rate (μmol CO_2 kg^{-1} soil s^{-1})			
Site	Before sucrose	2 h after sucrose	20 h after
Pristine forest	0.55 ± 0.06	1.22 ± 0.33	1.90 ± 0.06
Grazed pasture	0.40 ± 0.05	0.92 ± 0.15	1.06 ± 0.31
Isotope ratio of carbon in respired carbon dioxide (‰)			
Site	Before sucrose	2 h after sucrose	20 h after
Pristine forest	−21.0 ± 0.1	−17.8 ± 0.2	−13.9 ± 0.2
Grazed pasture	−23.7 ± 0.9	−17.0 ± 0.5	−17.2 ± 1.3

Discussion

Substrate quality

Compared with microorganisms in the forest soil, the pasture soil microbes had superior substrate. For energy, the pasture herbage's much lower lignin content meant greater potential enzymatic access to cellulose (Vance and Chapin, 2001). The extractable carbon content was nearly 4-fold greater. The soil pH, nitrogen and phosphorus levels were managed to be non-limiting to pasture growth. Substrate supply rate by rapid cycling in the pasture soil was estimated to be around twice that in the forest soil (data not shown). However, compared with plant tissue and soil, microbes had the most favourable C-to-N ratio reflecting the potential of endo-cellular reserves as substrate (DeNobili et al., 2001).

Timing and magnitude of respiratory response to added carbohydrate

For the forest soil, a sucrose application of 0.002 mol kg^{-1} caused soil microbial respiration rate to nearly double within 2 h. Over the sampling depth of 0 to 0.1 m, the soil's bulk density was 80 kg m^{-3} (expressed on a sampled volume basis) so that this dose was equivalent to 2 kg C ha^{-1}. In a Swedish pine forest, soil respiration rate also doubled only one hour after sucrose application, but the dose was more than 1000 times greater (3.45 t C ha^{-1}; Ekblad and Nordgren, 2002). By contrast, in the laboratory,

microbes in arable soil samples were 'triggered' into activity by glucose applications that were 3 orders of magnitude smaller than ours (De Nobili et al., 2001). Soil microbes are indeed poised to seize food opportunities.

In situ field experiments, including an undisturbed rhizosphere, are compelling. However, the fate or efficacy of sucrose application can be uncertain in the field. Aerobic respiration produces 12 moles of CO_2 per mole of sucrose, while fermentation only 4 mol mol^{-1}. However, it is challenging to determine a soil's aerobic and anaerobic fractions. Other possible fates for sucrose applied in the field include immobilisation and loss into drainage water. In the laboratory, for aerobic soil samples, efficacy of the sucrose application is more certain. However, our root-free soil samples were disturbed, and it was relatively warm and dry during the laboratory experiments. Before sucrose application, the forest's root-free soil had a respiration rate that was only 8% less than that of the intact cores that included soil, rhizosphere and roots. This unexpected result suggested that forest soil's root respiration rate was very low or the root-free soil rate was somehow enhanced by the disturbance of root removal. We suspect that this result came from a combination of the two possibilities.

For Okarito silt loam soil, sampled 5 years after land use change from native pakahi (scrub) vegetation to grazed pasture and sieved (2 mm mesh) to remove the roots, microbial respiration rate measured in the laboratory was 0.10 μmol CO_2 kg^{-1} s^{-1} at 25 °C according to Speir and Ross (1983). Following Lloyd and Taylor (1994) to adjust their rate downwards to our incubation temperature of 20 °C, the same microbial respiration rate was obtained as we measured for the root-free pasture soil, before sucrose application. This supported the validity of our comparison of Okarito peaty silt loam and Waimairi peaty loam under pristine forest and grazed pasture, respectively.

For the pasture soil, the microbial respiration response was dose dependent and it did not change over time. These microbes seemed able to utilise added substrate almost immediately and, for our doses, without limitation evident in the forest soil's saturation response. The former characterises soil microbial r-strategists, r for rapid catabolism of fresh organic matter, according to Fontaine et al. (2003).

Tracing the fate of added carbon using δ^{13} CO_2

The similarity of sucrose application effects on soil respiration and relative δ^{13}C enrichment of the respired carbon was striking. The increase in enrichment of $\delta^{13}CO_2$ after addition of C_4 sucrose to C_3 pasture and forest soils clearly indicates metabolism of the applied carbohydrate. The large change in $\delta^{13}CO_2$ after just 2 h for the pasture soil supports data from the dose response experiments suggesting pasture soil microbes were able to rapidly metabolise the simple added carbohydrate. Smaller changes in $\delta^{13}CO_2$ at 2 h compared to 20 h after sucrose addition to the forest soil also support the dose response work indicating that microbes from the forest soil respond more slowly to added carbohydrate.

A number of researchers have exploited naturally occurring variation in the δ^{13}C of plant organic material to trace the fate of added carbon in addition studies. These studies have applied mass balance models to calculate the proportion of respired carbon from native and applied sources, ie to quantify the priming effect. However, the model requires the δ^{13}C of native carbon to be known. Carbon in soils is present in many different forms (Murata et al., 1995), which are likely to have a wide range of δ^{13}C values. Uncertainty in the source of native carbon utilised by soil microbes means that assigning a single δ^{13}C value (Bowling et al., 2003), and so applying the mass balance model, is difficult. There is also uncertainty about C fractionation associated with the biochemistry of mitochondrial respiration (Henn and Chapela, 2000, 2001; Henn et al., 2002; Lin and Ehleringer, 1997). However, respiration includes the catabolism of simple sugars derived from soil organic matter or recently transported from plants (Ekblad and Högberg, 2001). Consequently, the isotope technique has value in qualitative interpretation of the fate of carbon if, as we found, the difference in δ^{13}C between native and applied carbon is large enough for changes in $\delta^{13}CO_2$ to be clear.

Plants with C_4 metabolism discriminate against $^{13}CO_2$ during carbon fixation to a much lesser extent than plants with the more common C_3 metabolism (Bender, 1968), resulting in large differences (up to 20‰) in the δ^{13}C of organic material from the two groups of plants. In this study we added sucrose extracted from the C_4 plant sugar cane ($\delta^{13}C = -11.9$‰) to soils supporting C_3 plants (leaf litter $\delta^{13}C = -30.7$ and -31.2‰ for the forest and pasture soil, respectively). Such a large difference in δ^{13}C al-

lows the fate of the applied carbon to be interpreted, at least qualitatively, from variation in the $\delta^{13}C$ of evolved CO_2.

The problems associated with assigning a $\delta^{13}C$ value for native carbon, discussed above, are clearly demonstrated in the current work. Leaf litter, which should be one pool of metabolisable carbon in the soil systems studies, was significantly more depleted in ^{13}C (9.7‰) compared to the CO_2 collected in the laboratory experiment prior to sucrose addition.

We suggest the difference in $\delta^{13}C$ between pretreatment CO_2 and leaf litter for both pasture and forest soils may be caused by either fractionation during respiration and/or by the sampling technique employed. Recent literature reports conflicting results as to both whether fractionation occurs during respiration, and to the extent of this fractionation. Lin and Ehleringer (1997) found that $\delta^{13}C$ of respired CO_2 released by incubated leaf protoplasts was not significantly different to $\delta^{13}C$ of source carbohydrate, while Duranceau et al. (1999) and Ghashghaie et al. (2001) observed that CO_2 respired from intact leaves in the dark was 3 to 6‰ more enriched than leaf sucrose (although sucrose may not have been the only respiratory substrate). Similarly variable results have been reported for field-based studies. Ekblad et al. (2002) added C_3, C_4 and ^{13}C-labelled sucrose to a natural forest soil and compared the $\delta^{13}C$ of CO_2 released before and after treatment. They concluded that any fractionation during microbial respiration was minor. However, a growing body of work on fungal uptake of carbon and subsequent respiration (Henn and Chapela, 2000; 2001; Henn et al., 2002) reports that CO_2 respired by both saprotrophic and mycorrhizal fungi was up to 8‰ more enriched than substrate sucrose. The Okarito Forest soil samples included root nodules of arbuscular mycorrhizal fungi (Russell et al., 2002). Fractionation has also been reported for plant residue decomposition *in situ* (Feng et al., 1999; Natelhoffer and Fry, 1988; Schweizer et al., 1999). Indeed, Feng (2002) takes an assumed respiratory fractionation as a starting point for a model describing $\delta^{13}C$ of soil organic matter.

Pataki et al. (2003) correctly point out that fractionation is very difficult to assess in natural systems, where source carbon is likely to be variable in $\delta^{13}C$. Comparing $\delta^{13}C$ of ecosystem-respired CO_2 ($\delta^{13}C_R$, from Keeling plot intercepts; Keeling, 1961) with $\delta^{13}C$ of sun and shade leaves, Pataki et al. (2003) found that $\delta^{13}C_R$ could be between -1 and $+6$‰ different to shade leaf $\delta^{13}C$. Bowling et al. (2003) have also recently concluded that $\delta^{13}C$ of leaves, roots and soil organic material are not good indicators of $\delta^{13}C$ of ecosystem CO_2 fluxes, but were reluctant to suggest that this is a result of fractionation during respiration. The difference between $\delta^{13}C$ of pretreatment CO_2 and leaf litter presented here (9.7 and 7.5‰ for forest and pasture soils, respectively) are higher than the upper value of 3‰ reported by Natelhoffer and Fry (1988), but close to the maximum fractionation during carbon uptake and release by fungi reported by Henn and Chapela (2001). Soils are generally dominated by fungal respiration (e.g., Laughlin and Stevens, 2002) and the difference between native source carbon and pretreatment $\delta^{13}CO_2$ may be due to fractionation during fungal respiration.

The second possible reason for the difference between leaf litter $\delta^{13}C$ and pretreatment $\delta^{13}CO_2$ may relate to the experimental technique employed. Cerling et al. (1991) point out that soil-respired CO_2 is distinct from CO_2 in soil pores, both in concentration and $\delta^{13}C$. At steady-state $\delta^{13}C$ of CO_2 in soil pores ($\delta^{13}C_{sp}$) varies with depth in the soil profile and the rate of soil respiration. $\delta^{13}C_{sp}$ is close to atmospheric values (about -8‰) near the soil surface and decreases with depth to a value 4.4‰ more enriched than $\delta^{13}C$ of soil-respired CO_2 due to diffusional fractionation. Disruption of the steady-state condition of the soil (by significantly changing the soil respiration rate, or by altering the CO_2 concentration gradient between the soil and the atmosphere) could result in significant changes in the CO_2 concentration and $\delta^{13}C$ profile within the soil, and could potentially change $\delta^{13}C$ of soil-respired CO_2 (Dudziak and Halas, 1996). Bowling et al. (2003) suggest that sampling systems that disturb the profile could result in $\delta^{13}C$ of soil-respired CO_2 being up to 4.4‰ in error. If such a situation occurred in our system, $\delta^{13}C$ of CO_2 sampled pretreatment could be corrected to -25.4 and -28.1‰, values much closer to $\delta^{13}C$ of organic matter in the soils.

Conclusions

Compared with microorganisms in a pristine forest soil, a grazed pasture's soil microbes had superior substrate. For the forest soil, supra-trace sucrose application resulted in microbial respiration rate nearly doubling. The peak response occurred one day after application, and saturation occurred at a relatively modest dose. For the pasture soil, microbial respiration responded differently to sucrose application. The

response was nearly immediate, constant over time and dose dependent. There was up to a 9-fold increase in the pasture soil's microbial respiration rate. This was indicative of soil microbial r-strategists, and their rapid catabolism of fresh organic matter. After application, the relative increase in enrichment of respired δ^{13}C clearly indicated metabolism of the applied carbohydrate. Although increasingly popular, two uncertainties kept us from pursuing further quantitative analysis, including the so-called priming effect of fresh organic matter input to the soils. The fraction of native carbon utilised by soil microbes and fractionation during microbial respiration are not yet known with confidence. Nevertheless, the similarity of sucrose application effects on soil respiration and relative δ^{13}C enrichment of the respired carbon was striking and encouraging.

Acknowledgements

We thank S Wood (ANU) and Gordon Brailsford (NIWA) for isotopic analyses, and Colin Meurk, Roger Parfitt and Des Ross for valuable discussions. Richard Sedcole advised us about statistical analysis. Peter Almond and Trevor Webb advised us about history and classification of the soils. Murray Stone generously allowed us to extract soil samples from his farm, and explained his soil management and cattle grazing regimes.

References

Bender M M 1968 Mass spectrometric studies of carbon-13 variations in corn and other grasses. Radiocarb. 10, 468–472.

Bingeman C W, Varner J E and Martin W P 1953 The effect of the addition of organic materials on the decomposition of an organic soil. Soil Sci. Soc. Am. Proc. 29, 692–696.

Bowling D R, Pataki D E and Ehleringer J R 2003 Critical evaluation of micrometeorological methods for measuring ecosystem-atmosphere isotopic exchange of CO_2. Agric. For. Met. 116, 159–179.

Cerling T E, Solomon D K, Quade J and Bowman J R 1991 On the isotopic composition of carbon in soil carbon dioxide. Geochim. Cosmo. Acta 55, 3403–3405.

Cox J E 1978 Soils and Agriculture of Part Paparua County, Canterbury, New Zealand. Soil Bureau Bull. 34, N. Z. Dept. Sci. Ind. Res., Wellington, 128 pp.

De Nobili M, Contin M, Mondini C and Brookes P C 2001 Soil microbial biomass is triggered into activity by trace amounts of substrate. Soil Biol. Biochem. 33, 1163–1170

Dudziak A and Halas S 1996 Diurnal cycle of carbon isotope ratio in soil CO_2 in various ecosystems. Plant Soil 183, 291–299.

Duranceau M, Ghashghaie J, Badeck F-W, Deleens E and Cornic G 1999 δ^{13}C of CO_2 respired in the dark in relation to δ^{13}C of leaf carbohydrates in *Phaseolus vulgaris* L. under progressive drought. Plant Cell Envir. 22, 515–523.

Ekblad A and Högberg P 2001 Natural abundance of ^{13}C in CO_2 respired from forest soils reveals speed of link between tree photosynthesis and root respiration. Oecolog. 127, 305–308.

Ekblad A and Nordgren A 2002 Is growth of soil microorganisms in boreal forests limited by carbon or nitrogen availability? Plant Soil 242, 115-122.

Ekblad A, Nyberg G and Högberg P 2002 ^{13}C-discrimination during microbial respiration of added C_3-, C_4- and ^{13}C-labelled sugars to a C_3-forest soil. Oecologia 131, 245–249.

Feng X 2002 A theoretical analysis of carbon isotope evolution of decomposing plant litters and soil organic matter. Glob. Biogeo. Cycles 16, 1119–1129.

Feng X, Peterson J C, Quideau S A, Virginia R A, Graham R C, Sonder J and Chadwick O A 1999 Distribution, accumulation and fluxes of soil carbon in four monoculture lysimeters at San Dimas Experimental Forest, California. Geochim. Cosmo. Acta 63, 1319–1333.

Ferretti D F, Lowe D C, Martin R J and Brailsford G W 2000 A new gas chromatograph-isotope ratio mass spectrometry technique for high-precision, N_2O-free analysis of δ^{13}C and δ^{18}O in atmospheric CO_2 from small air samples. J. Geophy. Res. 105 D5, 6709–6718.

Fontaine S, Mariotti A and Abbadie L 2003 The priming effect of organic matter: a question of microbial competition? Soil Biol. Biochem. 35, 837–843.

Ghashghaie J, Duranceau M, Badeck F-W, Cornic G, Adeline M-T and Deleens E 2001 δ^{13}C of CO_2 respired in the dark in relation to δ^{13}C of leaf metabolites: Comparison between *Nicotiana sylvestris* and *Helianthus annuus* under drought. Plant Cell Environ. 24, 505–515.

Henn M R and Chapela I H 2000 Differential C isotope discrimination by fungi during decomposition of C_3- and C_4-derived sucrose. Appl. Environ. Microbiol. 66, 4180–4186

Henn M R and Chapela I H 2001 Ecophysiology of ^{13}C and ^{15}N isotopic fractionation in forest fungi and the roots of the saprotrophic-mycorrhizal divide. Oecologia 128, 480–487.

Henn M R, Gleixner G and Chapela I H 2002 Growth-dependent stable carbon isotope fractionation by Basidiomycete fungi: δ^{13}C pattern and physiological process. Appl. Environ. Microbiol. 68, 4956–4964.

Hobbie E A, Watrud L S, Maggard S, Shiroyama T and Rygiewicz P T 2003 Carbohydrate use and assimilation by litter and soil fungi assessed by carbon isotopes and BIOLOG® assays. Soil Biol. Biochem. 35, 303–311.

Högberg P and Ekblad A 1996 Substrate-induced respiration measured *in situ* in a C_3-plant ecosystem using additions of C_4-sucrose. Soil Biol. Biochem. 28, 1131–1138.

Keeling C D 1961 The concentrations and isotopic abundances of atmospheric carbon dioxide in rural and marine air. Geochim. Cosmo. Acta 24, 277–298.

Laughlin R J and Stevens R J 2002 Evidence for fungal dominance of denitrification and codenitrification in a grassland soil. Soil Sci. Soc. Am. J. 66, 1540–1548.

Law B E, Kelliher F M, Baldocchi D D, Anthoni P M, Irvine J, Moore D and Van Tuyl S 2001 Spatial and temporal variation in respiration in a young ponderosa pine forest during a summer drought. Agric. For. Meteor. 110, 27–43.

Lin G and Ehleringer J R 1997 Carbon isotope fractionation does not occur during dark respiration in C_3 and C_4 plants. Plant Phys. 114, 391–394.

Lloyd J and Taylor J A 1994 On the temperature dependence of soil respiration. Func. Ecol. 8, 315–323.

Löhnis F 1926. Nitrogen availability of green manures. Soil Sci. 22, 253–290.

McGlone M S, Salinger M J and Moar N T 1993 Paleovegetation studies of New Zealand's climate since the Last Glacial Maximum. *In* Global Climates since the Last Glacial Maximum. Ed. H E Wright. pp. 294–317. University of Minnesota Press.

McGlone M S, Anderson A J and Holdaway R N 1994 An ecological approach to ploynesian settlement of New Zealand. *In* The Origins of the First New Zealanders. Ed. D G Sutton. pp 136–163. Auckland University Press, Auckland.

McLaren R G and Cameron K C 1996 Soil Science, 2nd edn. Oxford University Press, Melbourne. 304 pp.

Murata T, Nguyen M L and Goh K M 1995 The effects of long-term superphosphate application on soil organic matter content and composition from an intensively managed New Zealand pasture. Europ. J. Soil Sci. 46, 257–264.

Natelhoffer K J and Fry B 1988 Controls on natural nitrogen-15 and carbon-13 abundances in forest soil organic matter. Soil Sci. Soc. Am. J. 52, 1633–1640.

Pataki D E, Ehleringer J R, Flanagan L B, Yakir D, Bowling D R, Still C J, Buchmann N, Kaplan J O and Berry J A 2003 The application and interpretation of Keeling plots in terrestrial carbon cycle research. Global Biogeo. Cycles 17, 1022–1034.

Price S J, Sherlock R R, Kelliher F M, McSeveny T M, Tate K R and Condron L M 2003 Pristine New Zealand forest soil is a benchmark methane sink. Global Change Biol. 10, 16–26.

Ross D J, Kelliher F M and Tate K R 1999 Microbial processes in relation to carbon, nitrogen and temperature regimes in litter and a sandy mineral soil from a central Siberian *Pinus sylvestris* L. forest. Soil Biol. Biochem. 31, 757–767.

Rowland A P and Roberts J D 1994 Lignin and cellulose fractionation in decomposition studies using acid-detergent fibre methods. Commun. Soil Sci. Plant Anal. 25, 269–277.

Russell A J, Bidartondo M I and Butterfield B G 2002 The root nodules of the Podocarpaceae harbour arbuscular mycorrhizal fungi. New Phytol. 156, 283–295.

Schweizer M, Fear J and Cadisch G 1999 ^{13}C fractionation during plant residue decomposition and its implications for soil organic matter studies. Rapid Commun. Mass Spectr. 13, 1284–1290.

Sparling G P, Feltham C W, Reynolds J, West A W and Singleton P L 1990 Estimation of soil microbial C by a fumigation-extraction method: use on soils of high organic matter content, and a reassessment of the k_{EC}-factor. Soil Biol. Biochem. 22, 301–307.

Speir T W and Ross D J 1983 Biochemical properties with pasture development in a west coast wet land soil: A note. N. Z. J. Sci. 26, 505–508.

Stuart A and Ord K 1987 Kendall's advanced theory of statistics. Vol. 1. Distribution Theory, 5th edn. Charles Griffith & Company, London. 604 pp.

Tate K R, Ross D J, O'Brien B J and Kelliher F M 1993 Carbon storage and turnover, and respiratory activity, in the litter and soil of an old-growth southern beech (*Nothofagus*) forest. Soil Biol. Biochem. 25, 1601–1612.

Vance E D and Chapin III F S 2001 Substrate limitations to microbial activity in taiga forest floors. Soil Biol. Biochem. 33, 173–188.

Vandergoes M J and Fitzsimons S J 2003 The last glacial-interglacial transition (LGIT) in South Westland, New Zealand: Paleoecological insight into mid-latitude southern hemisphere climate change. Quat. Sci. Rev. 22, 1461–1476.

Section editor: S. Recous