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Soil Microbial Community-Level Physiological Profiling as Related to Carbon and Nitrogen Availability Under Different Land Uses

María Celina ZABALOY[1,*], Jay L. GARLAND[2], Marco ALLEGRINI[3] and Elena del Valle GOMEZ[3]

[1] *Centro de Recursos Naturales Renovables de la Zona Semiárida, CONICET, Bahía Blanca 8000 (Argentina)*
[2] *Microbiological and Chemical Exposure Assessment Research Division, Environmental Protection Agency, Cincinnati OH 45268 (USA)*
[3] *Facultad de Ciencias Agrarias Universidad Nacional de Rosario, Zavalla 2125 (Argentina)*

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ABSTRACT

The goal of this work was to assess soil microbial respiration, determined by the assay of community-level physiological profiling in an oxygen-sensitive microplate (O_2-CLPP), in response to endogenous C and several individual C substrates in the soils with different organic C contents (as a function of soil type and management practice). We also used the O_2-CLPP to determine the respiratory response of these soils to endogenous C and amended C substrates with N addition. A respiratory quotient (RQ) was calculated based on the ratio of the response to endogenous soil C *vs.* each C-only substrate, and was related to total organic carbon (TOC). For assessing N availability for microbial activity, the effect of N supplementation on soil respiration, expressed as N_{ratio}, was calculated based on the response of several substrates to N addition relative to the response without N. Soils clustered in 4 groups after a principal component analysis (PCA), based on TOC and their respiratory responses to substrates and endogenous C. These groups reflected differences among soils in their geographic origin, land use and C content. Calculated RQ values were significantly lower in natural forest soils than in managed soils for most C-only substrates. TOC was negatively correlated with RQ ($r = -0.65$), indicating that the soils with higher organic matter content increased respiratory efficiency. The N addition in the assay in the absence of C amendment (*i.e.*, only endogenous soil C present) had no effect on microbial respiration in any soil, indicating that these soils were not intrinsically N-limited, but substrate-dependent variation in N_{ratio} within soil groups was observed.

Key Words: community-level physiological profiling, N limitation, oxygen biosensor system, soil organic C, soil respiration

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Land use and associated plant communities can influence belowground microbial communities through multiple factors, including the type and amount of C and nutrient inputs (Liao and Boutton, 2008). Soil organic matter (SOM) comprises animal detritus, plant litter and exudates, and decaying microbial biomass. It is widely recognized that the availability of organic C regulates the turnover and activities of heterotrophic microbial communities in soils (Degens *et al.*, 2000) and, at the same time, microbial community composition, size and physiology may affect the degradation rates of SOM (Waldrop and Firestone, 2004; Cleveland *et al.*, 2014). Therefore, deeper understanding of the microbial role in soil C and nutrient cycling is needed to better predict ecosystem responses to human-mediated changes in land use, climate and nutrient availability (van Hees *et al.*, 2005; Hartmann and Richardson, 2013).

Soil microbiota mineralizes SOM mainly *via* the respiratory process, which consumes O_2 and releases CO_2 to the atmosphere (Dilly, 2003; van Hees *et al.*, 2005). The soil microbiota is generally substrate-limited under natural conditions, which is reflected by the strong increase in microbial respiratory activity after the addition of readily available C such as glucose (Dilly, 2005). In turn, C turnover closely depends on the availability of major nutrients like N and P for microbial activity because microbes need to maintain a stoichiometric balanced composition of C and nutrients (Manzoni *et al.*, 2012). Thus, C input may alter the physiological balance of the microbial community, triggering nutrient limitations or shifting the C partitioning in the cells (Schimel and Weintraub, 2003; Orwin *et al.*, 2006). N limitation either inhibits the uptake of substrate C or no inhibition occurs but excessive C is routed to overflow respiration, with concomitant decrease in C use efficiency (*i.e.*, the proportion of added C that is respired *vs.* retained in the cell

*Corresponding author. E-mail: mzabaloy@uns.edu.ar.

biomass, Manzoni *et al.*, 2012).

A generally accepted index of C use efficiency is the metabolic quotient (qCO_2, defined as respiration rate per unit biomass). This variable has value as a relative measure of how efficiently soil microbial biomass is utilizing C resources, and of the degree of substrate limitation for soil microbial biomass (Wardle and Ghani, 1995; Ohtonen *et al.*, 1999). Cheng *et al.* (1996) defined a C availability index as the ratio of basal respiration rate to substrate-induced respiration (SIR), which is analogous to the qCO_2 if microbial biomass is determined by the SIR approach and soil is analyzed without preconditioning for stabilization of the respiration rate (Dilly, 2005). Soil type, litter quality (Dilly and Munch, 2004), and management practices (*e.g.*, tillage systems, crop rotations, pastures, organic farming, applications of agrochemicals and land-use changes) (Kaschuk *et al.*, 2010; Lupatini *et al.*, 2013) are known to affect both indices. Ultimately, all these factors may affect the proportion of zymogenous microorganisms (*i.e.*, *r*-strategists species with high qCO_2) to autochthonous microbes (*i.e.*, *K*-strategists species with low qCO_2) (Montecchia *et al.*, 2011).

The capacity of a microbial community to mineralize specific C substrates likely reflects the *in situ* activity, which in turn may mirror differences in the type, abundance and bioavailability of C sources present in the organic matter pool (Orwin *et al.*, 2006; Banning *et al.*, 2012). Garland and Mills (1991) introduced an approach for visualizing heterotrophic microbial communities as a multivariate profile of responses to multiple sole C sources, or community-level physiological profiling (CLPP). A microtiter-based assay containing an O_2-sensitive fluorophore as detection system (Becton-Dickinson Oxygen Biosensor System®, BDOBS; Wodnicka *et al.*, 2000) allows measurement of basal soil respiration and substrate-induced responses, *i.e.*, community-level physiological profiling in an oxygen-sensitive microplate (O_2-CLPP), at low amendment levels (∼100 μg C g^{-1} soil) (Zabaloy *et al.*, 2008), limiting the selective enrichment bias of previous CLPP methods (Garland *et al.*, 2012). Other than the examination of the physiological profiling of microbial communities (Väisänen *et al.*, 2005; Montecchia *et al.*, 2011), this approach has also proved suitable to examine nutrient (mainly for N and P) limitations in a wide range of soils (Väisänen *et al.*, 2005; Brown *et al.*, 2009; Gomez and Garland, 2012). The difference in O_2 consumption in soils with and without N addition in the assay transiently decreased following fertilization of laboratory microcosms (Zabaloy *et al.*, 2008) and agricultural soils (Garland *et al.*, 2010). Moreover, the response to N addition in the assay followed extractable inorganic N contents in a field soil (Garland *et al.*, 2010). Previous work, thus, suggests that the O_2-CLPP approach provides an integrated means of evaluating soil microbial respiration, which involves functional potential of substrate use, metabolic efficiency and detection of N limitation.

The goal of this work was to assess soil microbial respiration, determined by the assay of O_2-CLPP, in response to endogenous C and several individual C substrates in the soils with different organic C contents (as a function of soil type and management practice). Additionally, the O_2-CLPP was used to determine the respiratory response of these soils to endogenous C and amended C substrates with N addition.

MATERIALS AND METHODS

Site description

El Bolsón (EB) (41° 58′ S, 71° 32′ W) is located in the southwest of Río Negro Province, Argentina at 350 m above sea level and in the mountainous region called Cordillera Andino-Patagónica. The weather is cold and humid, Mediterranean-type (precipitation concentrated in autumn and winter), with a mean annual rainfall of 1 000 mm and mean annual temperature of 9 °C. The soils are mainly allophanic (derived from volcanic ashes), classified as Andisols (USDA Soil Taxonomy) and Typic Udivitrand (0–30 cm horizon: sandy loam texture, organic matter of 60.0–80.0 g kg^{-1}, and pH in water of 5.9–6.2).

Zavalla (ZA) (32° 43′ S, 60° 55′ W) and Venado Tuerto (VT) (33° 44′ S, 61° 58′ W) are located in the southeast of Santa Fe Province in the humid, rolling Pampa region of Argentina, at 50 and 111 m above sea level, respectively. The weather is mild humid to sub-humid with a mean annual rainfall of 987 mm, concentrated in autumn and spring, and mean annual temperature of 17 °C. The soils are classified as Mollisols (USDA Soil Taxonomy), Vertic Argiudoll for ZA (0–15 cm horizon: silty loam texture, organic matter of 46.5 g kg^{-1}, and pH in water of 6.9), and Typic Argiudoll for VT (0–15 cm horizon: loam texture, organic matter of 34.6 g kg^{-1}, and pH in water of 6.7).

Sampling was carried out at 8 sites in El Bolsón, 8 sites in ZA (within the Experimental Station of the Faculty of Agronomy, University of Rosario), and 6 sites in VT (in Estancia La Unión). Characteristics of the sampling sites are provided in Table I. Both managed and undisturbed sites were sampled in EB and ZA. Samples from VT were all collected from field fertilization treatments (0, 90, and 180 kg ha^{-1} urea, re-

TABLE I

Characteristics of the sampling sites in El Bolsón (Typic Udivitrand), Zavalla (Vertic Argiudoll), and Venado Tuerto (Typic Argiudoll), with soil samples collected at a depth of 0–5 cm

Study area	Soil sample code[a)]	Sampling site	Land use	TOC[b)]
				g C kg^{-1} soil
El Bolsón (EB)	EB-1	Mallín Ahogado	Hop (*Humulus lupulus*)	50.8
	EB-2	Mallín Ahogado	Hop (*Humulus lupulus*)	28.6
	EB-3	Mallín Ahogado	Currant (*Ribes rubrum*)	55.4
	EB-4	Mallín Ahogado	Raspberry (*Rubus idaeus*)	45.4
	EB-5	Mallín Ahogado	Raspberry (*Rubus idaeus*)	33.0
	EB-6	Reserva Municipal Cerro Amigo	*Austrocedrus* forest	75.4
	EB-7	Camino Los Nogales	Raspberry (*Rubus idaeus*)	48.6
	EB-8	Parque Nacional Lago Puelo	*Austrocedrus* forest	115.8
Zavalla (ZA)	ZA-1	Experimental station	Spring vetch (*Vicia sativa*)	29.5
	ZA-2	Experimental station	Aged planted pasture	31.5
	ZA-3	Experimental station	Planted pasture	27.0
	ZA-4	Experimental station	Ploughed	19.9
	ZA-5	Experimental station	Wheat	60.9
	ZA-6	University campus	Oak wood	54.4
	ZA-7	University campus	Park	80.3
	ZA-8	Experimental station	Maize stubble	22.6
Venado Tuerto (VT)	VT-1	Plots without fertilization, high TOC	Wheat	28.3
	VT-2	Plots without fertilization, low TOC	Wheat	27.0
	VT-3	Plots with 90 kg ha^{-1} urea, high TOC	Wheat	27.8
	VT-4	Plots with 90 kg ha^{-1} urea, low TOC	Wheat	25.3
	VT-5	Plots with 180 kg ha^{-1} urea, high TOC	Wheat	29.5
	VT-6	Plots with 180 kg ha^{-1} urea, low TOC	Wheat	26.4

[a)] Representing distinct sampling sites.
[b)] Total organic carbon, detected in 0–15 cm horizon. At Venado Tuerto sampling sites, low TOC is < 27 g C kg^{-1} soil and high TOC is > 27 g C kg^{-1} soil.

spectively) in plots under precision agriculture with total organic carbon (TOC) > 27 g C kg^{-1} soil (high) or TOC < 27 g C kg^{-1} soil (low). These two levels of TOC were related to differences detected in yield maps. Usual fertilization rate, before the trial was established in June 2010, was 120 kg ha^{-1}.

Soil sampling and analysis

Soil samples composed of 15 sub-samples were collected from the upper horizon (0–5 cm) at each of the sites described in the above section. At the undisturbed forest sites, the upper organic layer was carefully removed before collecting mineral soil cores. All the soils were sieved (< 2 mm), stored in plastic bags and kept at 4 °C until analysis (within 2 weeks). A portion of each soil sample was air-dried, sieved through a 2-mm sieve, and analyzed for TOC by dry combustion using an LECO CR12 C analyzer (LECO Corp., Saint Joseph, USA) by a service laboratory (LABSPA-CONICET, Bahía Blanca, Argentina).

Soil respiratory analysis by O_2-CLPP

BDOBS plates were prepared as described previously (Zabaloy *et al.*, 2008), with an un-amended control (no C added), and 7 C substrates (CS) selected to cover a range of ecologically-relevant C sources with different chemical groups, namely carbohydrates (mannose, sucrose), carboxylic acids (propionic acid, acetate), phenolic acids (vanillic, coumaric), and amino acid asparagine (a C + N substrate). Briefly, 40 µL of each CS stock solution (300 mg L^{-1}) was dispensed to give a final concentration of 50 mg L^{-1} (equivalent to 12 µg of CS $well^{-1}$). Similarly, N was added as $(NH_4)_2SO_4$ in one half of the plate by dispensing 40 µL of a stock solution (60 mg L^{-1}) to achieve a final inorganic N amendment concentration of 10 mg L^{-1} (equivalent to 2.4 µg of N source amendment $well^{-1}$). Soil slurries were prepared by mixing 5 g fresh soil with 12.5 mL sterile distilled water and 5 mL of glass beads in a 50 mL-Falcon tube, shaken by hand for 1 min and immediately dispensed (160 µL) in the plates, to a final volume of 240 µL. Assuming homogenous mixing of the soil slurries and no soil settling, the mass loaded was 64 mg of soil $well^{-1}$. The CS and N quantities represent amendment levels of 187.5 µg g^{-1} soil and 37.5 µg g^{-1} soil, respectively.

Plates were incubated for 24 h at 30 °C in a microplate fluorescence reader (Polarstar Omega, BMG LabTech, Germany), equipped with a 485-nm excitation filter and a 620-nm emission filter, in bottom rea-

ding mode. Fluorescence readings were normalized by dividing the relative fluorescence units by the reading at 1 h. The normalized relative fluorescence units were plotted against time of reading and several parameters were calculated from the resulting curve (Garland *et al.*, 2003). The maximum reading or peak in fluorescence was recorded as $F_{\max}$. The integrated area under the curve (AUC) within 6 h was calculated for the respiration of each C substrate and the basal respiration, using Sigmaplot 10.0 (Systat Software, Inc., San Jose, USA). A respiratory quotient (RQ), analogous to the metabolic quotient (qCO_2) (Anderson and Domsch, 1985) and C availability index (Cheng *et al.*, 1996), was calculated by dividing the AUC of the endogenous soil C (*i.e.*, basal respiration, AUC_{BR}) by the AUC of each C-only source (AUC_{CS}). AUC_{CS} reflects the biomass instantaneously responding to specific C sources (Garland *et al.*, 2012, Lehman *et al.*, 2013). The effect of N supplementation on soil respiration, expressed as N_{ratio}, was evaluated for each substrate by calculating the ratio between $F_{\max}$ with N to the $F_{\max}$ without N as proposed by Lehman *et al.* (2013). According to Garland *et al.* (2012) and Lehman *et al.* (2013), the comparison of respiratory responses with and without N in the BDOBS assay reflects N availability, with $N_{ratio} \leq 1$ standing for no N limitation and $N_{ratio} > 1$ for N limitation of microbial respiration.

Data analysis

TOC and $F_{\max}$ data were included in a principal component analysis (PCA) to assess variables that better discriminate among soils. Soils were assigned to different groups as observed after the PCA, excluding those soils that did not clearly cluster in any group.

The RQ data for the different substrates were analyzed by Kruskal-Wallis test at $P = 0.05$, to examine differences among soil groups. Pairwise comparisons were done with Mann-Whitney U test. Simple linear regression analysis was calculated for correlations between RQ and TOC. Potentially influential observations were removed from the analysis after computing regression (leave-one-out deletion) diagnostics (Crawley, 2007).

N_{ratio} data for the different substrates and endogenous C were analyzed by one-way analysis of variance (ANOVA) at $P = 0.05$, to examine differences among soil groups resulting from PCA. Normality and homogeneity of variances were checked prior to the ANOVA, and data were transformed if needed to meet the test assumptions. Pairwise comparisons were performed after a significant ANOVA result by Tukey's HSD test.

All statistical analyses were performed using R version 2.15.0 (R Development Core Team, 2013).

RESULTS

Physiological profiling of soils

PCA indicated 3 clearly separated groups based on the first two components, which accounted for 81% of the total variance (Fig. 1). PC 1 was correlated with the response to endogenous soil C (0.82), mannose (0.82), sucrose (0.78) and acetate (0.85). Asparagine had the highest loading (0.91) on PC 2, followed by propionic acid (0.74). Group I included the cropped soils from VT and EB-7 from a farm in EB. They were characterized by moderate response to endogenous soil C, mannose, sucrose and acetate and low response to asparagine and propionic acid. Group II was composed of the soils of contrasting origin (EB and ZA), which showed relatively low response to endogenous soil C, mannose, sucrose and acetate and strong response to asparagine and propionic acid. Group III soils were characterized by relatively high response to endogenous soil C, mannose, sucrose and acetate and moderate to high response to asparagine and propionic acid. PC 3, which explained 11.5% of the remaining variance, was associated with TOC (−0.88). The inclusion of PC 3 (Fig. 1) in the analysis further distinguished soils from VT with relatively lower TOC than the EB-7, as well as most managed soils from EB and ZA, with relatively high and low TOC, respectively. Therefore, 4 soil groups were obtained from PCA and defined as VT (I), EB (II), ZA (II) and EB+ZA (III), while 4 samples (ZA-5, EB-5, EB-7, and EB-8) did not clearly cluster in any of these groups. The managed and undisturbed sites from the EB and ZA areas were generally separated into two distinct groups, with the managed sites into EB (II) and ZA (II) and undisturbed sites into EB+ZA (III).

RQ as related to TOC

The RQ calculated for carbohydrates and phenolic acids were significantly different ($P < 0.05$) among soil groups, while RQ for carboxylic acids were marginally significant ($P < 0.10$). The undisturbed forest soils of Group III had the lowest RQ values, while no differences were observed among the managed soils (Table II). TOC was highly and negatively correlated to RQ for mannose ($r = -0.69$, $P < 0.001$), sucrose ($r = -0.66$, $P < 0.01$), and vanillic acid ($r = -0.65$, $P < 0.01$) (Fig. 2), but not to RQ for carboxylic acids nor coumaric acid (data not shown), although negative correlations were observed for these substrates before the potentially influential points were removed

from the analysis (data not shown). Regression analysis showed that TOC explained more than 42% of variation in RQ (Fig. 2).

Microbial respiration with added N in the assay

N_{ratio} ranged between 0.85 ± 0.13 (for asparagine)

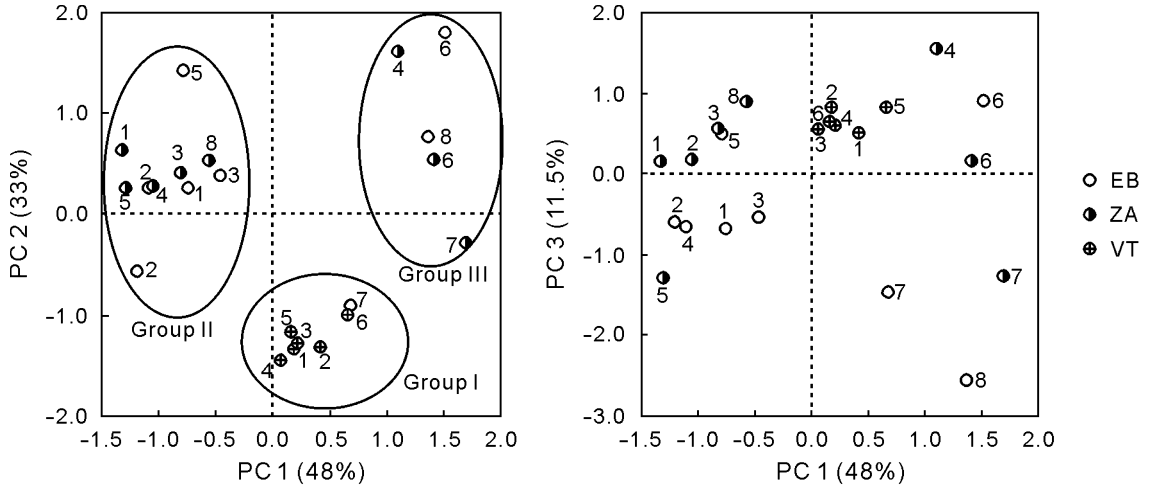

Fig. 1 Principal component (PC) analysis of peak fluorescent response data of microbial communities from managed and undisturbed soils of El Bolsón (EB), Zavalla (ZA) and Venado Tuerto (VT), Argentina. Numbers indicate soil sample codes as shown in Table I.

TABLE II

Mean respiratory quotient (RQ) calculated for the 7 C substrates, including mannose (Man), sucrose (Suc), propionic acid (Pro), acetate (Ace), coumaric acid (Cou), vanillic acid (Van), and asparagine (Asp), used in the assay of community-level physiological profiling in an oxygen-sensitive microplate for 4 soil groups

Soil group[a)]	RQ_{Man}	RQ_{Suc}	RQ_{Pro}	RQ_{Ace}	RQ_{Cou}	RQ_{Van}	RQ_{Asp}
VT (I)	0.97 ± 0.02[b)]a[c)]	0.87 ± 0.04ab	0.98 ± 0.02a	0.95 ± 0.04a	1.00 ± 0.01a	1.02 ± 0.01a	0.71 ± 0.09a
EB (II)	0.97 ± 0.01a	0.91 ± 0.02a	0.90 ± 0.02a	0.92 ± 0.02a	0.97 ± 0.02a	0.97 ± 0.02b	0.77 ± 0.08a
ZA (II)	0.97 ± 0.01a	0.93 ± 0.01a	0.94 ± 0.03a	0.89 ± 0.03a	0.96 ± 0.02a	1.00 ± 0.02ab	0.83 ± 0.03a
ZA+EB (III)	0.75 ± 0.09b	0.70 ± 0.07b	0.78 ± 0.10a	0.70 ± 0.08a	0.74 ± 0.08b	0.73 ± 0.08c	0.60 ± 0.07a

[a)]Study areas of Venado Tuerto (VT), El Bolsón (EB), and Zavalla (ZA) were classified into 4 soil groups based on principal component analysis. The managed and undisturbed sites from the EB and ZA areas were generally separated into two distinct groups, with the managed sites into EB (II) and ZA (II) and undisturbed sites into EB+ZA (III).
[b)]Means ± standard errors ($n = 6$ for VT (I) and 4 for EB (II), ZA (II), and EB+ZA (III)).
[c)]Means followed by the same letter(s) within each column are not significantly different at $P < 0.05$ by a significant Kruskall-Wallis test.

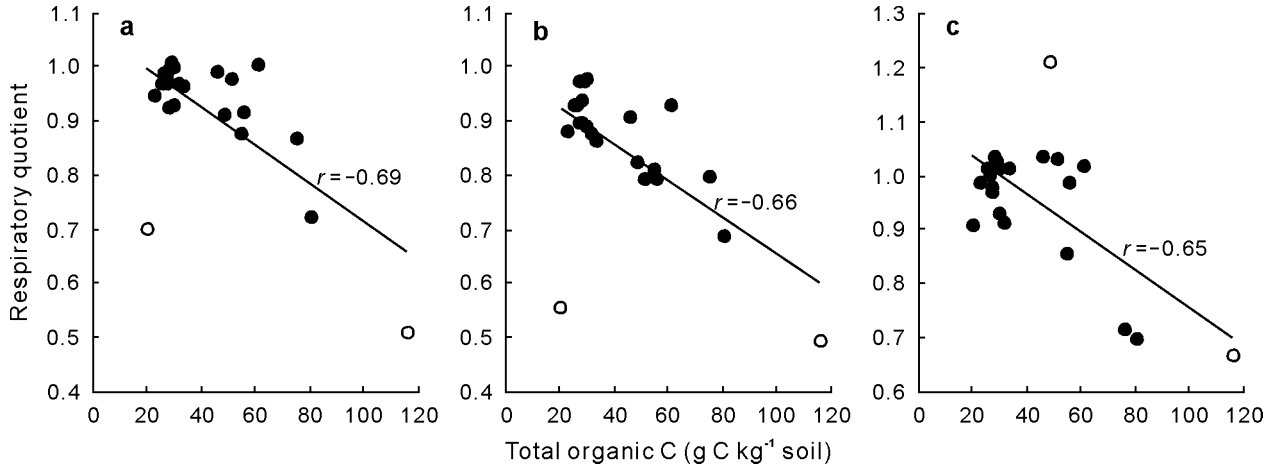

Fig. 2 Linear regression between total organic C and respiratory quotient for C-only substrates, *i.e.*, mannose (a), sucrose (b), and vanillic acid (c), used in the assay of community-level physiological profiling in an oxygen-sensitive microplate ($n = 22$). Potentially influential observations that were removed from the regression analysis are shown with white symbols.

and 1.28 ± 0.30 (for mannose) in average for all soils. In general, the N effect on the respiratory response to endogenous C and C-only substrates did not differ among soils (Fig. 3). The respiratory response to asparagine decreased with added N, and N_{ratio} was significantly lower in the EB (II) soils than in the VT (I) and undisturbed soils (III). The respiratory responses to sucrose and mannose were higher in the ZA (II) soils

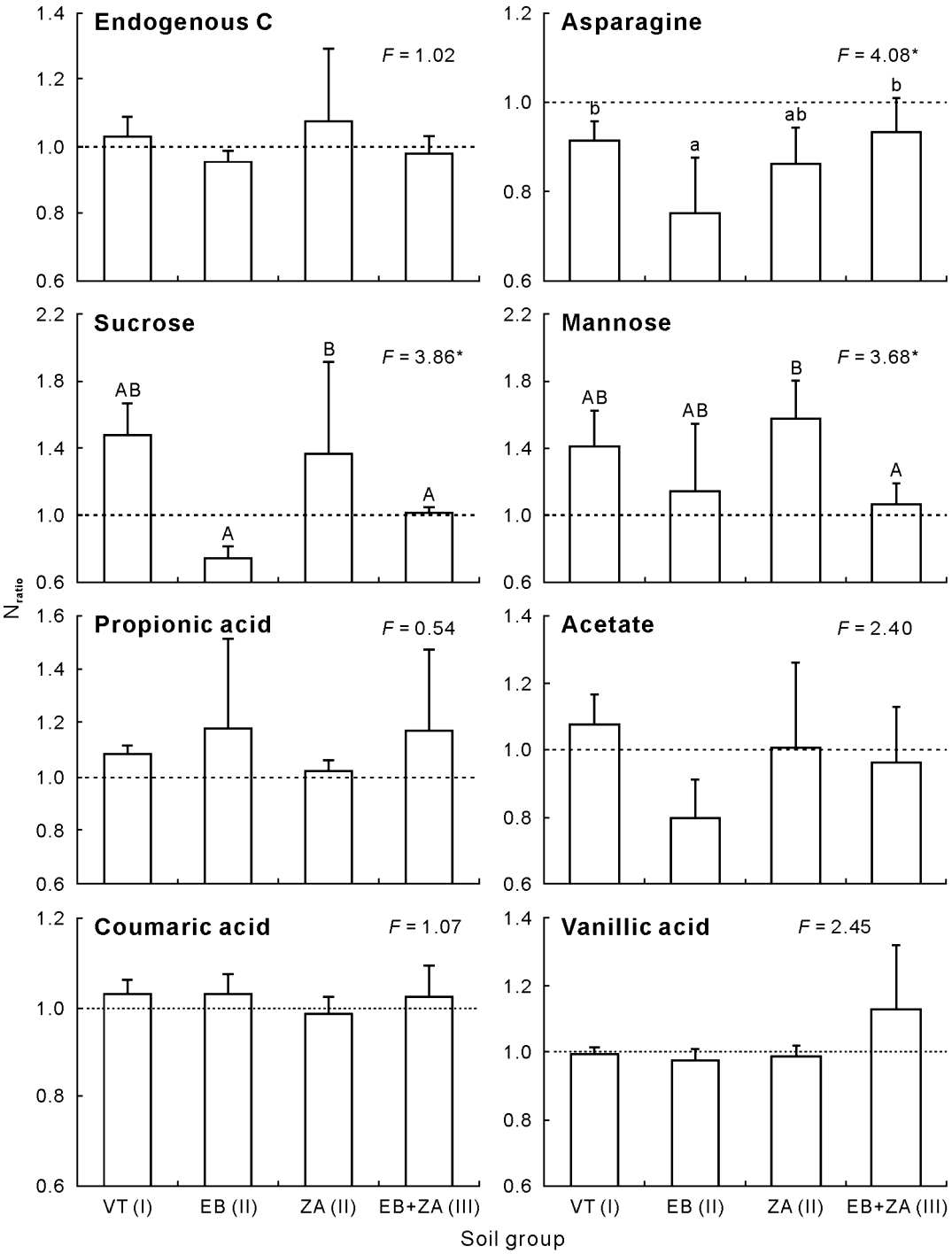

Fig. 3 Effect of N addition on microbial respiration of different C sources, including endogenous C and 7 C substrates, *i.e.*, mannose, sucrose, propionic acid, acetate, coumaric acid, vanillic acid, and asparagine, in 4 soil groups. The effect of N supplementation, expressed as N_{ratio}, was calculated as the ratio between the maximum reading in fluorescence (F_{max}) with N to the F_{max} without N. Vertical bars indicate standard deviations of the means ($n = 6$ for VT (I) and 4 for EB (II), ZA (II), and EB+ZA (III)). Bars with the same lowercase letter(s) are not significantly different at $P < 0.05$ among soil groups and those with the same uppercase letter(s) are not significantly different at $P < 0.10$ among soil groups by Tukey's HSD test. There is no significant difference among the bars without letters. The F statistic of analysis of variance test was obtained for each C source and the symbol * represents significant difference at $P < 0.05$. See Table II for the detailed descriptions of soil groups VT (I), EB (II), ZA (II), and EB+ZA (III).

soils than in the other soil groups, although these differences were only marginally significant. N_{ratio} was significantly higher than 1 for carbohydrates in the VT soils, and for mannose (but not for sucrose) in the ZA (II) soils. N_{ratio} for propionic acid was also higher than 1 in the VT soils, although the magnitude of increase in respiration with added N was relatively low (∼8%). N_{ratio} was significantly lower than 1 for sucrose and acetate use in the EB (II) soils.

DISCUSSION

Physiological profiling of the soils revealed the dual effects of contrasting land use (*e.g.*, managed *vs.* undisturbed) and geographic origin on microbial respiration. The two most distinct soil groups (*i.e.*, Groups II and III) generally separated managed and natural sites from the EB and ZA areas. The third major group contained the soils from the VT area, all of which were managed. The underlying basis for the distinctiveness of the VT soils, *i.e.*, why they did not group more closely with the other managed soils, including a soil from the ZA region similarly planted with wheat, is unclear from the present study, but may reflect biogeographical differences.

The soils from managed sites in EB and ZA included in Group II were all subjected to cultivation and agricultural management, while most soils from the same localities included in Group III were relatively undisturbed, natural forest sites. Stevenson *et al.* (2004) demonstrated the differences in the respiration responses of soil microbial communities of pastures and forests, but showed strong similarities within each vegetation class, despite the wide geographical spread, different soils, and plant species. In our study, the undisturbed forest soils exhibited high respiratory response to endogenous soil C, carbohydrates, carboxylic acids, and amino acid. Similarly, the results of Pignataro *et al.* (2012) showed significant increases in the utilization of different C sources (including amino acids, carbohydrates, phenolic acids, and carboxylic acids) for the MicroResp assay in an oak coppiced forest soil. Tree residues (through litter and rhizodeposition) provide a great availability of C compounds that may fuel both basal- and substrate-induced respiration (Pignataro *et al.*, 2012).

The inclusion of ZA-4 (ploughed site) in Group III may be explained by a relatively high and sudden availability of labile organic C caused by plowing. In this regard, Sun *et al.* (2014) found that substrate-induced respiration (*i.e.*, active biomass) was greater for the more intensively tilled treatments (conventional tillage, deep plowing). Gomez and Garland (2012) also reported an increase in both basal respiration and the ability of the community to respond to substrates in a chisel-plowed soil. Tillage promotes residue decomposition and mixing with whole soil, which in turn results in greater microbial colonization of the residues (Sun *et al.*, 2014), promotion of organic matter mineralization, and increase of readily available soil C due to enhanced aeration (Pikul Jr. *et al.*, 2001; Al-Kaisi *et al.*, 2005).

The similar respiratory response amongst all of the VT soils despite the different N fertilizer inputs was probably due to identical management and relatively uniform TOC values. Fertilization may exert a less consistent physiological response on soil microbes than tillage (Gomez and Garland, 2012) or organic amendments (Lazcano *et al.*, 2013) in dry land agricultural soils, as microbial activity is more often C limited than N limited (Aldén *et al.*, 2001; Allen and Schlesinger, 2004; Thiet *et al.*, 2006; Waring *et al.*, 2013).

The third axis (PC 3) discriminated the soils clustered in Groups I and II from different geographic origin on the basis of their TOC content. Soil organic C is the main source of C and energy limiting growth of heterotrophic microbes in soil (Demoling *et al.*, 2007; Sun *et al.*, 2014) and is one of the most important soil characteristics shaping microbial community structure and function (Pignataro *et al.*, 2012; Zhang *et al.*, 2013). While the relevance of TOC in determining microbial community function of soils was confirmed in our study, our results contrast with those reported by Nsabimana *et al.* (2004), who found that organic C was the main soil property accounting for 53% variability in the redundancy analysis of catabolic response profiles of different land use types (including maize, pastures, and tree plantations).

The respiratory quotient (RQ) calculated for all the soils and C substrates was close to 1 or even lower than 1, indicating an efficient use of available substrates by analogy with C availability index and qCO_2 (Cheng *et al.*, 1996; Dilly, 2005). The values of qCO_2 higher than 2 indicate an energetically less efficient microbial community (Anderson, 2003; Moscatelli *et al.*, 2005). The forest soils of Group III had the lowest RQ values as could be predicted for natural, undisturbed soils and mature ecosystems (Dilly, 2003; Batisda *et al.*, 2008). Montecchia *et al.* (2011) also found evidence of greater respiratory efficiency in naturally vegetated sites with high soil organic content relative to agricultural sites in the same region. The RQ values calculated for carbohydrates and vanillic acid were inversely related to the C content of soils. In other words, those soils with higher soil organic C contents were more ef-

ficient in the use of available C in the assay. Similarly, a negative correlation between qCO_2 and soil organic C was reported in an acidic Ultisol amended with industrial and agricultural by-products (Li *et al.*, 2014).

The N addition in the assay in the absence of C amendment (*i.e.*, only endogenous soil C present) had no effect on microbial respiration in any soil, indicating that these soils were not intrinsically N limited. Negligible microbial activity in response to N inputs in the absence of C addition has been previously reported (Aldén *et al.*, 2001; Allen and Schlesinger, 2004). Soils responded similarly to N addition for all C-only substrates, and only differed in asparagine use. However, substrate-dependent variation in N_{ratio} for a given soil was observed, suggesting that the assay may reflect differences in the degree of N limitation amongst microbial guilds in the soil community (Garland *et al.*, 2012). A recent study by Lehman *et al.* (2013) showed a significant N limitation ($N_{ratio} > 1$) for carbohydrates respiration, which decreased with increasing concentrations of inorganic soil nitrate and ammonium. Gómez and Garland (2012) also reported significant increase in basal respiration and respiration of C-only substrates (mannose, acetate, coumaric acid, and carboxy-methyl cellulose) with addition of N to the assay. However, N addition in the plate caused a reduction in basal respiration and in the response to carbohydrates in the soil from fertilized plots (Gomez and Garland, 2012), as was also the case with sucrose and acetate use in the soils from EB (II) in our study ($N_{ratio} < 1$).

While the increase in the respiratory response with added N ($N_{ratio} > 1$) may be indicative of N limitation for microbial activity and growth (Schimel and Weintraub, 2003; Garland *et al.*, 2012), the interpretation of respiration decrease with N addition ($N_{ratio} < 1$) is less clear. Ramírez *et al.* (2010) also observed consistent reduction of microbial respiration rates with a range of inorganic N fertilizers and urea applied to both grassland and forest soils, but they provided no mechanistic basis for the apparent suppression of respiration. A significant reduction of the respiratory response to certain substrates with added N may result from a decrease in energy-dependent, carrier-mediated uptake of organic forms of N (*e.g.*, amino acids, peptides, and amino sugars) when NH_4^+ is made available in the assay (Geisseler *et al.*, 2010; Garland *et al.*, 2012). When the availability of NH_4^+ is high, enzyme systems for the utilization of alternative N sources are repressed (Geisseler *et al.*, 2010), consequently the respiratory demand, measured as the rate of O_2 consumption, is reduced (Garland *et al.*, 2012). Another plausible explanation is that N addition may redirect C from overflow metabolism (waste respiration) into producing microbial biomass (Schimel and Weintraub, 2003; Manzoni *et al.*, 2012). According to the mechanistic model developed by Schimel and Weintraub (2003), a decrease in respiration with N addition to soil could be evidence that microbes are, in fact, N limited. In practice, the O_2-CLPP approach does not measure the efficiency of the substrate metabolism. However, the value of $N_{ratio} < 1$ suggests that C use efficiency comes into play, indicating that addition of inorganic N stimulates assimilatory use. Future work should focus on measuring assimilation and respiration of the same substrates using radiolabelled C sources, to determine whether $N_{ratio} < 1$ is associated with more efficient use (*i.e.*, greater assimilation to respiration ratio) as a result of increased inorganic N availability.

In summary, the respiratory response to several C substrates and endogenous C assayed with O_2-CLPP allowed us to discriminate among soils based on their land use (*i.e.*, managed *vs.* undisturbed soils) and geographic origin. These microbial physiological responses were more sensitive to land use differences than a single soil chemical property (*i.e.*, TOC). The O_2-CLPP provided an integrated approach to evaluate the influence of land use on C and N availability for microbial respiration as well as to estimate the efficiency in the use of those resources. The ease of use of this approach allows for high throughput assessment of C and nutrients response patterns to evaluate impacts of land use changes and to predict potential negative effects.

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