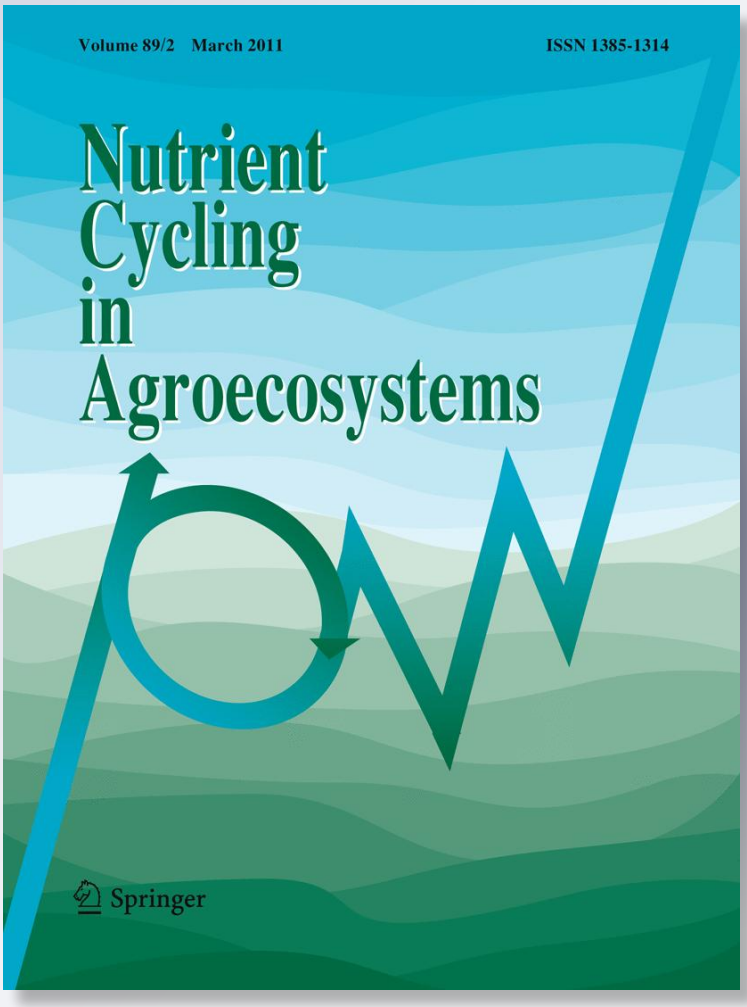
Microbial nitrogen dynamics in south central Chilean agricultural and forest ecosystems located on an Andisol

Nutrient Cycling in Agroecosystems
(formerly Fertilizer Research)

ISSN 1385-1314
Volume 89
Number 2

Nutr Cycl Agroecosyst (2010)
89:175-187
DOI 10.1007/
s10705-010-9386-0



Springer

Nutr Cycl Agroecosyst (2011) 89:175–187
DOI 10.1007/s10705-010-9386-0

ORIGINAL ARTICLE

Microbial nitrogen dynamics in south central Chilean agricultural and forest ecosystems located on an Andisol

Dries Huygens · Dries Roobroeck · Lynn Cosyn · Francisco Salazar · Roberto Godoy · Pascal Boeckx

Received: 28 January 2010 / Accepted: 6 July 2010 / Published online: 15 July 2010

Abstract The natural soil N supply in volcanic soils (Andisols) can be a significant source of plant-available N for agro-ecosystems. Nevertheless, intensive farming systems in south Chile apply high fertilization rates, which lead to high production costs and involve a risk for adverse ecosystem effects. In order to achieve sustainable land management, a better understanding of the processes that govern soil N availability and loss, and their external drivers, is required. In this study, we selected a winter-cropland, a summer crop-winter fallow rotation, and a forest, used as a reference ecosystem. Gross N transformations (^{15}N isotope dilution) and microbial community structure (phospho-lipid fatty acid analysis) in the topsoil were determined. Gross N mineralization was about ten times lower in the agro-ecosystems than in the forest, while gross nitrification was low in all sites. Gross N immobilization equalized or exceeded the gross inorganic N production in all sites. Microbial biomass was 3–5 times more abundant in the forest than in the agro-ecosystems. A positive relationship between the ratio fungi/bacteria and total microbial biomass was observed in these Andisols. We suggest that the reduction in fungal biomass induced a lower extracellular enzyme production and limited soil organic matter depolymerisation in the agro-ecosystems. We conclude that soil N cycling was unable to provide a significant N input for the croplands, but also the risk for ecosystem N losses was low, even under fallow soil conditions. Current fertilization practices appropriately anticipated the soil N cycling processes, but further research should indicate the potential of alternative land management to reduce fertilizer cost.

Keywords Andisol · ^{15}N isotope dilution · Phospho-lipid fatty acid (PLFA) · Fungi · Chile · Cropland

D. Huygens · D. Roobroeck · L. Cosyn · P. Boeckx
Laboratory of Applied Physical Chemistry, ISOFYS, Faculty of Bioscience Engineering, Ghent University, Coupure 653, Ghent, Belgium

D. Huygens (✉)
Institute of Soil Science and Agricultural Engineering, Faculty of Agricultural Sciences, Universidad Austral de Chile, Casilla 567, Valdivia, Chile
e-mail: dries.huygens@ugent.be

F. Salazar
National Institute for Agricultural Research, INIA-Chile, P.O. Box 24-O, Osorno, Chile

R. Godoy
Institute of Botany, Faculty of Sciences, Universidad Austral de Chile, Valdivia, Chile

Introduction

Andisols cover a main part of the agricultural and forest land in south central Chile (71–73°W, 39–44°S, 4.5 million ha). The soil organic matter (SOM) of these Andisols is typically predominated

by humic acids with the highest degree of humification (type A humic acid), and is complexed with active Al and Fe in the soils (Nanzyo et al. 1993; Nierop et al. 2005). These characteristics explain why Andisols have high carbon (C) and nitrogen (N) contents, but low rates of C turnover, relative to other soil types (Torn et al. 1997). Nevertheless, Shoji et al. (1993) indicated that the natural soil N supply in Andisols can be a significant source of plant-available N in cropping ecosystems. For example, soil derived N taken up by winter wheat and corn amounted to 62–101 kg N ha^{-1} in Japanese Andisols.

In volcanic soils of south central Chile, agricultural practices, including fertilization and cropland rotation, are known to reduce the quantity of soil C and N relative to native forest, without altering the SOM quality. Borie et al. (2002) and Heredia et al. (2007) indicated that the contribution of different SOM fractions (humin, humic acids, fulvic acids, low molecular organic molecules) to total SOM varied only to a small extent across a wide range of volcanic soil series and land-use classes. In their study, topsoil N varied between 0.54 and 1.16%, but low molecular organic molecules only made up a small percentage of total SOM (<20%, Borie et al. 2002). These results indicate that, independent of land-use, total soil N contents are high, but most of the N is stabilized in recalcitrant and complexed SOM fractions. Next to changes in SOM, land management also exerts a strong influence on the microbial soil community, which may feed back on functional processes in the N cycle (Zak et al. 2003). Hence, microbial soil N cycling processes such as N mineralization, N nitrification, and N immobilisation may be altered as a result of land management (Rhoades and Coleman 1999).

In south central Chile, uncertainty in both plant N demand in relation to its growth potential and soil N supply leads some farmers to adopt high fertilization strategies. Intensively managed south Chilean agro-ecosystems are typically fertilized with N rates in the range of 100–300 kg N ha^{-1} $year^{-1}$ (Bernier and Undurraga 2009; Cartes et al. 2009). As such, fertilizer cost amounts up to one-third of total expenses for farmers (Campillo et al. 2007). These fertilizer N supplies are currently even further increasing because of agricultural intensification (FAO 2009). In many industrialised countries, the increased fertilizer N use has led also to a number of environmental problems (Galloway et al. 2003). As a result, increased N leaching, associated loss of soil nutrients, acidification of soils and water bodies, elevated greenhouse gas emissions, contamination of ground water, and transfer of N to estuaries and coastal oceans have frequently been documented (Matson et al. 1997). Also in south central Chile, adverse ecosystem effects have already been documented. E.g. Alfaro et al. (2006) indicated leaching losses of up to 261 kg N ha^{-1} $year^{-1}$ after inorganic fertilizer additions in intensively managed grassland ecosystems (but see Salazar et al. 2008 for contrasting results). Mora et al. (2007) documented significant decreases in soil pH as a result of urea fertilizer applications. Finally, Oyarzún and Huber (2003) indicated that the contribution of NO_3^- to total stream N concentrations was higher in watersheds used for agriculture (73%) than in watersheds covered by native forest vegetation (50%).

In order to achieve sustainable land management, a better understanding of the processes that govern soil N availability and loss, and their external drivers, is required for these volcanic soils. To the best of our knowledge, no information on gross soil N transformation pathways has been documented for south Chilean agro-ecosystems. Even worldwide, published results of gross N fluxes in agro-ecosystems located on volcanic Andisols are scarce. Notwithstanding, detailed information on soil N cycling in south Chilean forest ecosystems is available (Perakis and Hedin 2001; Huygens et al. 2008). Studies in these ecosystems indicated very high gross NH_4^+ production (up to 42 μg N g^{-1} day^{-1}), but also a suppression of autotrophic nitrification as a result of strong competition for available NH_4^+ by abiotic immobilization processes and NH_4^+ assimilating heterotrophic microorganisms (Huygens et al. 2007). As a result, inorganic N losses via leaching or gaseous N emissions are negligible in these forest ecosystems. Nevertheless, Perakis et al. (2005) observed that when external N inputs were increased to levels above 160 kg N ha^{-1} $year^{-1}$, N retention in the forest soil matrix strongly declined, leading to substantial NO_3^- leaching losses. This effect was attributed to a reduced assimilation of inorganic N by microbial biomass and/or a decrease in abiotic reactions between inorganic N and SOM. All this indicates the potential of south Chilean volcanic soils as a significant source of plant N supply for agro-ecosystems, but also the risk for significant N losses when external N inputs are increased.

This study aims to bring a first assessment of soil N cycling in agro-ecosystems and pristine forests, located in south central Chile (a low-fertilized winter cropland (WC), a summer crop—winter fallow rotation with a history of high fertilizer N application during summer (CF), and a pristine forest (PF)). We compare site-differences in gross N cycling rates as a function of inherent microbial community structure, assessed by phospho-lipid fatty acid (PLFA) analyses. The latter analysis allows determining the abundance of microbial groups which have a differential microbial physiology and functional role in the soil N cycle. We aspire to provide information that helps to improve agricultural management decisions and to limit adverse ecosystem effects such as N leaching.

Materials and methods

Study sites

All study sites are located in central south Chile on the same soil type (Diaz et al. 1960; Tosso 1985), classified as Typic Hapludands (Soil-Survey-Staff 2006) or Silandic Andosols (IUSS-Working-Group-WRB 2006). The natural vegetation of south central Chile used to be temperate rainforest, with *Laurelia* spp. and *Nothofagus* spp. as main emergent tree species. No exact details of forest-to-agricultural land conversion have been documented, but most old-growth forest clearing in this region occurred about 150 years ago during the German colonization period. The low-fertilized winter cropland site (WC) is located at the experimental site "La Pampa" (40°52′S, 73°12′W, 91 m.a.s.l) of the National Institute for Agricultural Research (INIA-Chile). The average annual temperature equals 11.0°C. Mean annual precipitation is 1600 mm, with 70% of the rain concentrated in winter (April–September). Long-term site history, recorded in INIA logbooks, documents crop-grass rotational management, including potato, wheat and annual ryegrass rotation. Winter wheat (*Triticum aestivum* L.) covered the surface at the moment of soil sampling (September, beginning of Austral spring). The site was fertilized just before crop establishment (May, 2007) with about 20 kg urea-N ha^{-1}. Over the last years, fertilization N supply averaged about 100 kg N ha^{-1} $year^{-1}$, typically applied as urea. The summer crop—winter fallow rotation site (CF) is located at the experimental study site "Remehue" of the INIA (40°31′S, 73°03′W, 65 m.a.s.l.), with similar climate conditions as WC. CF history documents rotational long-term management of wheat, barley, potato, corn and oat. At the time of sampling (September, beginning of Austral spring), the soil surface was left fallow after summer cultivation of forage corn (*Zea mays* L.). The CF site has a long-term NPK fertilization (>10 years) history with an average annual N supply of 180 kg N ha^{-1} $year^{-1}$. The fertilizer supply (50% urea, 50% NH_4NO_3) is added in two doses over the year; about one-third just before crop establishment (November), and the remaining two-thirds during late summer (February). The pristine forest site (PF) is located in the National Park Puyehue (40°47′S, 72°12′W, 400 m.a.s.l.), at the foot of the Andes mountain range. This National Park constitutes one of the few remnants of old-growth rainforest ecosystems in the region. Due to the higher altitude, climate conditions are somewhat different from WC and CF; average precipitation equals about 3000 mm, while mean annual temperature is about 8°C. This forest is locally known as a Valdivian rainforest, and is dominated by evergreen trees such as *L. philippiana*, *N. dombeyi*, *Saxegothaea conspicua*, and *Drimys winteri*.

Soil sampling

Three sampling plots (10 × 10 m) were established at each study area (200 × 200 m site). A composite sample, consisting out of three randomly selected soil subsamples, was taken in each plot. The composite soil samples were considered as replicates ($n = 3$ for each site). Samples were taken from the top soil layer (0–10 cm) at the beginning of the Austral spring (September) in all study sites. In PF, the recognisable plant material ($O_i + O_e$ layer) was removed before sampling. Gravimetric soil water contents of field-wet soil samples varied between 43 and 55% (m/m). Bulk densities were determined using steel cylinders (Klute 1986). After transport to the laboratory, soils were homogenized and recognizable plant and stone material was removed by hand-picking. For each site and replicate, the samples were divided into four subsamples. Subsample (a) was oven-dried (65°C, 48 h) for the determination of gravimetric soil water content and total recovery of ^{15}N material, (b) was air-dried and used for the determination of physico-

chemical soil characteristics, (c) was freeze-dried for PLFA determination; (d) was gradually dried (~5 days) to a soil water content of 50% water-filled pore space (WFPS) for ^{15}N incubation experiments. Soils were stored during 1–8 days at 5°C before assessing gross N cycling rates.

Phospholipid fatty acids (PLFA) determination

The PLFA extraction and derivatization method used in this study has been described in Denef et al. (2007). Briefly, total lipids were extracted from 6 g of freeze-dried soil using phosphate buffer/chloroform/methanol at a 0.9:1:2 ratio and partitioned into neutral, glyco- and phospho-lipids by solid phase extraction. Phospholipids were methylated by mild alkaline methanolysis (using methanolic KOH) to form fatty acid methyl esters (FAMEs), which were analyzed by capillary gas chromatography–combustion-isotope ratio mass spectrometry (GC–c-IRMS) via a GC/C III interface (GC, Thermo Scientific, Germany; IRMS, DeltaPLUS XP, Thermo Scientific, Germany) as described by Denef et al. (2007). The maximum number of PLFA peaks that were detected was 26, but only 18 were selected for quantification because of their known presence in bacteria and fungi. We determined the ratios of the peak area of each individual PLFA to that of 16:0, a universal PLFA occurring in the membranes of all organisms. PLFA ratios less than 0.02 were excluded from the data set (Drijber et al. 2000). Total PLFA-C was used as in indicator for total microbial biomass (Zelles 1999). PLFA 18:1ω9c and 18:2ω6,9 were used as an indicator of fungal biomass (Zelles 1999; Chung et al. 2007), while a summation of PLFAs 14:0, i14:0, 15:0, i15:0, a15:0, i16:0, 16:1ω7c, Me10–16:0, 17:0, i17:0, a17:0, Me10–17:0, cy17:0, 18:0, Me10–18:0, and 18:1ω7c was used as an indicator for bacterial biomass (Kroppenstedt 1985, 1992; Bååth et al. 1992; Frostegård and Bååth 1996; Pennanen et al. 1996). Within the bacterial group, i14:0, i15:0, a15:0, Me10–16:0, Me10–17:0 i17:0, and a17:0 and Me10–18:0i16:0 were specifically assigned to gram-positive bacteria (Kroppenstedt 1985, 1992; Bååth et al. 1992; Frostegård and Bååth 1996), while 16:1ω7c, 16:1ω7t, cy17:0, and 18:1ω7c represent the gram-negative bacteria (Bååth et al. 1992; Frostegård and Bååth 1996). Some bacterial biomarkers (14:0, 15:0, 17:0, and 18:0) were not assigned to a specific group.

^{15}N isotope dilution experiments

We assessed gross N cycling on disturbed soil samples in the laboratory, not the field, to separate microclimate effects (e.g. temperature, soil moisture content) from inherent microbial community characteristics as drivers for gross N cycling (e.g. Billings and Gaydess 2008; McKinley et al. 2008). PVC tubes (7 cm diameter, 10 cm height) were filled with field-wet soil, equivalent to 150 g dry soil. Four different cores were incubated for each site replicate. PVC tubes were labelled two by two with a NH_4Cl–KNO_3 solution, of which one of the two N moieties was ^{15}N labelled (98 atom% excess), introducing the 'mirrored' moiety at natural abundance (i.e. two tubes $^{15}NH_4$–NO_3 labelled, two tubes NH_4–$^{15}NO_3$ labelled). 2.5 μg ^{15}N g^{-1} soil was added to each core in a 5 ml aqueous solution. Homogeneous ^{15}N distribution was obtained by injecting the 5 ml ^{15}N solution (Terumo Europe NV, Leuven, Belgium) at 5 different spatial points over the total length of the soil core (Huygens et al. 2008). One core for each ^{15}N treatment was extracted (1 M KCl, 60 min) 15 min after ^{15}N application (T_0) to determine initial ^{15}N enrichments, while the second core were extracted 24 h later (T_1). The 1-day incubation experiment was carried out at a constant temperature of 15°C. Soil extracts were frozen immediately at −22°C, and shipped to the Laboratory of Applied Physical Chemistry (Ghent University, Belgium) for analysis on NH_4^+ and NO_3^- concentration and ^{15}N enrichment. Samples were shipped in frozen state using express courier service (4 day period). While samples thawed during shipment, they were never exposed to high temperatures. Gross N mineralization, nitrification fluxes, and NH_4^+ and NO_3^- consumption fluxes were calculated according to Davidson et al. (1991). Gross NH_4^+ immobilization was calculated as the difference between NH_4^+ consumption and gross nitrification.

Chemical analysis

Ammonium in the soil extracts was determined colorimetrically by the salicylate-nitroprusside method (Mulvaney 1996). Nitrate was determined colorimetrically via NO_2^- reduction and subsequent imidazole buffered reaction with N-1-napthylethylenediamine. The ^{15}N contents of NH_4^+ and NO_3^- were analyzed after conversion to N_2O (Hauck 1982;

Stevens and Laughlin 1994), and measured using a trace gas preparation unit (ANCA-TGII, PDZ Europa, UK), coupled to an Isotope Ratio Mass Spectrometer (IRMS) (20-20, SerCon, UK). Solid soil samples were ground with a planetary ball mill (PM400, Retsch, Germany) for total nitrogen (TN) and total carbon (TC) analysis with an elemental analyzer (ANCA-SL, PDZ Europa, UK), coupled to an IRMS (20-20, SerCon, UK). Olsen P was determined by extraction with 0.5 M $NaHCO_3$ at pH 8.5 (Olsen et al. 1954). Olsen P is a measure for plant available inorganic P levels. Soil texture was determined using the pipette method (Day 1965). Soil pH_{H2O} was determined in a 1:2.5 soil:solution ratio (05669 -20, Cole Palmer, USA).

Statistical analysis

Statistical analyses were conducted in SPSS (SPSS Inc., version 16.0, Chicago, USA) or the R language and environment (R software, R Development Core Team 2009). Statistical differences of means ($P < 0.05$, $n = 3$) were distinguished using ANOVA followed by a Duncan's multiple range post-hoc test. Principal component analysis (PCA) was used to identify the most discriminatory effects between multivariate PLFA microbial community composition data of the four different sites. At the same time, a reduction in the data variance (from 18 PLFA biomarkers to three PCA scores) was obtained in order to perform MANOVA analysis [condition: (number of multivariate parameters) < (number of sites, including replicates—1)]. MANOVA analysis (Hotelling Lawley) on the PCA scores of the three axes determined significant differences in microbial community composition between sites. Correlations between gross N transformation fluxes and TC, TN, and total microbial biomass were performed using two-tailed Pearson test.

Results

Site characteristics

Total carbon (TC) was almost two times greater in PF than CF and WC (Table 1). Total nitrogen (TN) was greater in PF than in CF (+52%) and WC (+73%), respectively (Table 1). TC and TN contents varied

Table 1 Physico-chemical site characteristics of the different agricultural and forest sites

Site	Total carbon (%)	Total nitrogen (%)	C/N (–)	NH_4^+ (μg g^{-1})	NO_3^- (μg g^{-1})	Olsen P (μg g^{-1})	pH_{H2O} (–)	Texture (–)	Bulk density (g cm^{-3})	CEC* (cmol kg^{-1})
WC	7.6 (0.4)^b	0.64 (0.05)^b	11.9 (0.3)^{ab}	1.5 (0.5)^b	3.9 (0.4)^b	41.8 (6.2)^a	6.0 (0.3)^a	Silt loam	0.78 (0.12)^a	58.2
CF	8.3 (0.6)^b	0.73 (0.06)^b	11.4 (0.5)^b	1.7 (0.6)^b	3.2 (0.4)^b	46.5 (5.3)^a	5.5 (0.2)^{ab}	Silt loam	0.75 (0.07)^a	53.2
PF	15.6 (1.4)^a	1.11 (0.26)^a	14.4 (2.2)^a	11.1 (3.3)^a	6.1 (1.4)^a	4.7 (1.8)^b	5.1 (0.2)^b	Loam	0.57 (0.10)^b	49.9

CF summer crop—winter fallow rotation, *WC* winter cropland, *PF* pristine forest; standard deviations between brackets; values in the same column not followed by a different letter are significantly different

* Cation exchange capacity, NH_4OAc (pH = 7) extractable base cations and Al^{3+}, indicative for these soil series (adopted from Tosso 1985)

between 15.6 and 7.6%, and 1.11 and 0.64%, respectively. The C/N ratio was significantly greater in PF (14.4) than in CF (11.4), while WC (11.9) showed an intermediate value (Table 1). Also, NH_4^+ was about 7 times greater in PF than in CF and WC, while NO_3^- was about 2 times greater in PF than in CF and WC. Olsen P was about an order of magnitude greater in the fertilized sites CF and WC than in PF. This effect could be attributed to P fertilization in the agro-ecosystems. Soil pH was significantly greater in WC than in PF, while CF showed an intermediate value. Historic liming practices might have increased natural soil pH in the agricultural sites. Texture was classified as silt loam for the agricultural sites, and as loam for the PF. Bulk density was significantly lower in the PF (0.57 g cm^{-3}) than in WC and CF, which showed similar values (0.75–0.78 g cm^{-3}). Cation exchange capacity (CEC) was relatively high at all sites, with values varying between 49.9 and 58.2 cmol(+) kg^{-1}.

Soil microbial biomass and community structure

Total microbial biomass was about 3–5 times greater in PF (12.7 μg PLFA-C g^{-1}) than in CF (3.6 μg PLFA-C g^{-1}) and WC (2.5 μg PLFA-C g^{-1}) (Table 2). When normalized for TC, microbial biomass (C-normalized microbial biomass) was greater in PF (80.9 μg PLFA-C g^1 C) than in WC (43.6 μg PLFA-C g^{-1} C) and CF (33.4 μg PLFA-C g^1C). A similar trend was observed for N-normalized microbial biomass. The contribution of fungi to total microbial biomass was significantly greater in PF (7.3 mol% C) than in CF (6.0 mol% C) and WC (5.2 mol% C) (Table 2). Bacteria dominated over fungi in all sites, with a contribution to total microbial biomass varying between 92.7 mol% C (PF) and 94.8 mol% C (WC) (Table 2). Gram-negative bacteria showed a greater mol% C in PF (43.0) than in CF (18.5) and WC (13.2). The ratio of fungi to bacteria was significantly different between the three study sites, with PF showing the greatest value (0.08), followed by CF (0.06) and WC (0.05) (Table 2). PCA analysis with the data of the 18 PLFA biomarkers indicated that the first and second PCA axes explained in total 83.5% of the total variance between sites (Fig. 1). The location of the microbial communities of both agricultural sites (CF and WC) are plotted on the negative site of the first PCA axis, while the three replicates of the PF are plotted on the positive site of PCA 1 (Fig. 1). The negative site of the PCA 1 axis is dominated by bacteria, with gram-positive bacteria (i16:0, i14:0, 10Me17:0) having the greatest weight. The positive site of PCA1 is especially dominated by fungi (18:2ω6,9) and gram-negative bacteria (18:1ω7c). The different replicates of the sites WC and CF are especially separated along the PCA2 axis, which explained 11.2% of the total variance (Fig. 1). MANOVA analysis indicated that the microbial community composition is significantly different between WC and PF ($P < 0.001$), and CF and PF ($P = 0.004$). The agricultural sites WC and CF show no significant differences in microbial community composition ($P = 0.27$).

Significant correlations (99% level) were observed between total microbial biomass and TC ($r = 0.96$) and TN ($r = 0.93$) (data not shown). In addition, a clear positive relationship was observed between the

Table 2 Soil microbial biomass and community structure for the different agricultural and forest sites

	WC	CF	PF
Total microbial biomass (μg PLFA-C g^{-1})	2.5 (0.7)^b	3.6 (1.4)^b	12.7 (4.5)^a
C-normalized microbial biomass (μg PLFA-C g^{-1} C)	43.6 (19.2)^b	33.4 (11.1)^b	80.9 (13.7)^a
N-normalized microbial biomass (μg PLFA-C g^{-1} N)	5.0 (2.2)^b	4.0 (1.4)^b	11.5 (0.1)^a
Fungi (mol% C)	5.2 (0.7)^b	6.0 (0.3)^b	7.3 (0.7)^a
Bacteria (mol% C)	94.8 (0.7)^a	94.0 (0.3)^a	92.7 (0.3)^b
G+ (mol% C)	74.6 (2.6)^a	68.0 (3.6)^a	44.7 (0.6)^b
G− (mol% C)	13.2 (0.5)^b	18.5 (6.2)^b	43.0 (0.1)^a
Fungi/bacteria ratio (−)	0.05 (0.01)^b	0.06 (0.00)^b	0.08 (0.01)^a

G+ gram-positive bacteria, *G−* gram-negative bacteria, *CF* summer crop—winter fallow rotation, *WC* winter cropland, *PF* pristine forest; standard deviations between brackets; values in the same row not followed by a different letter are significantly different

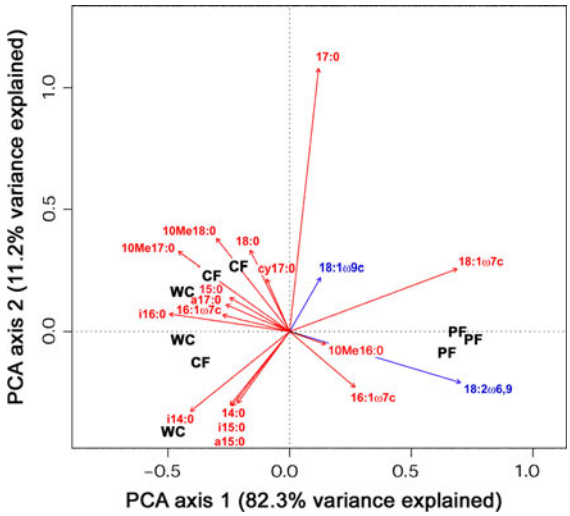


Fig. 1 The location of the different agricultural and forest replicates on a biplot generated via principal component analysis (PCA) of a multivariate data-set of 18 phospho-lipid fatty acid (PLFA) biomarkers (*red arrows* = bacterial PLFA biomarkers, *blue arrows* = fungal PLFA biomarkers; *CF* summer crop—winter fallow rotation, *WC* winter cropland, *PF* pristine forest)

fungi/bacteria ratio and C- and N-normalized microbial biomass (Fig. 2).

Gross N transformations

Recoveries of $^{15}NH_4^+$ and $^{15}NO_3^-$ as dissolved inorganic N equalled 38–53% and 26–58%, respectively. The rapid removal of inorganic N shortly after ^{15}N application is a commonly observed phenomenon (e.g. Nömmik and Vahtras 1982; Davidson et al. 1991; Roing et al. 2006; Russow et al. 2008). Probably, the explanation is a very fast and strong abiotic adsorption reaction to clay–humus complexes within soils (Russow et al. 2008). Nevertheless, the use of the ^{15}N isotope dilution technique in our study can be justified because:

(1) The adsorption of inorganic N on clay minerals is a fast abiotic reaction, occurring within minutes after ^{15}N additions (Nömmik and Vahtras 1982). We accounted for this effect by extracting a subset of soil cores 15 min after

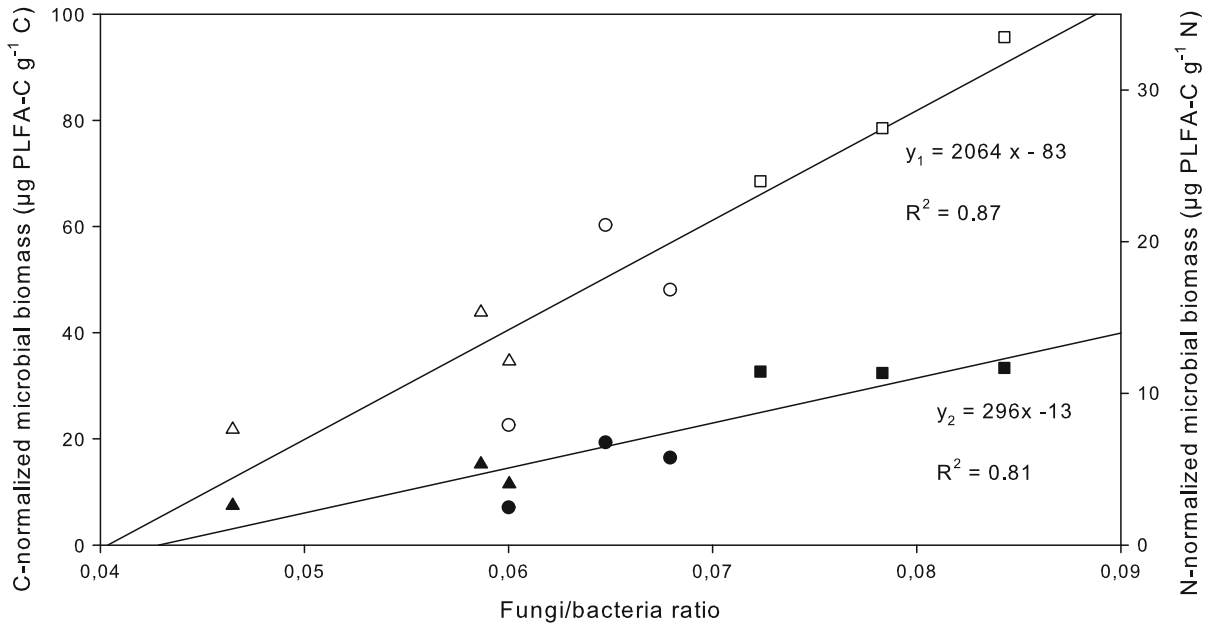


Fig. 2 The relationship between C- and N-normalized microbial biomass and fungi/bacteria ratio; data points include three replicates of the different agricultural and forest sites (*open symbol* for C-normalized microbial biomass (left *Y* axis), *filled symbols* for N-normalized microbial biomass (right *Y* axis); *circles* winter cropland, *triangles* summer crop—winter fallow rotation, *squares* pristine forest)

^{15}N addition (T_0 data), once abiotic adsorption has taken place. N fluxes were then calculated based on differences in ^{15}N enrichments and N concentrations between T_1 data (24 h after ^{15}N addition) and T_0 data (Davidson et al. 1991). According to theory (Davidson et al. 1991), it is assumed that N transformation processes during this period (T_1–T_0) are dominantly executed through microbial processes.

(2) The release of non-exchangeable inorganic N is dominantly microbial (i.e. microbial N mineralization) (Drury et al. 1991; Trehan 1996; Lin et al. 2004; Russow et al. 2008). The microbial release process is typically rather slow (Drury and Beauchamp 1991; Trehan 1996), and thus unlikely to occur in significant amounts in a 1-day incubation experiment.

(3) The non-exchangeable inorganic N is a large soil pool in many soil types (up to 18% of total soil N; Young and Aldag 1982; Liang et al. 1999). This is without a doubt also the case in Andisols, characterized by a high CEC and SOM content. This will result in a large dilution of the 'recently fixed' ^{15}N (2.5 μg g^{-1} soil) into the bigger 'native' non-exchangeable inorganic N (at natural abundance). Hence, if any re-mineralization already takes place, it will occur at ^{15}N enrichment values close to natural abundance. This is in agreement with the condition that all N pools, other than the labelled one, should remain at values close to natural abundance during ^{15}N isotope dilution studies (Kirkham and Bartholomew 1954).

NH_4^+ turnover was about 10 times faster in PF than in CF and WC (Table 3). Gross N mineralization and NH_4^+ consumption rates were about an order of magnitude greater in PF than in CF and WC, which showed no significant differences (Table 3). Gross nitrification fluxes were greater in PF and WC than in CF (Table 3). No significant difference in NO_3^- consumption fluxes were observed between the different sites. The gross N mineralization flux was significantly correlated with total microbial biomass ($r = 0.96$), TC ($r = 0.86$), and TN ($r = 0.84$) at the 99% significance level (Table 4). Gross NH_4^+ consumption was significantly correlated with gross N mineralization ($r = 0.71$, $P = 0.03$, data not shown). In addition, a significant (95% level) correlation was observed between gross NH_4^+ consumption and TC ($r = 0.91$), total microbial biomass ($r = 0.83$), and TN ($r = 0.71$) (Table 4). No significant correlations were found between gross nitrification or gross NO_3^- consumption fluxes and TC, TN or total microbial biomass (Table 4), and gross N mineralization or NH_4^+ consumption (data not shown).

Table 4 Pearson correlation coefficient between gross N transformation fluxes and total N, total C, and total microbial biomass

	Total N	Total C	Total microbial biomass
Gross N mineralization	0.84**	0.86**	0.96**
Gross NH_4^+ consumption	0.71*	0.91*	0.83*
Gross nitrification	0.18	0.40	0.44
Gross NO_3^- consumption	−0.43	−0.24	−0.39

** Correlation significant at the 0.01 level, * Correlation significant at 0.05 level, $n = 9$

Discussion

Soil organic matter availability is a main factor limiting microbial biomass growth in natural and managed soil ecosystems (Singh and Singh 1995;

Table 3 Gross N transformation fluxes for the different agricultural and forest sites

	WC	CF	PF
Gross N mineralization (μg N g^{-1} day^{-1})	0.45 (0.06)[b]	0.40 (0.27)[b]	3.81 (0.38)[a]
Gross NH_4^+ consumption (μg N g^{-1} day^{-1})	0.53 (0.16)[b]	0.30 (0.45)[b]	4.90 (0.79)[a]
Gross nitrification (μg N g^{-1} day^{-1})	0.89 (0.34)[a]	0.21 (0.16)[b]	1.10 (0.20)[a]
Gross NO_3^- consumption (μg N g^{-1} day^{-1})	1.06 (0.34)[a]	0.60 (0.59)[a]	0.58 (0.59)[a]

CF summer crop—winter fallow rotation, *WC* winter cropland, *PF* pristine forest; standard deviations between brackets; values in the same row not followed by a different letter are significantly different

Booth et al. 2005). In agreement with Booth et al. (2005), our results also indicate a positive correlation between microbial biomass, and soil C and N substrate availability across forest and agro-ecosystems. At a global scale, the SOM contents of agro-ecosystems are about 42% lower than in native forests (Guo and Gifford 2002). Our agricultural soils showed a lower TC (−47 to −51%) and TN (−34 to −42%) concentration than the pristine forest soils, which is in agreement with previous studies in south Chilean Andisols (Borie et al. 2002; Heredia et al. 2007). Notwithstanding the lower soil C and N concentrations relative to forests, the soil C (7.6–8.3%) and N (0.64–0.73%) in our agro-ecosystems are still located at the upper-end of worldwide topsoil SOM concentrations across agro-ecosystems, grasslands and forest ecosystems (Booth et al. 2005). Other nutrients that might limit microbial growth in volcanic soils, such as available P (Borie and Zunino 1983), are clearly higher in the agricultural sites as a result of long-term fertilization management. Land use and vegetation have the potential to alter the SOM quality in volcanic soils (Nanzyo et al. 1993; Verde et al. 2008; but see Nierop et al. 2005, 2007 for contrasting results). Borie et al. (2002) and Heredia et al. (2007) indicated that the internal balance of C and N associated with labile and stable SOM fractions was largely similar between land use classes in south Chilean volcanic soils.

A clear difference in microbial community composition was observed between the agro-ecosystems and forest ecosystems. Considering the similarity in SOM composition between forests and agro-ecosystems, we attribute the lower relative contribution of fungi to total microbial biomass in the agro-ecosystems to the different vegetation (Zak et al. 2003), and land management practices (Nodar et al. 1992; Beare et al. 1997; Bardgett and Shine 1999). The higher abundance of gram-negative bacteria in forests can be attributed to the higher organic matter content and microbial substrate availability (Zelles et al. 1992; Bossio et al. 1998). On the other hand, the dominance of gram-positive bacteria in the agricultural sites can be ascribed to the (temporal) absence of a vegetation cover in some periods of the year (Brant et al. 2006). Additionally, soil microbial community composition may have been influenced by soil texture and associated water availability in the soil pores (Fierer et al. 2003; Tippkötter et al. 2009).

Notwithstanding the fair correlation between soil C and N and microbial biomass, a clear difference in C- and N-normalized microbial biomass was observed between the forest and agricultural sites (Table 2; Fig. 2). Hence, for the same unit of SOM substrate present, microbial biomass abundance is significantly greater in the forest than in the agricultural sites. These results stand in contrast with results obtained by Singh and Singh (1995) and Yang et al. (2008) who found that C- and N-normalized microbial biomass was greater or equal in agricultural soils than in SOM-rich forest soils.

Since soil microbes rely on low molecular weight compounds for their growth, SOM depolymerisation is the bottleneck process regulating microbial growth in soil ecosystems (Schimel and Bennett 2004). Extracellular enzymes catalyze this initial, rate-limiting step of SOM decomposition (Sinsabaugh 1994; Schimel and Bennett 2004). The activities of extracellular enzymes may change if land management shifts the microbial groups that produce specific enzymes or alters microbial allocation to enzyme production (Sinsabaugh and Moorhead 1994). We believe that a reduction in the extent of SOM depolymerisation also contributes to the lower microbial biomass and N production observed in our agro-ecosystems. Microbial populations differ in the types of extracellular enzymes produced (Kirk and Farrell 1987). Many groups of fungi are known to produce hydrolases and oxidases that degrade complex polymers, characterized by interlinked C and N compounds, and convert organically bound nutrients into forms that are available to plants and microbes (Dighton 2003; Allison et al. 2008). The positive relationship between fungi/bacteria ratio and substrate normalized microbial biomass in our study (Fig. 2) provides evidence that a decrease in fungal abundance limits the production of low molecular weight compounds and associated microbial growth in our agro-ecosystems. In the forest ecosystems, the higher availability of low molecular N compounds as a result of fungal processing might stimulate further microbial growth. As such, especially bacterial groups such as gram-negative bacteria, prevailing in sites with a high substrate availability (Zelles et al. 1992; Bossio et al. 1998), could benefit. These results are in agreement with Allison et al. (2008) and Muruganandam et al. (2009) who observed that land management (N fertilization and soil tillage,

respectively) affected fungal biomass and associated production of main extracellular enzymes involved in the breakdown of complex organic compounds. The direct effect of microbial community changes on extracellular enzyme activity, and altered capabilities to degrade macromolecular compounds, has also been documented by Waldrop et al. (2000). On the other hand, existing microbial groups might also shift their metabolism as a result of external drivers. As a result of N addition, microbes may invest more in the acquisition of C than in the already available N, causing a reduction in N-degrading enzyme productivity (Allison and Vitousek 2005). The latter may result in a decrease in internal soil N production. Chu et al. (2007) indicated shifts in soil enzyme activities between balanced and nutrient-deficiency fertilization without accompanied changes in microbial community composition. However, since any shift in metabolic activities as a result of long-term land management goes often hand in hand with changes in microbial community composition, it is difficult to separate direct effects on enzyme activities from indirect effects through altered microbial community structure. Soils of finer texture have often been documented to house a larger microbial biomass due to reduced fluctuations in soil water content or protection of microorganisms from faunal grazing (Rutherford and Juma 1992; Hassink et al. 1993). Although texture was somewhat finer in the agro-ecosystems than in the forest, biomass concentrations were greater in PF than in WC and CF. This indicates that texture was a less important driver of microbial biomass growth and their function in the soil N cycle than microbial community composition.

Gross N mineralization fluxes in the studied agro-ecosystems (0.40–0.45 μg g^{-1} day^{-1}) are relatively low compared to worldwide fluxes in soils under the same land use (0.3–10 μg N g^{-1} day^{-1}, Burger and Jackson 2003; Booth et al. 2005). We believe that the combination of humified recalcitrant soil material and low fungal abundance limits gross N mineralization in the agro-ecosystems. Our data indicate that, besides total microbial biomass and substrate concentration (Booth et al. 2005), also microbial community composition is a main player involved in the N mineralization in these Andisols. Next to differences in microbial community structures, the greater N mineralization rates in the forest than in the agro-ecosystems may potentially be fomented by the slightly coarser soil texture in the forest site. Some studies have indicated a negative relationship between N mineralization rates and clay content (Hassink et al. 1993). In fine-textured soils, SOM may experience a high degree of physical protection, resulting in an increased N mineralization rate per unit of microbial biomass (Franzluebbers et al. 1996). However, it should be noted that many studies did not observe a significant correlation between soil texture and N mineralization (Motavalli et al. 1995; Giardina et al. 2001).

Across sites, gross nitrification did not show a significant correlation with NH_4^+ transformation fluxes, or soil C and N. This observation can be ascribed to the fact that distinct NO_3^- production pathways take place in the different sites. Specialist heterotrophic organisms play a main role in the nitrification process of the forest ecosystem (Huygens et al. 2008), while these organisms are less abundant in managed agricultural soils, typically dominated by autotrophic nitrifiers (Kurakov et al. 2001). Gross NH_4^+ and NO_3^- immobilization fluxes equalize or exceed the gross N mineralization and nitrification fluxes in all sites. The combination of abiotic adsorption on clay minerals in these Andisols, rich in positively and negatively charged soil minerals (Tosso 1985), and microbial immobilization explains the high consumption of NH_4^+, even in absence of plant roots (Huygens et al. 2007).

Ecosystem implication and further perspectives

To the best of our knowledge, this is the first report of gross N cycling rates in agro-ecosystems located on volcanic Andisols. This study indicates that internal soil N cycling, characterized by low inorganic N production and strong inorganic N retention, is unable to provide a sufficient N input for south Chilean winter crops. On the other hand, the risk for N losses towards aqueous water bodies or atmosphere is small due to the low inorganic N (especially NO_3^-) availability, even under fallow soil conditions during the rainy winter period. This indicates that the current farming management practices, including often high fertilization rates, anticipate in an appropriate manner the internal soil N cycling processes in these south Chilean agro-ecosystems. However, the close relationship between N bioavailability, total SOM

accumulation and fungal biomass points towards opportunities for alternative agricultural land management that might reduce the excessive fertilizer cost. Organic additions (e.g. farmyard input or residue incorporation) or reduced tillage are known to stimulate the proliferation of fungal hyphae and promote microbial growth, with beneficial effects on extracellular enzyme activities and soil N mineralization (Doran 1980; Roldán et al. 2005; Stark et al. 2007). Considering that N depolymerisation by extracellular enzymes is a main process limiting N mineralization in these Andisols, such land management options might have a high potential to improve internal soil N productivity in these soils. Future research exploring microbial patterns and N biogeochemistry with study designs that do not confound vegetation and land management should be performed to address this hypothesis.

Acknowledgments Dries Huygens is a postdoctoral fellow of the Fund for Scientific Research (FWO, Flanders). This research was supported by the National Commission for Scientific and Technological Research—Chile (FONDECYT, N°1090455), and the Dirección de Investigación y Desarrollo—Universidad Austral de Chile (DID-UACh). Eric Gillis, Katja Van Nieuland, and Jan Vermeulen are acknowledged for PLFA and isotope analyses.

References

Alfaro MV, Salazar F, Endress DB, Dumont JCL, Valdebenito AB (2006) Nitrogen leaching losses on volcanic ash soil as affected by the source of fertilizer. J Soil Sci Plant Nutr 6:54–63

Allison SD, Vitousek PM (2005) Responses of extracellular enzymes to simple and complex nutrient inputs. Soil Biol Biochem 37:937–944

Allison SD, Czimczik CI, Treseder KK (2008) Microbial activity and soil respiration under nitrogen addition in Alaskan boreal forest. Glob Change Biol 14:1156–1168

Bååth E, Frostegård Å, Fritze H (1992) Soil bacterial biomass, activity, phospholipid fatty acid pattern, and pH tolerance in an area polluted with alkaline dust deposition. Appl Environ Microbiol 58:4026–4031

Bardgett RD, Shine A (1999) Linkages between plant litter diversity, soil microbial biomass and ecosystem function in temperate grasslands. Soil Biol Biochem 31:317–321

Beare MH, Hu S, Coleman DC, Hendrix PF (1997) Influences of mycelial fungi on soil aggregation and organic matter storage in conventional and no-tillage soils. Appl Soil Ecol 5:211–219

Bernier R, Undurraga P (2009) Fertilización de praderas permanentes para la producción de leche. In: Navarro H, Siebald E, Celis SR (eds) Manual de producción de leche para pequeños y medianos productores, Boletín Inia No 148. Instituto de Investigaciones Agropecuarias, Centro Regional de Investigación Remehue, Osorno, pp 12–18

Billings SA, Gaydess EA (2008) Soil nitrogen and carbon dynamics in a fragmented landscape experiencing forest succession. Landscape Ecol 23:581–593

Booth M, Stark JM, Rastetter E (2005) Controls on nitrogen cycling in terrestrial ecosystems: a synthetic analysis of literature data. Ecology 75:139–157

Borie F, Zunino H (1983) Organic matter P associations as a sink in P-fixation processes in allophanic soils of Chile. Soil Biol Biochem 15:599–603

Borie G, Peirano P, Zunino H, Aguilera SM (2002) N-pool in volcanic ash-derived soils in Chile and its changes in deforested sites. Soil Biol Biochem 34:1201–1206

Bossio DA, Scow KM, Gunapala N, Graham KJ (1998) Determinants of soil microbial communities: effects of agricultural management, season, and soil type on phospholipid fatty acid profiles. Microb Ecol 36:1–12

Brant JB, Myrold DD, Sulzman EW (2006) Root controls on microbial community structure in forest soils. Oecologia 148:650–659

Burger M, Jackson LE (2003) Microbial immobilization of ammonium and nitrate in relation to ammonification and nitrification rates in organic and conventional cropping systems. Soil Biol Biochem 35:29–36

Campillo RR, Jobet CF, Undurraga PD (2007) Optimization of nitrogen fertilization for high-yielding potential wheat on Andisols at the Araucanía Region, Chile. Agric Técn 67:281–291

Cartes P, Jara AA, Demanet R, Mora ML (2009) Urease activity and nitrogen mineralization kinetics as affected by temperature and urea input in southern Chilean Andisols. J Soil Sci Plant Nutr 9:69–82

Chu H et al (2007) Soil microbial biomass, dehydrogenase activity, bacterial community structure in response to long-term fertilizer management. Soil Biol Biochem 39:2971–2976

Chung HG, Zak DR, Reich PB, Ellsworth DS (2007) Plant species richness, elevated CO_2, and atmospheric nitrogen deposition alter soil microbial community composition and function. Glob Change Biol 13:980–989

Davidson EA, Hart SC, Shanks CA, Firestone MK (1991) Measuring gross nitrogen mineralization, immobilization, and nitrification by ^{15}N isotopic pool dilution in intact soil cores. J Soil Sci 42:335–349

Day PR (1965) Particle fractionation and particle size analysis. In: Black CA (ed) Methods of soil analysis, 2nd edn. American Society of Agronomy, Madison, pp 562–566

Denef K et al (2007) Community shifts and carbon translocation within metabolically-active rhizosphere microorganisms in grasslands under elevated CO_2. Biogeosciences 4:769–779

Diaz C, Bravo A, Aranda G (1960) Reconocimiento de suelos de las provincias de Osorno y Llanquihue. Agric Técn 19–20:125–226

Dighton J (2003) Fungi in ecosystem processes. Marcel Dekker, New York

Doran JW (1980) Soil microbial and biochemical changes associated with reduced tillage. Soil Sci Soc Am J 44:765–771

Drijber RA, Doran JW, Parkhurst AM, Lyon DJ (2000) Changes in soil microbial community structure with tillage under long-term wheat-fallow management. Soil Biol Biochem 32:1419–1430

Drury CF, Beauchamp EG (1991) Ammonium fixation, release, nitrification and immobilization in high- and low-fiwing soils. Soil Sci Soc Am J 55:125–129

Drury CF, Voroney RP, Beachamp EG (1991) Availability of NH_4^+-N to microorganisms in soils with varying NH_4^+ fixation capacities. Soil Biol Biochem 23:165–169

FAO (2009) Fertilizers. http://faostat.fao.org/. Retrieved on November 11, 2009

Fierer N, Schimel JP, Holden PA (2003) Variations in microbial community composition through two soil depth profiles. Soil Biol Biochem 35:167–176

Franzluebbers AJ, Haney RL, Hons FM, Zuberer DA (1996) Active fractions of organic matter in soils with different texture. Soil Biol Biochem 28:1367–1372

Frostegård A, Bååth E (1996) The use of phospholipid fatty acid analysis to estimate bacterial and fungal biomass in soil. Biol Fert Soils 22:59–65

Galloway JN et al (2003) The nitrogen cascade. Bioscience 53:341–356

Giardina CP, Ryan MG, Hubbard RM, Binkley D (2001) Tree species and soil textural controls on carbon and nitrogen mineralization rates. Soil Sci Soc Am J 65:1272–1279

Guo LB, Gifford RM (2002) Soil carbon stocks and land use change: a meta analysis. Glob Change Biol 8:345–360

Hassink J, Bouwman LA, Zwart KB, Bloem J, Brussaard L (1993) Relationships between soil texture, physical protection of organic matter, soil biota, and C and N mineralization in grassland soils. Geoderma 57:105–128

Hauck RD (1982) Nitrogen isotope ratio analysis. In: Page AL, Miller RA, Keeney DR (eds) Methods of soil analysis. ASA and SSSA, Madison, pp 735–779

Heredia W, Peirano P, Borie G, Zunino H, Aguilera M (2007) Organic carbon balance in Chilean volcanic soils after human intrusion and under different management practices. Acta Agric Scand Sect B-Soil Plant Sci 57:329–334

Huygens D, Rütting T, Boeckx P, Van Cleemput O, Godoy R, Müller C (2007) Soil nitrogen conservation mechanisms in a pristine south Chilean *Nothofagus* forest ecosystem. Soil Biol Biochem 39:2448–2458

Huygens D et al (2008) Mechanisms for retention of bioavailable nitrogen in volcanic rainforest soils. Nature Geosci 1:543–548

IUSS-Working-Group-WRB (2006) World reference base for soil resources. IUSS Working Group WRB. FAO, Rome

Kirk KT, Farrell RL (1987) Enzymatic 'combustion': the microbial degradation of lignin. Ann Rev Microbiol 41:465–505

Kirkham D, Bartholomew WV (1954) Equations for following nutrient transformations in soil, utilizing tracer data. Soil Sci Soc Am Proc 18:33–34

Klute A (1986) Methods of soil analysis, part 1. Physical and mineralogical methods, 2nd edn. American Society of Agronomy, Soil Science Society of America, Madison

Kroppenstedt R (1985) Fatty acid and menaquinone analysis of actinomycetes and related organisms. In: Goodfellow M, Minnikin D (eds) Chemical methods in bacterial systematics. Academic Press, London, pp 173–199

Kroppenstedt R (1992) The genus *Nocardiopsis*. In: Balows A (ed) The prokaryotes: a handbook on the biology of bacteria ecophysiology, isolation, identification, applications. Springer, New York, pp 1139–1156

Kurakov AV, Evdokimov IV, Popov AI (2001) Heterotrophic nitrification in soils. Eurasian Soil Sci 34:1116–1124

Liang BC, Mackenzie AF, Gregorich EG (1999) Measurement of fixed ammonium and nitrogen isotope ratios using dry combustion. Soil Sci Soc Am J 63:1667–1669

Lin S, Dittert K, Wu WL, Sattelmacher B (2004) Added nitrogen interaction as affected by soil nitrogen pool size and fertilization-significance of displacement of fixed ammonium. J Plant Nutr Soil Sci 167:138–146

Matson PA, Parton WJ, Power AG, Swift MJ (1997) Agricultural intensification and ecosystem properties. Science 277:504–509

McKinley DC, Rice CW, Blair JM (2008) Conversion of grassland to coniferous woodland has limited effects on soil nitrogen cycle processes. Soil Biol Biochem 40: 2627–2633

Mora ML, Cartes P, Nuñez P, Salazar M, Demanet R (2007) Movement of NO_3^-–N and NH_4^+–N in an Andisol and its influence on ryegrass production in a short term study. J Soil Sci Plant Nutr 7:46–64

Motavalli PP, Palm CA, Elliot ET, Frey SD, Smithson PC (1995) Nitrogen mineralization in humid tropical forest soils: mineralogy, texture, and measured nitrogen fractions. Soil Sci Soc Am J 59:1168–1175

Mulvaney RL (1996) Nitrogen-inorganic forms. In: Sparks DL (ed) Methods of soil analysis. ASA and SSSA, Madison, pp 1123–1184

Muruganandam S, Israel DW, Robarge WP (2009) Activities of nitrogen-mineralization enzymes associated with soil aggregate size fractions of three tillage systems. Soil Sci Soc Am J 73:751–759

Nanzyo M, Dahlgren RA, Shoji S (1993) Chemical characteristics of volcanic ash soils. In: Shoji S, Nanzyo M, Dahlgren RA (eds) Volcanic ash soils: genesis, properties and utilization. Elsevier, Amsterdam

Nierop KGJ, van Bergen PF, Buurman P, van Lagen B (2005) NaOH and $Na_4P_2O_7$ extractable organic matter in two allophanic volcanic ash soils of the Azores Islands—a pyrolysis GC/MS study. Geoderma 127:36–51

Nierop KGJ, Tonneijck FH, Jansen B, Verstraten JM (2007) Organic matter in volcanic ash soils under forest and Páramo along an Ecuadorian altitudinal transect. Soil Sci Soc Am J 71:1119–1127

Nodar R, Acea MJ, Carballas T (1992) Microbiological response to $Ca(OH)_2$ treatments in a forest soil. FEMS Microb Lett 86:213–219

Nömmik H, Vahtras K (1982) Retention and fixation of ammonium and ammonia in soils. In: Stevenson SJ (ed) Nitrogen in agricultural soils. ASA-CSSA-SSSA, Madison, pp 123–172

Olsen SR, Cole CV, Watanabe FS, Dean LA (1954) Estimation of available phosphorus in soils by extraction with sodium bicarbonate, USDA Circular 939. United States Department of Agriculture, Washington DC

Oyarzún CE, Huber A (2003) Nitrogen export from forested and agricultural watersheds of southern Chile. Gayana Bot 60:63–68

Pennanen T, Frostegard A, Fritze H, Baath E (1996) Phospholipid fatty acid composition and heavy metal tolerance of soil microbial communities along two heavy metal-polluted gradients in coniferous forests. Appl Environ Microbiol 62:420–428

Perakis SS, Hedin LO (2001) Fluxes and fates of nitrogen in soil of an unpolluted old-growth temperate forest, southern Chile. Ecology 82:2245–2260

Perakis SS, Compton JE, Hedin LO (2005) Nitrogen retention across a gradient of ^{15}N additions to an unpolluted temperate forest soil in Chile. Ecology 86:95–105

R Development Core Team (2009) R: a language and environment for statistical computing. R Foundation for Statistical Computing, Vienna

Rhoades CC, Coleman DC (1999) Nitrogen mineralization and nitrification following land conversion in montane Ecuador. Soil Biol Biochem 31:1347–1354

Roing K, Andren O, Mattsson L (2006) 'Non-exchangeable' ammonium in soils from Swedish long-term agricultural experiments: mobilization and effects of fertilizer application. Acta Agric Scand Sect B-Soil Plant Sci 56:197–205

Roldán A, Salinas-Garcia JR, Alguacil MM, Diaz E, Caravaca F (2005) Soil enzyme activities suggest advantages of conservation tillage practices in sorghum cultivation under subtropical conditions. Geoderma 129:178–185

Russow R, Spott O, Stange CF (2008) Evaluation of nitrate and ammonium as sources of NO and N_2O emissions from black earth soils (Haplic Chernozem) based on ^{15}N field experiments. Soil Biol Biochem 40:380–391

Rutherford PM, Juma NG (1992) Influence of soil texture on protozoa-induced mineralization of bacterial carbon and nitrogen. Can J Soil Sci 72:183–200

Salazar F, Alfaro M, Ramírez L, Pinochet D, Ibarra C (2008) Lixiviación de nitrógeno en una pradera permanente fertilizada en otoño. In: Gallo C (ed) XXXIII reunión anual de la sociedad Chilena de producción animal. Facultad de Ciencias Veterinarias y Ciencias Agrarias, Universidad Austral de Chile, Valdivia, pp 71–72

Schimel JP, Bennett J (2004) Nitrogen mineralization: challenges of a changing paradigm. Ecology 85:591–602

Shoji S, Nanzyo M, Dahlgren R (1993) Productivity and utilization of volcanic ash soils. In: Shoji S, Nanzyo M, Dahlgren R (eds) Volcanic ash soils—genesis, properties and utilization. Elsevier, Amsterdam, pp 209–244

Singh S, Singh JS (1995) Microbial biomass associated with water-stable aggregates in forest, savanna and cropland soils of a seasonally dry tropical region, India. Soil Biol Biochem 27:1027–1033

Sinsabaugh RL (1994) Enzymic analysis of microbial pattern and process. Biol Fert Soils 17:69–74

Sinsabaugh RL, Moorhead DL (1994) Resource allocation to extracellular enzyme production: a model for nitrogen and phosphorus control on litter decomposition. Soil Biol Biochem 26:1305–1311

Soil-Survey-Staff (2006) Keys to soil taxonomy, 10th edn. United States Department of Agriculture & Natural Resources Conservation Service, Washington, DC

Stark C, Condron LM, Stewart A, Di HJ, O'Callaghan M (2007) Influence of organic and mineral amendments on microbial soil properties and processes. Appl Soil Ecol 35:79–93

Stevens RJ, Laughlin RJ (1994) Determining ^{15}N in nitrite or nitrate by producing nitrous oxide. Soil Sci Soc Am J 58:1108–1116

Tippkötter R, Eickhorst T, Taubner H, Gredner B, Rademaker G (2009) Detection of soil water in macropores of undisturbed soil using microfocus X-ray tube computerized tomography (mu CT). Soil Till Res 105:12–20

Torn MS, Trumbore SE, Chadwick OA, Vitousek PM, Hendricks DM (1997) Mineral control of soil organic matter carbon storage and turnover. Nature 389:170–173

Tosso J (1985) Suelos volcánicos de Chile. Instituto de Investigaciones Agropecuarias, Santiago

Trehan SP (1996) Immobilisation of $^{15}NH_4^+$ in three soils by chemical and biological processes. Soil Biol Biochem 28: 1021–1027

Verde JR, Buurman P, Martinez-Cortizas A, Macias F, Arbestain MC (2008) NaOH extractable organic matter of andic soils from Galicia (NW Spain) under different land use regimes: a pyrolysis GC/MS study. Eur J Soil Sci 59:1096–1110

Waldrop MP, Balser TC, Firestone MK (2000) Linking microbial community composition to function in a tropical soil. Soil Biol Biochem 32:1837–1846

Yang LL, Zhang FS, Mao RZ, Ju XT, Cai XB, Lu YH (2008) Conversion of natural ecosystems to cropland increases the soil net nitrogen mineralization and nitrification in Tibet. Pedosphere 18:699–706

Young JL, Aldag RW (1982) Inorganic forms of nitrogen in soil. In: Stevensson FJ (ed) Nitrogen in agricultural soils. American Society of Agronomy, Madison, pp 43–66

Zak DR, Holmes WE, White DC, Peacock AD, Tilman D (2003) Plant diversity, soil microbial communities, and ecosystem function: are there any links? Ecology 84:2042–2050

Zelles L (1999) Fatty acid patterns of phospholipids and lipopolysaccharides in the characterisation of microbial communities in soil: a review. Biol Fert Soils 29:111–129

Zelles L, Bai QY, Beck T, Beese F (1992) Signature fatty acids in phospholipids and lipopolysaccharides as indicators of microbial biomass and community structure in agricultural soils. Soil Biol Biochem 24:317–332