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Priming and microbial nutrient limitation in lowland tropical forest soils of contrasting fertility

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Abstract Priming is an increase in soil organic carbon decomposition following input of labile organic carbon. In temperate soils where biological activity is limited commonly by nitrogen availability, priming is expected to occur through microbial acquisition of nitrogen from organic matter or stimulated activity of recalcitrant-carbon degrading microorganisms. However, these priming mechanisms have not yet been assessed in strongly weathered tropical forest soils where biological activity is often limited by the availability of phosphorus. We examined whether microbial nutrient limitation or community dynamics drive priming in three lowland tropical forest soils of contrasting fertility ('low', 'mid' and 'high') by applying C_4-sucrose (alone or in combination with nutrients; nitrogen, phosphorus and potassium) and measuring (1) the $\delta^{13}C$-signatures in respired CO_2 and in phospholipid fatty acid (PLFA) biomarkers, and (2) the activities of enzymes involved in nitrogen (*N*-acetyl β-glucosaminidase), phosphorus (phosphomonoesterase) and carbon (β-glucosidase, cellobiohydrolase, xylanase, phenol oxidase) acquisition from organic compounds. Priming was constrained in part by nutrient availability, because priming was greater when sucrose was added alone compared to when added with nutrients. However, the greatest priming with sucrose addition alone was detected in the medium fertility soil. Priming occurred in parallel with stimulated activity of phosphomonoesterase and phenol oxidase (but not *N*-acetyl β-glucosaminidase); when sucrose was added with nutrients there were lower activities of phosphomonoesterase and phenol oxidase. There was no evidence according to PLFA $\delta^{13}C$-incorporation that priming was caused by specific groups of recalcitrant-carbon degrading microorganisms. We conclude that priming occurred in the intermediate fertility soil following microbial mineralization of organic nutrients (phosphorus in particular) and suggest that priming was constrained in the high fertility soil by high nutrient availability and in the low fertility soil by the low concentration of soil organic matter amenable to priming. This first study of priming mechanisms in tropical forest soils indicates that input of labile carbon can result in priming by microbial mineralization of organic nutrients, which has important implications for understanding the fate of organic carbon in tropical forest soils.

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Introduction

Inputs of labile organic compounds to soil can stimulate microbial mineralization of pre-existing organic matter through 'priming effects' (Fontaine et al. 2004). Priming effects have the potential to feedback positively on climate change by contributing an additional source of atmospheric CO_2. A net input of atmospheric CO_2 would arise if environmental change increases plant productivity and labile carbon (C) inputs to soils, and these inputs are then exceeded by soil CO_2 emissions due to priming. Despite the large potential for priming to affect the global C cycle they remain poorly understood, especially in tropical forest soils. Tropical forests contain 30% of global soil C (Jobbagy and Jackson 2000) and may already be subject to increased inputs of labile C due to increased aboveground production resulting from environmental change (Phillips et al. 2008), yet no studies have investigated priming mechanisms in these ecosystems. Furthermore, priming mechanisms in tropical soils may differ markedly to temperate soils because they are generally more strongly weathered, with consequences for the nutrient limitation to soil microorganisms (Walker and Syers 1976; Cleveland et al. 2006).

Two mechanisms have been hypothesized to lead to priming. First, priming may occur when microorganisms become nutrient-limited during the degradation of new labile C and co-metabolize pre-existing soil organic matter to meet their nutrient demands, leading to soil C being mineralized and released as 'primed' CO_2 (Blagodatskaya and Kuzyakov 2008). Supporting evidence for this mechanism is provided by laboratory experiments on temperate soils, which measured increased activity of organic nitrogen (N)-degrading enzymes during priming (Asmar et al. 1994) and a reduction in priming when N was added with labile C, due to a switch from soil C to added labile C as the preferred substrate ('preferential substrate utilization'; Hagedorn et al. 2003; Blagodatskaya et al. 2007).

Second, priming may occur when microorganisms that specialize in the degradation of stable soil C are able to compete with other microorganisms and utilize part of the new labile C. This mechanism is thought to occur in nutrient-poor soils where slow-growing soil C-specialist microorganisms are able to gain a competitive advantage over fast-growing labile C-specialist microorganisms. According to this theory, the soil C-specialists increase in abundance by utilizing some of the new labile C and persist to degrade stable soil C when the labile C becomes diminished (Fontaine et al. 2003). Supporting evidence for this mechanism is provided by studies that measured prolonged priming after supply of added labile C is exhausted (Fontaine et al. 2004), and priming alongside an increased dominance of the microbial community by fungi (Carney et al. 2007).

A predictive understanding of priming remains elusive in part because few studies have measured changes in microbial community composition and biochemical activity during priming (Blagodatskaya and Kuzyakov 2008). Priming due to co-metabolism of organic matter by nutrient-limited microorganisms can be identified by an increase in the microbial synthesis of extracellular enzymes to liberate nutrients from organic matter (Kuzyakov et al. 2000; Schimel and Weintraub 2003; Blagodatskaya and Kuzyakov 2008). The enzyme *N*-acetyl β-glucosaminidase is mostly present in soils in extracellular forms (Parham and Deng 2000) and can be produced by microorganisms in response to N-deficiency to acquire N from organic matter (Sinsabaugh and Moorhead 1994; Olander and Vitousek 2000; Muruganandama et al. 2009). Phosphomonoesterase is released by microorganisms in response to phosphorus (P) deficiency to hydrolyze ester bonds between C and P in organic matter and liberate orthophosphate (Quiquampoix and Mousain 2005). Activity of these enzymes can indicate microbial mineralization of nutrients from organic matter due to N or P demand (Olander and Vitousek 2000; Allison et al. 2007; Sinsabaugh et al. 2008) and consequently may provide a useful indication of nutrient limitation during priming. Priming due to increased abundance of recalcitrant-C degrading microorganisms can be identified by a shift in microbial community composition (e.g. increase in fungal:bacterial ratios; Carney et al. 2007), prolonged priming following the exhaustion of the added substrate, and greater priming in low fertility soils (Fontaine et al. 2003, 2004).

Priming has been suggested as the cause of reductions in soil C in temperate forest grown under experimentally elevated CO_2, despite increased inputs of plant-C to soils (Carney et al. 2007; Langley et al. 2009). The parallel loss of soil C and increase in N mineralization in the study by Langley et al. (2009) suggests that priming occurred due to microbial 'co-metabolism' of organic matter to acquire N (Kuzyakov et al. 2000), which is often considered the limiting nutrient in temperate forests. In contrast, studies performed in tropical forests suggest that decomposition is limited by the availability of P (Hobbie and Vitousek 2000; Kaspari et al. 2008). Many tropical forest soils are strongly weathered and, while they have abundant available N (Martinelli et al. 1999), contain little available P due to its occlusion within secondary minerals and immobilization within organic matter (Walker and Syers 1976; Vitousek and Sanford 1986). Although the generalization is unlikely to hold true for all tropical forests due to varying rates of rock weathering and dust deposition (Porder and Hilley 2011), P-limitation of microbial C metabolism has been demonstrated for numerous tropical forests where experimental P-addition increased microbial mineralization of dissolved organic matter (Cleveland et al. 2006) and increased soil CO_2 efflux (Cleveland and Townsend 2006), and where elevated P concentration in litter increased decomposition rates (Hobbie and Vitousek 2000). Another study concluded P-limitation of microbial metabolism of C in a tropical soil when CO_2 efflux increased more rapidly following an addition of C with P compared to C with N (Gnankambary et al. 2008).

We asked whether priming effects occur due to (1) co-metabolism of soil organic matter due to microbial nutrient limitation, and/or (2) increased abundance of a specific group of soil C-degrading microorganisms. To investigate this, we assessed priming effects following sucrose additions with and without mineral nutrients to soils of contrasting fertility from three different lowland tropical forests in the Republic of Panama.

Materials and methods

Soils

Soils were collected from three 1 ha lowland tropical forest plots from the Center for Tropical Forest Science (CTFS) network in the Republic of Panama (Pyke et al. 2001; Turner and Engelbrecht 2011). We selected sites at Rio Paja ('low-fertility'—plot 26 in Turner and Engelbrecht 2011), Pipeline Road ('mid-fertility'—plot 15 in Turner and Engelbrecht 2011) and Campo Chagres ('high-fertility') due to their marked variation in nutrient status; total C, N and P increased in the order: Rio Paja < Pipeline Road < Campo Chagres (Table 1; plotted in context to other CTFS and RAINFOR forest sites in Fig. 1). Detailed information on the P composition of the soils is reported elsewhere (Turner and Engelbrecht 2011). Soils at Rio Paja are derived from fine grained rhyolitic tuff, soils at Pipeline Road are derived from marine sedimentary parent material of the Gatuncillo Formation, and soils at Campo Chagres are derived from calcareous sandstone of the Alajuela Formation. For each site, surface soil (0–10 cm) was collected from 30 random locations using a 2.5 cm diameter soil corer. Within 24 h of collection, soils were returned to the laboratory and visible stones and roots were removed by hand. Soils were then mixed, sieved (<2 mm) to isolate the fine earth fraction, weighed (500 g air-dry weight) into PVC containers (16 cm diameter, 10 cm height), adjusted to 60% water holding capacity and pre-conditioned at 22°C for 10 days prior to experimental treatments. The temperature and moisture content (by daily watering) were then maintained throughout the experiment.

Experimental design

Treatments were a sugar-cane sucrose solution and a combined nutrient solution (N in NH_4^+ and NO_3^-, P and K in KH_2PO_4) applied as follows: control (no addition; CTL), nutrient addition (NPK), sucrose addition (C) and sucrose and nutrient addition (CNPK). Additions of sucrose and nutrients on the basis of dry soil were: 4000 mg C kg^{-1}, 100 mg N kg^{-1}, 100 mg P kg^{-1}, and 130 mg K kg^{-1}. Additions of C were within the range of annual input of C to tropical forest soils. For example, nearby tropical forest soils in Panama receive 470–650 g litterfall-C ha^{-1} $year^{-1}$ (Wieder and Wright 1995; Kaspari et al. 2008), equivalent to 2300–3300 mg C kg^{-1} soil $year^{-1}$ from litterfall alone (assuming that the majority is mineralized within the top 20 cm of soil with a bulk density of 1 g cm^{-3}).

Treatments were allocated according to a randomized block design with four replicates of each treatment and four controls and stratified according

Table 1 Site locations, total soil carbon, total nutrients, pH and soil texture for untreated soils

	Low-fertility	Mid-fertility	High-fertility
C (g kg^{-1})	28.1	40.1	104.0
N (g kg^{-1})	2.1	3.5	8.9
P (mg kg^{-1})	71	200	696
C/N	13.4	11.5	11.7
C/P	396	201	149
N/P	30	18	13
pH	4.4	6.3	6.8
Sand (%)	4	38	32
Silt (%)	62	27	21
Clay (%)	33	36	47

Soils were designated: 'low-fertility' (Rio Paja), 'mid-fertility' (Pipeline Road) and 'high fertility' (Campo Chagres). Data are single analyses of pooled sub-samples; soil texture data are from Turner and Engelbrecht (2011)

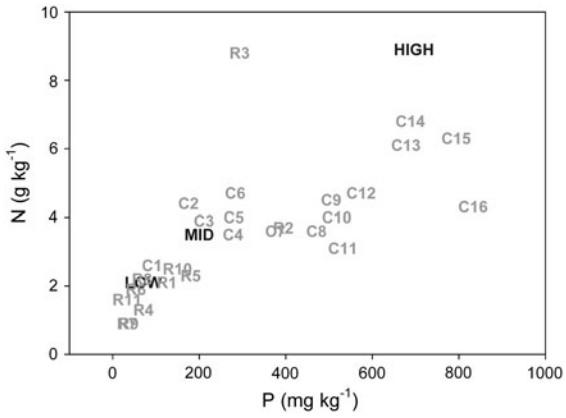

Fig. 1 Tropical forest sites in Panama (including sites from this study: LOW, MID, HIGH; and other CTFS sites: C1–C16) and across the Amazon (RAINFOR sites: R1–R11), distributed according to soil fertility as determined by total concentration of nitrogen and phosphorus (measured at 0–10 cm depth). CTFS site descriptions are given in Turner and Engelbrecht (2011): LOW (Rio Paja P26), MID (Pipeline P15), HIGH (Campo Chagres), C1 (Rio Paja P25), C2 (Pipeline P09), C3 (Sherman P02), C4 (Pipeline P08), C5 (Buena Vista P12), C6 (Santa Rita P32), C7 (Albrook), C8 (Pipeline P17), C9 (Mocambo), C10 (Cerro Torre), C11 (Las Cruces P27), C12 (Buena Vista P13), C13 (Sherman P01), C14 (BCNM P18), C15 (Gamboa P24), C16 (Cerro Galera). RAINFOR site descriptions are given in Quesada et al. (2011): R1 (SUC-02), R2 (CUZ-03), R3 (HCC-21), R4 (CHO-01), R5 (BOG-02), R6 (ELD-12), R7 (CAX-02), R8 (TAP-04), R9 (SIN-01), R10 (JUR-01), R11 (MAN-12)

to untreated soil CO_2 efflux. Sucrose and nutrients were applied once in 20 ml solutions (20 ml C solution and/or 20 ml NPK solution) during daily watering of soils. Measurements of CO_2 efflux and $\delta^{13}C$ values in CO_2 efflux were made on 10 occasions for the low-fertility soil (days 0, 1, 2, 3, 4, 6, 8, 10, 14, 18); seven occasions for the mid-fertility soil (days 0, 1, 2, 3, 4, 6, 8); and nine occasions for the high-fertility soil (days 0, 1, 2, 3, 4, 6, 8, 10, 12). Measurements for the low-fertility soil continued for 18 days because of the unexpectedly long time it took for the added sucrose to decompose and measurements for the mid-fertility soil discontinued at 8 days because of equipment problems.

Soil samples (0–2 cm depth) were taken 3 days after treatments were imposed and analyzed for extractable nutrients, microbial nutrients, enzyme activities, and PLFAs. We sampled soil 3 days after treatments were imposed to quantify chemical and biological properties during priming effects, rather than during the peak of priming, which could only be determined retrospectively. Soils were extracted for nutrients on the day of sampling to minimize the rapid changes in nutrients that can occur during storage (Turner and Romero 2009).

Soil CO_2 efflux and $\delta^{13}C$ determination

Soil CO_2 efflux was measured using a Li-8100 soil respiration system (infra red gas analyzer; Li-Cor, Lincoln, Nebraska, USA). To sample soil CO_2 efflux for determination of $\delta^{13}C$ we used 4-l static chambers constructed from thick PVC and fitted over each container to give an air tight seal (Bertolini et al. 2006). Air samples were collected during a near-linear increase in chamber CO_2 concentration, characterized in preliminary tests at different rates of soil CO_2 efflux, with the first sample extracted 3 min after chamber placement and the final sample extracted at 2–3 times ambient CO_2. Samples for determination of $\delta^{13}C$ values were collected in 12 ml exetainers, wax-sealed, shipped to Lancaster, UK and analyzed within approximately 3 months. The $\delta^{13}C$ values of CO_2 samples were determined by isotope ratio mass spectrometry using a Micromass TraceGas Pre-concentrator coupled to an Isoprime isotope ratio mass spectrometer (Micromass, Wythenshawe, UK). A further 20 ml of soil chamber air was extracted and analyzed for CO_2 concentration using a gas chromatograph (Shimadzu GC-14B, Columbia, MD, USA) equipped with an electron capture detector (Loftfield et al. 1997) which was calibrated with four

standard gases (360, 706, 1505 and 5012 ppm CO_2 Deuste Steininger GmbH, Mühlhausen, Germany).

Soil nutrients, enzymes and microbial biomass

Soil inorganic N (in $NO_3^-$and NH_4^+) and microbial C and N were determined by K_2SO_4 extraction. Microbial C and N were determined as the difference between chloroform-fumigated and unfumigated soil samples following a 24 h fumigation period (Vance et al. 1987) and corrected for efficiency of the extraction procedure with *k*-factors of 0.45 for C (Wu et al. 1990) and 0.54 for N (Joergensen and Mueller 1996). Total C and N in the extracts were determined by combustion and gas chromatography using a Thermo-Electron Flash 1112 Elemental Analyzer (CE Elantech, Lakewood, NJ). Readily-exchangeable phosphate (extractable P) and microbial P were determined by extraction with anion-exchange membranes and hexanol fumigation based on the method described by Kouno et al. (1995). Phosphate was recovered from anion-exchange membranes by shaking for 1 h in 50 ml of 0.25 M H_2SO_4, with detection in the acid solution by automated molybdate colorimetry using a Lachat Quickchem 8500 (Hach Ltd, Loveland, CO, USA). Extractable P was determined by P recovered from unfumigated samples and microbial P was calculated as the difference between the fumigated and unfumigated samples.

Five enzymes involved in C and nutrient cycling were measured using microplate fluorimetric assays with 200 μM methylumbelliferone (MU)-linked substrates (Marx et al. 2001): *β*-glucosidase (degradation of labile C), cellobiohydrolase (degradation of cellulose), *N*-acetyl *β*-glucosaminidase (degradation of N-glycosidic bonds), phosphomonoesterase (degradation of monoester-linked simple organic phosphates) and xylanase (degradation of hemicellulose). Soil samples were collected from soil containers to 2 cm depth, stored at 3°C and assayed within 3 days, which does not appear to greatly alter observed activities in tropical forest soils (Turner and Romero 2010). On the day of the assay, 2 g soil (dry weight basis) was added to 200 ml 1 mM NaN_3 solution and dispersed by stirring on a magnetic stir plate. After 5 min and while stirring, 50 μl aliquots of soil suspension were removed using an 8-channel pipette and dispensed into a 96-well microplate containing 50 μl modified universal buffer solution (Tabatabai 1994) adjusted to soil pH. Each microplate included assay wells (soil solution plus 100 μl MU substrate), blank wells (soil solution plus 100 μl of 1 mM NaN_3) and quench wells (soil solution plus 100 μl MU standard). A further control plate was prepared with MU substrates and standards with no soil solution to determine quenching by soil solution in assay plates. There were eight analytical replicate wells for each assay. Microplates were incubated at 22°C for either 1 h (*β*-glucosidase, *N*-acetyl *β*-glucosaminidase, phosphomonoesterase) or 4 h (cellobiohydrolase, xylanase). Following incubation, 50 μl of 0.5 M NaOH was added to terminate the reaction and plates were immediately analyzed on a Fluostar Optima spectrofluorometer (BMG Labtech, Offenburg, Germany) with excitation at 360 nm and emission at 450 nm.

A further enzyme, phenol oxidase (degradation of phenolic compounds), was measured using 5 mM L-dihydroxyphenyalanine (L-DOPA) as substrate (e.g. Waldrop and Firestone 2004a). Briefly, 1 g soil (oven-dry basis) was added to 100 ml of 5 mM bicarbonate buffer and mixed well; 100 μl of 5 mM L-DOPA solution and 100 μl of soil solution were then added to a 96-well plate. Control plates were made using 100 μl of 5 mM bicarbonate buffer and 100 μl aliquots of soil solution. There were 16 analytical replicates and controls per soil sample. Plates were analyzed on a Fluostar Optima spectrofluorometer (BMG Labtech, Offenburg, Germany), with phenol oxidase activity calculated as the increase in absorbance at 450 nm over 1 h.

Phospholipid fatty acids (PLFA)

Phospholipid fatty acids were extracted from freeze dried soils using the method of Crossman et al. (2004). Following sampling (3 days after treatments were imposed), soils were stored at −35°C for 3–6 months and then freeze-dried approximately 1 month prior to PLFA extraction. PLFA fingerprints were identified and quantified by gas chromatography (GC). The $\delta^{13}C$ values of PLFA were determined using gas-chromatography-combustion-isotope ratio mass spectrometry (GC-C-IRMS). All analytical conditions are described in Chamberlain et al. (2006). Molecular structures of PLFA are described using standard nomenclature: the first number refers to the total number of C-atoms and the number after the colon refers to the number of double bonds.

A number following a 'ω' is the location of the first double bond relative to the aliphatic end of the molecule. Notations 'Me', 'OH' and 'cy' are, respectively, methyl, hydroxy, cyclopropane groups and notations 'i' and 'a' respectively are iso- and anteiso-branched fatty acids. PLFA biomarkers were grouped as Gram-positive bacterial (15:0, i15:0, a15:0, i16:0, i17:0, a17:0, 7Me17:0), Gram-negative bacterial (16:1ω5, 16:1ω7, 17:1ω8, 7,9cy17:0, 18:1ω7, 7,8cy19:0, 19:1), fungal (18:2ω6, 18:1ω9) and non-specific saturated (14:0, 16:0, 18:0; Frostegård and Bååth 1993; Zelles 1999).

Calculations and statistics

The percent of respired CO_2 originating from added sucrose was calculated according to:

$$\%\ C_{\text{sucrose-derived}} = \left[\frac{\delta_C - \delta_T}{\delta_C - \delta_L}\right] \times 100 \qquad (1)$$

where δ_C is the $\delta^{13}C$ value of the respired CO_2 from control soils, δ_T is the $\delta^{13}C$ value in respired CO_2 from treated soils and δ_L is the $\delta^{13}C$ value of the sucrose C (e.g. Balesdent et al. 1988). The increase in soil C mineralization (primed CO_2) was then calculated from the increase in soil respiration in treatments relative to controls minus the contribution of sucrose C (Kuzyakov et al. 2000). We also used a variation of Eq. 1 to calculate the mass of C within individual PLFAs at day three. The proportion of sucrose-derived C within each PLFA was calculated using Eq. 1, where δ_C is the $\delta^{13}C$ value of a specific PLFA from untreated control soils, δ_T is the $\delta^{13}C$ value for the same PLFA from treated soils and δ_L is the $\delta^{13}C$ value of the sucrose (e.g. Nottingham et al. 2009). Real priming effects (extra soil-derived CO_2 from soil organic matter) were distinguished from apparent priming effects (extra soil-derived CO_2 from substitution of soil-labeled with sucrose-labeled C in microbial biomass) by comparing total primed CO_2-C with microbial C and by examining changes of soil-labeled C within PLFA biomarkers. For each partitioned C-source we calculated standard errors from summed respective variances. Cumulative evolution of CO_2-C was calculated by integrating the area beneath the CO_2 efflux versus time curve.

We used repeated measures ANOVA to study the variation in total soil CO_2 efflux and partitioned components of soil CO_2 efflux with time between three treatments and a control. For all other analyses one-way ANOVAs were used with either soil type or treatment as the factor and respective response variables were: cumulative CO_2 evolution, PLFA concentration, quantity of sucrose-derived C or soil-derived C within PLFAs, concentration of PLFA biomarker groups (Gram-positive, Gram-negative, fungal) and fungal/bacterial ratios (according to the ratio of 18:2ω6,9/bactPLFAs; Bardgett et al. 1996). Pair-wise comparisons were made using post-hoc Tukey HSD analysis and significant differences were determined at $P \leq 0.05$. Treatment effects on the concentrations of soil and sucrose-derived C within PLFA were further examined using Principal Components Analysis (PCA) to construct new variables from multivariate PLFA data sets. Prior to analysis, data were tested for normality using a Ryan-Joiner test and non-normal data were log-transformed. All statistical analyses were performed using Minitab (version 15, Minitab Inc., PA, USA).

Results

Total, sucrose and soil-derived soil CO_2 efflux

Additions of C and CNPK led to significant increases in total soil CO_2 efflux for all soils ($P < 0.001$; Fig. 2). For CNPK compared to C additions, the peak in total soil CO_2 efflux was higher and occurred earlier for low-fertility and mid-fertility soils but was higher and occurred at the same time for the high-fertility soil (Fig. 2). Following C additions, total soil CO_2 efflux peaked after 10, 3 and 2 days and following CNPK additions after 3, 1, and 2 days for low-, mid- and high-fertility soils respectively (Fig. 2). For CNPK compared to C additions, the cumulative evolution of total C over 8 days was significantly higher for all soils ($P < 0.001$; Fig. 3). Addition of NPK alone had no significant effect on total soil CO_2 efflux for any soil ($P > 0.90$; data not shown).

Priming, identified by a significant increase in soil-derived CO_2 efflux in treated relative to control soils, occurred following C additions for all soils (for all comparisons, $P < 0.05$; Fig. 2). The peak in primed CO_2 efflux following C additions occurred over 0–10 days for low-fertility, 0–4 days for mid-fertility and 0–3 days for high-fertility soils, and was prior to

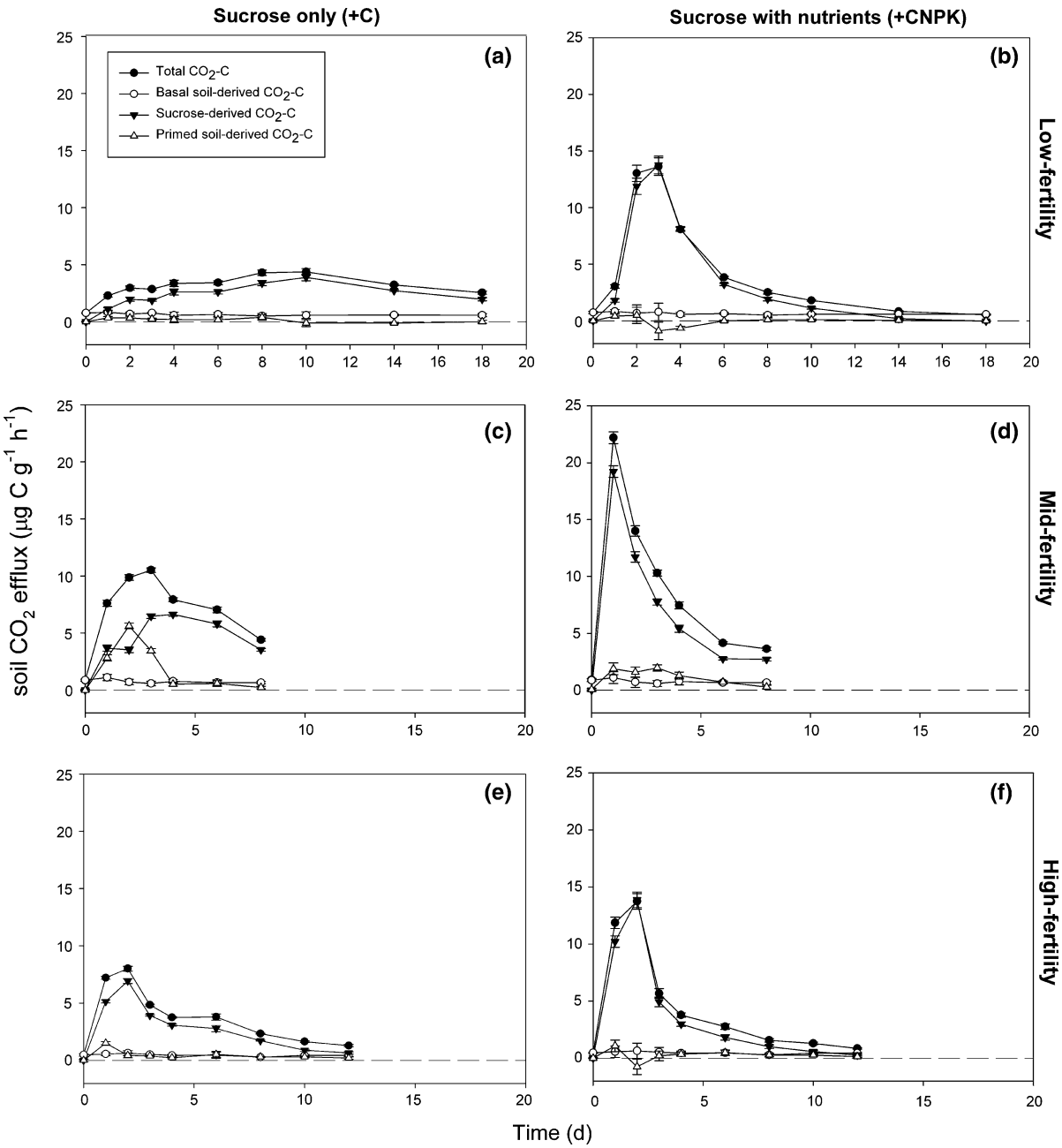

Fig. 2 Total CO_2-C efflux (μg CO_2-C g^{-1} soil h^{-1}) following treatments was partitioned into basal soil-derived C, sucrose-derived C, primed soil-derived C for low-fertility (**a**, **b**), mid-fertility (**c**, **d**) and high-fertility (**e**, **f**) soils following + C and + CNPK additions. Data are means ± 1 standard error (n = 4)

the peak in sucrose-derived CO_2 for all soils (Fig. 2). The cumulative evolution of primed CO_2-C over 8 days following C additions was higher for soils in the order low-fertility < high-fertility < mid-fertility (the portion of soil-derived CO_2-C in excess of basal CO_2-C in Fig. 3). For CNPK compared to C additions, primed CO_2 efflux was significantly lower for all soils (Figs. 2, 3).

The peak in sucrose-derived CO_2 efflux was higher for all soils and occurred earlier for low-fertility and

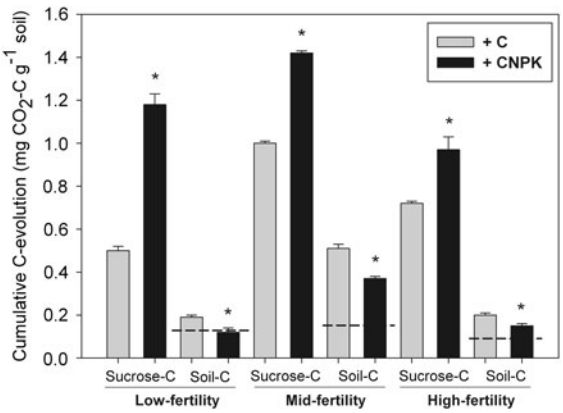

Fig. 3 Cumulative sucrose- and soil-derived CO_2-C evolution over 8 days following treatments. Each pot received 4 mg sucrose-C g^{-1} soil. Primed CO_2-C is the portion of soil-derived CO_2-C in excess of basal CO_2-C; basal CO_2-C, represented by the *dashed reference lines* through the soil-derived components, was constant for each soil and assumed equal to total CO_2-C in control (CTL) treatments. Significant differences in the evolution of sucrose- and soil-derived CO_2-C between treatments for each soil type are highlighted by *asterisks* ($P \leq 0.05$). Data are means ± 1 standard error (n = 4)

mid-fertility soils following CNPK compared to C additions (Fig. 2). Cumulative evolution of sucrose-C 8 days following the addition of 4 mg sucrose-C g^{-1} was higher for soils in the order low-fertility < high-fertility < mid-fertility, with 0.50, 0.72 and 1.00 mg CO_2-C g^{-1} respired respectively. Following CNPK additions, cumulative evolution of sucrose-derived C was higher for soils in the order high-fertility < low-fertility < mid-fertility, with respiration of 0.97, 1.18, 1.42 mg CO_2-C g^{-1}, respectively; significantly higher compared to C additions for all soils ($P < 0.01$; Fig. 3). Sucrose-derived CO_2-C evolution following addition of CNPK compared to C alone was proportionally higher for soils in the order high-fertility (35%) < mid-fertility (46%) < low-fertility (136%) (Fig. 3).

Soil nutrients

Concentrations of NO_3^- and extractable P in untreated soils were higher in the order low-fertility < mid-fertility < high-fertility, with significant differences between all soil types ($P < 0.05$; Table 2). There was an exception to this pattern for NH_4^+, which was higher in soils in the order low-fertility = high-fertility < mid-fertility, with significantly higher concentrations of NH_4^+ in mid-fertility compared to other soils ($P < 0.05$).

The concentrations of NO_3^-, NH_4^+ and extractable P were generally lower in soil where sucrose-C was added alone compared to all other treatments, with the exception of extractable P in the low-fertility soil, in which concentrations in untreated soils were already very low (Table 2). Following NPK and CNPK additions, there were significantly higher concentrations of NO_3^-, NH_4^+ and extractable P for all soils ($P < 0.01$) except NH_4^+ following CNPK addition in the low-fertility soil ($P = 0.28$), relative to controls (Table 2).

Soil pH was significantly different in some treated soils relative to controls: higher for low-fertility following CNPK additions, higher for the high-fertility soil following C and CNPK additions, and lower for the low-fertility soil following NPK additions (Table 2).

Microbial biomass

The concentrations of microbial C and microbial N in untreated soils increased in the order low-fertility < mid-fertility < high-fertility, with significant differences between all soils ($P < 0.05$; Fig. 4); while microbial P concentrations increased in the order: low-fertility = mid-fertility < high-fertility with significantly higher concentrations of microbial P in the high-fertility soil compared to other soils (for all comparisons, $P < 0.05$). It was notable that, although the low-fertility soil had a concentration of extractable P four-fold lower than the mid-fertility soil (0.7 cf. 2.9 mg kg^{-1}), there was no significant difference in microbial P between the two soils ($P = 0.20$).

Microbial C was significantly higher in CNPK compared to C treated soils for low- and high-fertility ($P < 0.05$), but not mid-fertility soils ($P > 0.05$; Fig. 4). Microbial N was significantly higher in the CNPK treated low-fertility soil, but was significantly lower in CNPK treated high-fertility and NPK treated mid-fertility soils relative to untreated controls (for all comparisons, $P < 0.05$; Fig. 4). Microbial P was significantly higher in all CNPK treated soils relative to untreated controls (for all comparisons, $P < 0.05$; Fig. 4). The ratio of microbial C:N was significantly higher in CNPK treated high-fertility soils ($P < 0.05$) and there was a trend of higher ratios of microbial C:P in all C treated soils, relative to controls.

Enzyme activities

There were many significantly lower enzyme activities in treated soils relative to control soils. In

Table 2 Nutrients and pH in surface soils measured 3 days following treatments

			Low-fertility	Mid-fertility	High-fertility
NO_3^-	mg N kg^{-1}	CTL	1.8 ± 0.4	8.0 ± 0.4	22.3 ± 0.7
		+ NPK	31.7 ± 1.9*	47.8 ± 1.3*	91.1 ± 3.0*
		+ C	0.2 ± 0.1	1.0 ± 0.1*	0.1 ± 0.1*
		+ CNPK	22.2 ± 2.2*	23.6 ± 07*	45.3 ± 1.1*
NH_4^+	mg N kg^{-1}	CTL	0.3 ± 0.1	1.7 ± 0.3	0.3 ± 0.1
		+ NPK	45.0 ± 2.2*	26.5 ± 1.2*	83.9 ± 3.4*
		+ C	0.1 ± 0.1	0.3 ± 0.1	0.2 ± 0.1
		+ CNPK	1.1 ± 0.7	7.0 ± 0.6*	14.5 ± 2.3*
Extractable P	mg P kg^{-1}	CTL	0.7 ± 0.1	2.9 ± 0.1	4.6 ± 0.2
		+ NPK	234.3 ± 24.5*	102.2 ± 11.4*	461.9 ± 33.0*
		+ C	0.7 ± 0.1	0.6 ± 0.2	0.9 ± 0.2
		+ CNPK	179.3 ± 14.9*	92.9 ± 22.8*	256.0 ± 20.1*
pH		CTL	4.4 ± 0.1	6.3 ± 0.1	6.8 ± 0.1
		+ NPK	4.2 ± 0.1	5.6 ± 0.1*	6.8 ± 0.1
		+ C	4.5 ± 0.1	6.1 ± 0.1	7.1 ± 0.1*
		+ CNPK	4.7 ± 0.1*	6.3 ± 0.1	7.0 ± 0.1*

Significant differences between treatments and controls are highlighted by asterisks (where $P \leq 0.05$). Data are means ± 1 standard error (n = 4)

particular, the low-fertility soil had lower enzyme activities following NPK addition (β-glucosidase, *N*-acetyl β-glucosaminidase, phosphomonoesterase, $P < 0.001$); following C addition (β-glucosidase, cellobiohydrolase, $P < 0.01$; phosphomonoesterase, xylanase, $P < 0.001$) and following CNPK addition (phosphomonoesterase, xylanase $P < 0.001$) compared to controls (Table 3). In the mid-fertility soil there was significantly lower xylanase activity following C addition ($P < 0.05$) and in the high-fertility soil significantly lower cellobiohydrolase and xylanase activity following C addition ($P < 0.05$) and β-glucosidase activity following CNPK addition ($P < 0.05$), compared to controls.

There were also several significantly higher enzyme activities in treated soils relative to control soils. In the low-fertility soil, phenol oxidase was higher following C addition ($P < 0.05$). In the mid-fertility soil, phosphomonoesterase and phenol oxidase were higher following C addition ($P < 0.05$) and *N*-acetyl β-glucosaminidase was higher following CNPK addition ($P = 0.05$). In the high-fertility soil there were significantly higher activities for β-glucosidase following NPK addition ($P < 0.05$), *N*-acetyl β-glucosaminidase following CNPK addition ($P = 0.05$), and phosphomonoesterase following C additions ($P < 0.001$).

PLFA concentration and carbon incorporation

Total concentration of PLFA was similarly high in low- and high-fertility soils; both were significantly higher than the mid-fertility soil ($P < 0.001$; Table 4). Fungal:bacterial ratios increased in soils in the order mid-fertility (0.08) < high-fertility (0.12) < low-fertility (0.16). Gram-positive bacteria PLFA concentrations increased in soils in the order low-fertility < mid-fertility < high-fertility, whereas Gram-negative bacteria PLFA and fungal PLFA concentrations increased in the order mid-fertility < high-fertility < low-fertility. There were no significant differences in total PLFA concentration following any treatments; the only microbial group that was significantly different following treatments was the higher concentration of fungal PLFA biomarkers in the low-fertility soil following CNPK additions ($P < 0.001$; Fig. 5; Table 4). Accordingly, fungal:bacterial ratios were not significantly different following treatments among soils except for low-fertility soil following CNPK additions, where they increased from 0.16 to 0.52 ($P < 0.001$; Table 4).

Following C addition, there was significant sucrose-derived C incorporation in the low-fertility soil for a saturated (18:0) biomarker; mid-fertility soil for

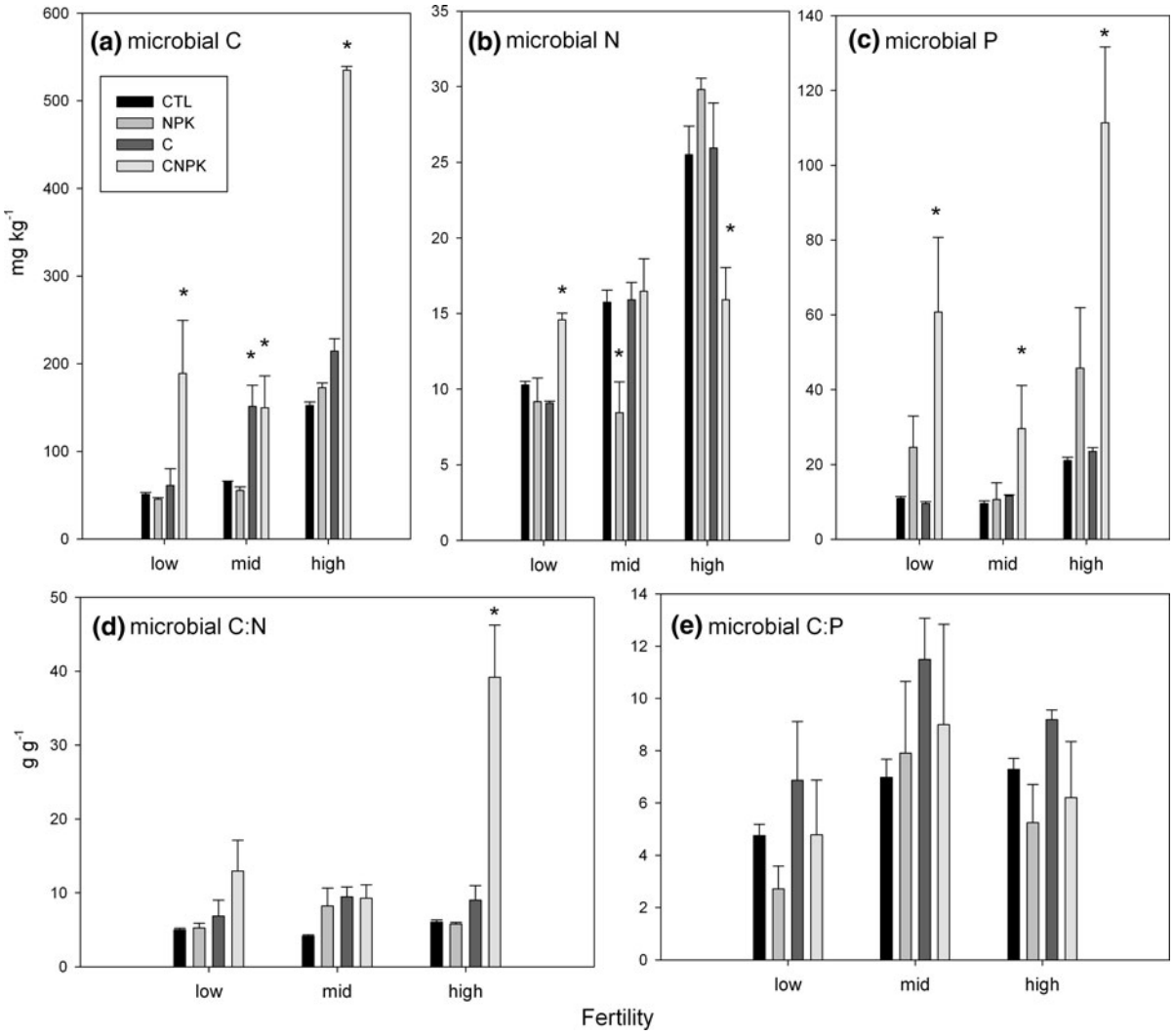

Fig. 4 Microbial carbon (**a**), nitrogen (**b**) and phosphorus (**c**); and the ratios of carbon/nitrogen (**d**) and carbon/phosphorus (**e**) in surface soils measured 3 days following treatments. Significant differences ($P \leq 0.05$) are shown by asterisks between +CNPK and +C treated soils. Data are means ± 1 standard error (n = 4)

Gram-positive (i15:0, 7Me17:0), Gram-negative (16:1ω7, 18:1ω7), saturated (16:0, 18:0) and fungal (18:1ω9) biomarkers; and high-fertility soil for Gram-positive (a15:0, i16:0, i17:0), Gram-negative (16:1ω7) saturated (16:0) and fungal (18:2ω6, 18:1ω9) biomarkers (for all comparisons, $P < 0.001$; Fig. 5). Following CNPK addition, sucrose-derived C was significantly incorporated into PLFA in the low-fertility soil for Gram-negative (16:1ω7, 19:1), saturated (16:0, 18:0) and fungal (18:1ω6, 18:1ω9) biomarkers with major incorporation into fungal biomarkers ($P < 0.001$; Fig. 5). In mid- and high-fertility soils CNPK additions led to significant sucrose-derived C incorporation in broad ranges of biomarkers: in Gram-positive (i15:0, a15:0, i16:0), Gram-negative (16:1ω7, 18:1ω7), saturated (16:0, 18:0) and fungal (18:2ω6, 18:1ω9) biomarkers in the mid-fertility soil; and in Gram-positive (i15:0, a15:0, i16:0, i17:0), Gram-negative (18:1ω7), saturated (16:0) and fungal (18:2ω6, 18:1ω9) biomarkers in the high-fertility soil ($P < 0.001$; Fig. 5).

Soil-derived C concentrations in PLFA for treated soils compared to controls were only significantly different following CNPK additions. There was significantly less soil-derived C in Gram-negative

Table 3 Enzyme activities in soils three days following treatments

	N-cycling	P-cycling	C-cycling			
	N-acetyl glucosaminidase (nmol MU min^{-1} g^{-1})	Phosphomonoesterase (nmol MU min^{-1} g^{-1})	β-Glucosidase (nmol MU min^{-1} g^{-1})	Cellobiohydrolase (nmol MU min^{-1} g^{-1})	Xylanase (nmol MU min^{-1} g^{-1})	Phenol oxidase (mg h^{-1} g^{-1})
Low-fertility						
CTL	3.42 ± 0.16	40.12 ± 1.05	3.05 ± 0.17	0.31 ± 0.01	1.43 ± 0.01	0.60 ± 0.08
+ NPK	2.02 ± 0.21*	26.79 ± 0.58*	1.72 ± 0.10*	0.33 ± 0.02	1.27 ± 0.05	0.77 ± 0.08
+ C	2.76 ± 0.19	33.35 ± 1.14*	1.93 ± 0.08*	0.26 ± 0.03*	1.00 ± 0.13*	0.90 ± 0.02*
+ CNPK	3.26 ± 0.08	31.45 ± 0.81*	2.69 ± 0.02	0.31 ± 0.05	0.96 ± 0.11*	0.86 ± 0.03
Mid-fertility						
CTL	1.82 ± 0.12	7.80 ± 0.45	1.03 ± 0.08	0.17 ± 0.01	0.52 ± 0.09	1.62 ± 0.03
+ NPK	1.75 ± 0.07	7.25 ± 0.27	1.01 ± 0.07	0.18 ± 0.01	0.49 ± 0.03	1.51 ± 0.03
+ C	1.76 ± 0.25	12.71 ± 0.79*	0.78 ± 0.05	0.15 ± 0.02	0.28 ± 0.02*	1.99 ± 0.06*
+ CNPK	2.29 ± 0.18*	6.56 ± 0.36	0.98 ± 0.05	0.17 ± 0.01	0.34 ± 0.01	1.82 ± 0.14
High-fertility						
CTL	1.43 ± 0.14	6.32 ± 0.27	2.59 ± 0.07	0.45 ± 0.01	0.85 ± 0.06	1.01 ± 0.08
+ NPK	2.02 ± 0.25	6.84 ± 0.37	2.99 ± 0.15*	0.43 ± 0.02	0.80 ± 0.02	1.10 ± 0.10
+ C	1.95 ± 0.09	12.28 ± 0.36*	2.29 ± 0.06	0.34 ± 0.04*	0.65 ± 0.02*	1.11 ± 0.04
+ CNPK	2.16 ± 0.19*	7.79 ± 0.58	2.15 ± 0.06*	0.39 ± 0.02	0.71 ± 0.08	1.18 ± 0.05

Significant differences between treatments and controls are highlighted by asterisks ($P \leq 0.05$). Data are means ± 1 standard error (n = 4)

Table 4 PLFA concentration within microbial groups 3 days following treatments

	Total (μg C g^{-1} soil)	Bacterial (μg C g^{-1} soil)	Gram-positive bacterial (μg C g^{-1} soil)	Gram-negative bacterial (μg C g^{-1} soil)	Fungal (μg C g^{-1} soil)	Saturated (μg C g^{-1} soil)	Fung/Bact
Low-fertility							
CTL	62.9 ± 10.1	42.1 ± 7.1	14.7 ± 1.8	27.4 ± 5.5	6.9 ± 1.1	13.9 ± 2.1	0.16 ± 0.01
+ C	55.7 ± 2.5	37.2 ± 1.8	12.9 ± 0.5	24.3 ± 1.3	6.2 ± 0.4	12.2 ± 0.4	0.2 ± 0.0
+ CNPK	63.7 ± 3.7	34.3 ± 0.9	10.9 ± 0.2	23.5 ± 0.8	18.0 ± 2.7*	11.4 ± 1.9*	0.52 ± 0.09*
Mid- fertility							
CTL	46.9 ± 3.0	35.2 ± 5.5	18.1 ± 3.0	17.1 ± 2.6	2.9 ± 0.4	8.8 ± 1.3	0.08 ± 0.01
+ C	39.1 ± 4.7	28.2 ± 3.6	13.6 ± 1.8	14.7 ± 2.0	2.4 ± 0.2	8.5 ± 1.0	0.1 ± 0.0
+ CNPK	47.3 ± 1.8	34.8 ± 0.7	15.8 ± 0.6	19.1 ± 1.3	2.6 ± 0.1	9.8 ± 1.3	0.07 ± 0.01
High-fertility							
CTL	63.9 ± 32	46.6 ± 2.7	22.4 ± 1.7	24.2 ± 1.6	5.6 ± 0.3	11.6 ± 0.8	0.12 ± 0.01
+ C	69.0 ± 4.9	50.1 ± 3.9	23.6 ± 2.3	26.5 ± 1.7	5.6 ± 0.4	13.3 ± 0.7	0.1 ± 0.0
+ CNPK	71.2 ± 5.8	55.8 ± 5.1	27.3 ± 2.7	28.5 ± 2.5	6.9 ± 0.6	8.5 ± 1.9	0.12 ± 0.01

Fungal/bacterial ratios are the ratio of 18:2ω6,9:bacterial PLFAs. Significant differences between treatments and controls are highlighted by asterisks ($P \leq 0.05$). Data are means with 1 SE (n = 4)

(17:1ω8) and fungal (18:1ω9) biomarkers in the low-fertility soil and significantly less soil-derived C in a fungal (18:2ω6) biomarker in the high-fertility soil ($P < 0.001$). There was significantly higher soil-derived C in a Gram-negative biomarker (16:1ω7) in both mid- and high-fertility soils following CNPK addition ($P < 0.001$).

The relatively large treatment and soil-fertility effects on sucrose-derived C within PLFA compared to the smaller effects on soil-derived C within PLFA were summarized by multivariate analyses (Fig. 6). The concentration of soil-derived C within PLFA varied widely among treatments and soils (Fig. 6a). In contrast, for the concentration of sucrose-derived C within PLFA (Fig. 6b) there was a clear separation along the X-axis according to treatment (explaining 34% of variation) and along the Y-axis according to soil-fertility (explaining 28% of variation).

Discussion

Priming and soil fertility

The largest release of additional soil-derived (i.e. primed) CO_2 following sucrose-C addition was for soil from the site of intermediate fertility, suggesting that priming and soil fertility were not linearly correlated. Microbial metabolism of sucrose-C, on the other hand, was limited by soil fertility, because respiration of sucrose-derived CO_2 was proportionally higher when added in combination with nutrients for soils in the order of decreasing fertility. Therefore, factors other than fertility constrained microbial metabolism of soil C through priming in the low-fertility soil. We hypothesize that priming was limited by both nutrient deficiency to microorganisms (Blagodatskaya et al. 2007) and the concentration and 'lability' of soil organic matter (Kuzyakov and Bol 2006). Thus, sucrose-C additions did not lead to significant priming in the high fertility soil because microbial nutrient limitation was insufficient, while in the low fertility soil priming was limited because the pool of soil C amenable to priming was insufficient. Priming may also have been influenced by soil pH and mineralogy. Soil pH can affect priming due to the adsorption of enzymes on soil surfaces, which occurs when soil pH is lower than the isoelectric point of the enzyme (Quiquampoix 2000); clay content can affect priming due to the stabilization of soil organic C within clay-mineral complexes, which can protect it from priming (Rasmussen et al. 2007). In the low-fertility soil where priming was lowest, both clay content and pH were relatively low, suggesting that pH rather than

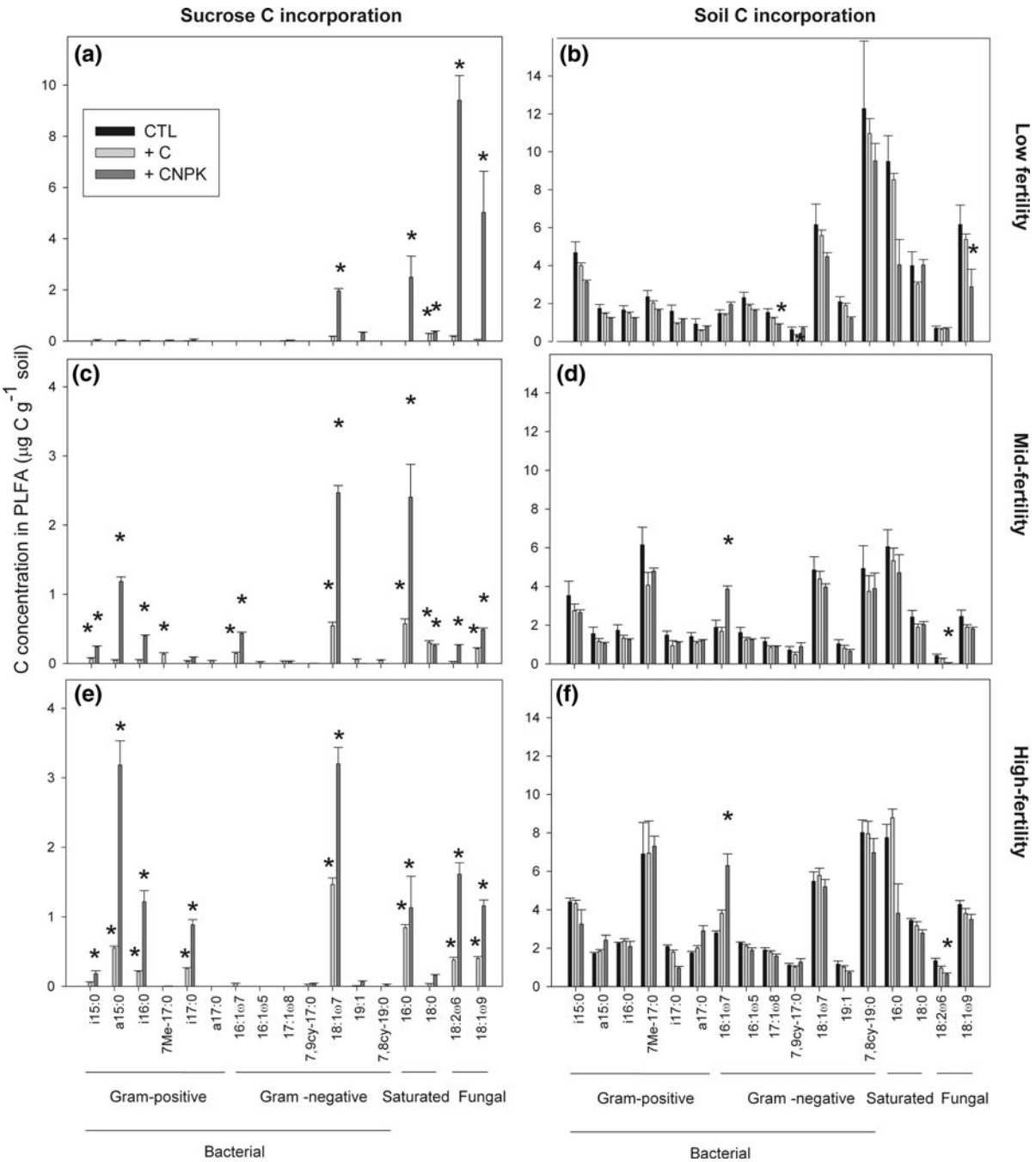

Fig. 5 Concentration of sucrose-derived C and soil-derived C within PLFA 3 days after + C and + CNPK and CTL treatments for low-fertility (**a**, **b**), mid-fertility (**c**, **d**) and high-fertility (**e**, **f**) soils. Significant incorporation of sucrose-derived C and significant differences in soil-derived C between treatments and controls are highlighted by asterisks (where $P \leq 0.05$). Data are means ± 1 standard error ($n = 4$)

mineralogy may have had some influence on priming. However, our hypothesis that sucrose-C addition did not induce significant priming because high nutrient demand in this soil had already led to depletion of the limited pool of C amenable to priming was supported by the high enzyme activities and low organic C

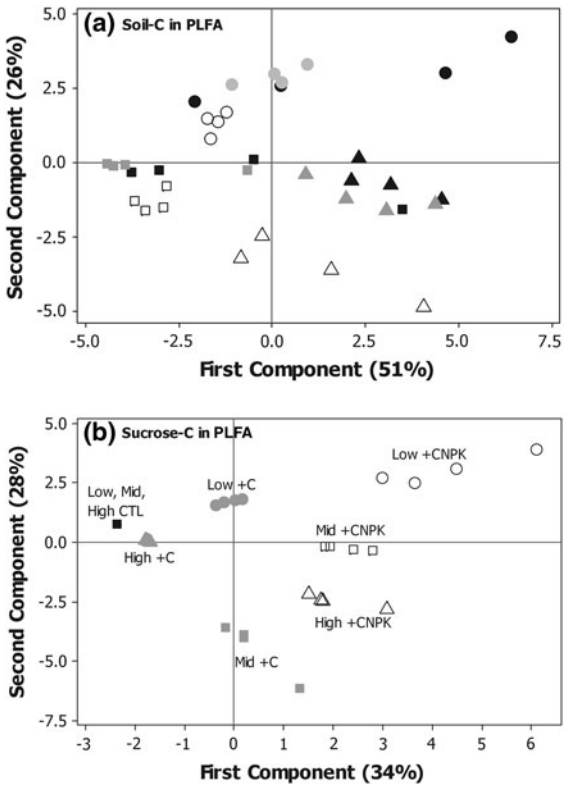

Fig. 6 The effect of sucrose-C and nutrient additions on concentration of soil-derived C and sucrose-derived C within microbial community composition as revealed by principal components analysis, showing **a** soil-derived C concentration in PLFA and **b** sucrose-derived C concentration in PLFA. Data are grouped into low-fertility (*circles*), mid-fertility (*squares*) and high-fertility (*triangles*) soils following sucrose-C addition (+C, *grey symbols*), sucrose and nutrient addition (+CNPK, *white symbols*) and no addition controls (CTL, *black symbols*)

concentration in the untreated low-fertility soil—it has the lowest C concentration of these three soils, and also of a much wider set of soils in the region (Table 1; Turner and Engelbrecht 2011). We suggest that soils at this site undergo constant and intense priming during the decomposition of plant residues, consistent with descriptions of priming as a phenomena that preferentially degrades labile fractions of soil organic matter (Kuzyakov and Bol 2006) and constrains the accumulation of C in surface soils (Fontaine et al. 2007).

Priming mechanisms

Our results suggest strongly that priming was due to microbial demand for nutrients required to mineralize the added sucrose-C. This was indicated by significant reductions in priming when C was added in combination with nutrients (100% for low-fertility, 33% for mid-fertility, 44% for high-fertility soils; Fig. 3), the depletion of NO_3^-, NH_4^+ and extractable P (Table 3), the increases in microbial C:N and C:P ratios (Fig. 4), and increases in phosphomonoesterase activity (Table 3). It seems likely that when sucrose-C was added alone, energy derived from its metabolism was used to degrade soil organic matter through production of extracellular enzymes to liberate additional nutrients, which simultaneously made soil C available for microbial metabolism ('co-metabolism'; Kuzyakov et al. 2000).

The rapid priming (the peak in primed-CO_2 occurred before the peak in sucrose derived-CO_2) due to microbial nutrient demand as indicated by our data is consistent with some studies of temperate soils (Zyakun and Dilly 2005; Blagodatskaya et al. 2007; Nottingham et al. 2009), but at odds with others that do not predict such rapid priming (Fontaine et al. 2004) or an increased production of extracellular enzymes (De Nobili et al. 2001). This suggests that different mechanisms may apply in different soils on different time scales. For example, priming through increased abundance and activity of slow-growing recalcitrant-C degrading microorganisms that persist after the added labile C is exhausted (Fontaine et al. 2004) is not consistent with our detection of short-term priming, which was greatest in the soil of intermediate fertility with no change in microbial community composition. Priming by increased growth of recalcitrant-C degrading microorganisms may be more important on longer time scales following addition of substrates that take longer to decompose (e.g. the long-term depletion of soil C and altered microbial community composition in Carney et al. (2007)). Similarly inconsistent with our results is 'apparent priming', either through turnover of soil microbial biomass (Wu et al. 1993), or a small increase in microbial mineralization of endo-cellular C reserves with no change in decomposition of soil organic matter (where microorganisms increase their metabolic activity following a trace C input in anticipation of a larger input in the near future; De Nobili et al. 2001). These apparent priming mechanisms are not consistent with the large release of soil-derived C from the mid-fertility soil (360 mg CO_2-C g^{-1} soil), which was 120% greater than the original concentration of microbial C. Further evidence that priming measured in our study was not 'apparent'

was that despite the cumulative release of 260 mg soil-derived CO_2-C g^{-1} soil over three days between sucrose-C addition and extraction of soil for PLFA analyses, there was no significant reduction in soil C within PLFA biomarkers (Fig. 5), which would be predicted if there was significant substitution of soil-labeled C by sucrose-labeled C within microorganisms (see Perelo and Munch 2005; Schneckenberger et al. 2008; Nottingham et al. 2009). Thus, apparent priming can only account for a minor part of the short-term increase in the efflux of soil-derived CO_2 measured in our study.

Microbial carbon utilization: enzyme activity and PLFA

Microbial priming of C from soil organic matter was supported in part by changes in activities of enzymes that degrade organic C. We measured higher activity of phenol oxidase during priming in low- and mid-fertility soils. This oxidative enzyme catalyzes the degradation of phenolic C compounds, including lignin and humus, and can limit the accumulation of soil organic matter (Sinsabaugh 2010). Oxidative enzymes have been linked to stimulated decomposition of recalcitrant-C in experimentally warmed soils (Waldrop and Firestone 2004b) and soils beneath a deciduous forest growing under elevated CO_2 through priming effects (Carney et al. 2007). The lack of increase in phenol oxidase activity when nutrients were added to soils alongside C (Table 3), a result similar to that found by Carreiro et al. (2000) following leaf-litter and nutrient additions to a temperate deciduous forest soil, suggests that its induction was in response to nutrient-deficiency and associated with microbial acquisition of nutrients from soil organic matter.

The cause of differences in the activities of hydrolytic C-degrading enzymes during priming was less clear. The addition of sucrose-C led to lower activities of several hydrolytic enzymes that catalyze the degradation of more labile forms of organic C; β-glucosidase (in low- and high-fertility soils), cellobiohydrolase (in low- and high-fertility soils), and xylanase (in all soils). These patterns can be explained by a depletion of labile forms of soil C following priming; the sucrose-stimulated microorganisms rapidly exhausted the organic-C forms that are degraded by these enzymes. A similar pattern was detected by Waldrop and Firestone (2004b) who attributed a 50% reduction in activity of hydrolytic cellulases and hemicellulases in experimentally warmed soils to a depletion of labile soil C. The generally lower activities may also be due to microbial metabolism of the enzymes themselves as a nutrient source following sucrose-C addition (e.g. as a N source; Treseder and Vitousek 2001), or of the soil organic matter to which these enzymes were adsorbed.

The absence of reduction in soil-derived C within PLFA during priming provided strong evidence that the 'primed' CO_2 efflux originated from soil organic matter rather than turnover of microbial biomass. However, whether priming resulted from the activity of specific microbial groups was inconclusive. The parallel stimulation of priming and activity of phenol oxidase suggested that priming was induced by actinomycetes or fungi, known producers of phenol oxidase (Kirk and Farrell 1987), but this was not reflected by stimulated incorporation of primed soil C into their PLFA biomarkers (Fig. 5; the PLFA biomarker, 10Me18:0 for actinomycetes was not detected; Frostegård and Bååth 1993). We found a higher concentration of soil-derived C within the Gram-negative biomarker 16:1ω7 (following CNPK addition to mid- and high-fertility soils; Fig. 5), which has been detected by other studies following substrate additions to temperate soils (Waldrop and Firestone 2004a; Nottingham et al. 2009). The soil C incorporated into this bacterial biomarker may originate from primed soil organic matter, but it was notable that in our study there was no increase in soil C-incorporation into this biomarker in soils when sucrose-C was added alone and priming was greatest. Further studies using different molecular techniques are required to resolve whether: (i) specific microorganisms caused priming and there was rapid release and re-assimilation of C within microorganisms following death of microorganisms to re-distribute soil-labeled C within PLFA biomarkers, or (ii) priming resulted from the activity of various functionally redundant microbial groups.

While there were no conclusive patterns in primed soil-derived C within microbial groups, there were clear patterns in sucrose-derived C (Fig. 6). Sucrose-derived C was incorporated into similar bacterial biomarkers (a15:0, 16:1ω7) for mid- and high-fertility soils, but for the low-fertility soil it was preferentially incorporated into fungal biomarkers (18:2ω6, 18:1ω9), which led to a large shift in

microbial community composition in CNPK-treated soils (Table 4). Fungal-based food webs are more retentive of nutrients than bacterial-based foods webs (Coleman et al. 1983), are associated with more acidic soils (Alexander 1964; low-fertility soils had pH of 4.4) and with later retrogressional and therefore less fertile stages of soil development (Allison et al. 2007; Wardle et al. 2008). A recent experiment on a soil devoid of organic matter similarly found that the metabolism of added sucrose-C was predominantly by fungi (Engelking et al. 2008). Thus, we hypothesize that due to low pH and low concentration of available nutrients (Table 3) nutrient cycling was more retentive and dominated by fungal activity in the low-fertility soil. Further evidence for this was the high enzyme activity in the untreated low-fertility soil (Table 4), an indication of high allocation of microbial resources into nutrient acquisition.

Microbial nutrient limitation and acquisition of nutrients

Priming most likely resulted from microbial utilization of organic nutrients, in particular P, with evidence for both increased demand and increased acquisition of P by microorganisms during priming. The increased acquisition of mineral nutrients during priming was reflected by lower concentrations of mineral N and P in sucrose-C treated soils (Table 2), although this was not reflected as a significant increase in microbial N or P (Fig. 4), which is not surprising given that concentrations of microbial nutrients were up to an order of magnitude higher than concentrations of mineral nutrients. An increased microbial demand for P was reflected by significantly higher phosphomonoesterase activity for both mid- and high-fertility soils following sucrose-C addition compared to controls, providing evidence that there was increased microbial production of phosphomonoesterase in response to P deficiency to access additional P from organic sources. In contrast, there was no change in *N*-acetyl β-glucosaminidase activity (to release N) following sucrose-C addition (Table 3), suggesting that N was less limiting than P or that other enzymes we did not measure were involved in N-acquisition (e.g. enzymes involved in the hydrolysis of amino acids; Acosta-Martinez and Tabatabai 2000). Our interpretation of up-regulated phosphomonoesterase activity is supported by several studies that have measured higher phosphomonoesterase activity in response to greater P deficiency in soils (Olander and Vitousek 2000; Allison et al. 2007; Sinsabaugh et al. 2008).

Priming and nutrient limitation in tropical forests

Our study provides further evidence that when soil microorganisms have sufficient energy, the availability of soluble soil nutrients regulates soil organic C mineralization by priming (Fontaine et al. 2011). Furthermore, our study suggests that the ultimate control on microbial respiration in moist and aerobic soils is the availability of labile C rather than nutrients, and that nutrients only limit microbial respiration when labile C is abundant (Fig. 2; NPK additions had no significant effect on CO_2 efflux, data not shown). While we are cautious in making 'real world' inferences based on results from our laboratory study, the implication that the controls on microbial sources of soil CO_2 efflux shift between labile C and nutrients, with priming occurring when there are large inputs of labile C relative to nutrients, is supported by observations in tropical forests. Two studies hypothesized a release of primed soil organic matter within soil CO_2 efflux following the addition of C to soils: Sayer et al. (2007) to explain increased soil CO_2 efflux from soils that received experimentally increased litterfall, and Cleveland et al. (2010) to explain increased soil CO_2 efflux from soils that received more concentrated dissolved organic matter following a drought treatment. Two further studies in tropical forests where nutrients were added without C further support our interpretation that priming occurs when there are large inputs of labile C relative to nutrients; both studies provide evidence in support of suppressed priming in the presence of excess nutrients. Koehler et al. (2009) observed no or negative responses of soil CO_2 efflux to N additions, while Cleveland and Townsend (2006) observed little response of soil CO_2 efflux to P addition, except during the dry-wet season transition when litter decomposition rates were highest and therefore the C limitation of microbial activity was lower.

Priming resulting from nutrient limitation in tropical forest soils, which can contain abundant organic N (Hedin et al. 2005) and organic P (Turner and Engelbrecht 2011; Dieter et al. 2010), would have large consequences for the tropical forest C balance under elevated CO_2 by increasing the pool of

nutrients available for plant assimilation and destabilizing soil organic C. In particular, we suggest that P limitation of heterotrophic activity in tropical forest soil (as demonstrated by Hobbie and Vitousek 2000; Cleveland et al. 2006; Cleveland and Townsend 2006; Kaspari et al. 2008), is alleviated in the presence of excess labile organic C to provide energy for mineralization of organic P in parallel with priming effects.

Conclusions

This is the first study to investigate priming effect mechanisms in tropical forest soils. Priming resulted from microbial nutrient limitation and appeared to be stimulated by microbial acquisition of P from organic matter. Priming appeared to be constrained in low fertility soils by low concentrations of organic C, and in high fertility soils by the abundance of available nutrients. This provides a framework for understanding the extent and distribution of priming effects in lowland tropical forests, and suggests that nutrient availability constrains soil C storage in such ecosystems. However, a better understanding of contrasting tropical forest soils and how their properties may influence priming intensity on different time scales is still required before this potentially important feedback mechanism can be included in C-cycle models.

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