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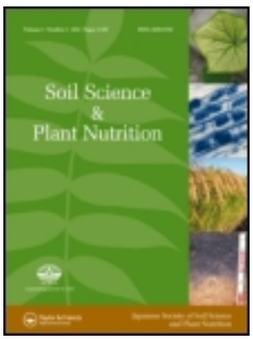

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ORIGINAL ARTICLE

Potential mineralization and nitrification in volcanic grassland soils in Chile

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Abstract

A proportion of the nitrogen (N) applied to grasslands as organic or inorganic fertilizers can be lost to water courses as nitrate and to the atmosphere as nitrous and nitric oxides. Volcanic soils from Chile are not generally prone to leaching, possibly due to net immobilization of nitrate and/or ammonium, and/or due to inhibition of nitrification by either chemical or physical processes. In laboratory studies we found large mineralization potentials in soils from three different Chilean soils after 17 weeks of incubation, totalling 215 and 254 mg kg^{-1} dry soil for two Andisols and 127 mg kg^{-1} dry soil in an Ultisol. Nitrification occurred after a short period, and was lowest in the Ultisol. In addition, microbial analysis showed nitrifiers to be present in all three soils. Adsorption of ammonium was two-fold stronger than for nitrate, ranging from 29 to 180 kg N ha^{-1}. The highest potential for N adsorption in the 0–60 cm soil profile was with the Ultisol (398 kg N ha^{-1}), but was similar in both Andisols (193 and 172 kg N ha^{-1}, respectively). The combination of ammonium retention together with delayed nitrification could account for the low leaching rates in these soils.

Key words: Volcanic soils, nitrification, mineralization, microbial processes, nitrogen losses.

INTRODUCTION

Grasslands are important ecosystems as they occupy about 50% of the Earth's surface (Snaydon 1981) across a range of latitudes, soils and climates. Grassland management involves a variety of practices that affect the soil's physical structure (e.g., machinery and the presence of livestock), nutrient balance (e.g., additions of nitrogen, N and carbon, C) and the capacity of the soil to store C (e.g., cultivation) affecting water, soil and air quality. A proportion of N added to grasslands as organic or inorganic fertilizer is lost to water courses as nitrite (NO_2^-), ammonium (NH_4^+), nitrate (NO_3^-) and organic N, and lost to the atmosphere as ammonia (NH_3), nitrous oxide (N_2O) and nitric oxide (NO) as well as nitrogen (N_2).

The main processes responsible for the production and removal of NO_3^- are nitrification and denitrification, which, as with many components of the soil N cycle, are driven by the activity of prokaryotes although the relative contribution of bacteria and archaea is uncertain. Different groups have the potential to reduce NO_3^- to N_2O or N_2, and denitrifying bacteria are universally present in soils, although active denitrification requires anaerobic conditions with a supply of NO_3^- and soil organic matter (OM). These microorganisms contain the enzymes to reduce NO_3^- to N_2O and N_2 via NO_2^- and NO (Zumft 1997). There are two forms of nitrite reductase found in bacteria, *nirK* and *nirS*. They appear to be mutually exclusive but interchangeable in bacteria, and the significance of which form is present in a particular group is unclear (Hallin *et al.* 2009). The N_2O

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reductase (*nosZ* enzyme) catalyzes the reduction of N_2O to N_2 as the end product of denitrification, but the gene responsible for its transcription is not found in all denitrifying bacteria (Philippot *et al.* 2007). In contrast, *amoA*, the gene for NH_3 oxidation which is a key step in nitrification, is found in only a few groups of autotrophic bacteria and archaea although these are present in most soils (Nicol and Schleper 2006).

We investigated some of the N cycling processes in volcanic grassland soils from Chile. These soils are characterized by low nutrient availability, high organic matter content, and the presence of allophane and other minerals which give a low bulk density and high phosphate retention (Matus *et al.* 2008). These soils are not generally prone to leaching in grassland (Alfaro *et al.* 2009; Salazar *et al.* 2011) and crops (Nissen *et al.* 1991). Losses as NO_3^--N measured in a Chilean grassland soil using the ceramic suction cup technique in grazed areas receiving inorganic N fertilizer varied between 8 and 17 kg N ha^{-1} yr^{-1}, over 3 yr under a range of stocking rates (Salazar *et al.* 2011). Specifically, rates for the soils used in this study for ungrazed pastures receiving 200 kg N ha^{-1} (see below) were 2.0, 3.0 and 2.5 kg N ha^{-1} for Osorno, Cudico and Chiloé respectively (Alfaro *et al.* 2011). Values for pastures in other locations after application of 200 kg N ha^{-1} are reported between 44 kg ha^{-1} yr^{-1} for permanent pasture in England (Scholefield *et al.* 1993) and 101 kg ha^{-1} yr^{-1} for dairy grazing systems in New Zealand (Ledgard *et al.* 1999).

The objective of this study was to investigate under controlled conditions the potential for mineralization and nitrification of two Chilean volcanic Andisols (high organic matter content) and one Ultisol (lower organic matter content) and the relationship of these potentials to the presence of soil microbial communities that are normally involved in these processes. We investigated the potential of these soils to produce NO_3^- and their capacity to retain NH_4^+ and NO_3^-. Microbial analysis provided quantification of the genes encoding nitrifying/denitrifying enzymes naturally present in these soils.

We hypothesize that the low leaching rates could be due to (1) high rates of immobilization of ammonium and nitrate in these soils (biotic and abiotic processes); and (2) that nitrification could be inhibited by specific soil properties related to either the chemical or physical processes in these soils; (3) that there is potential for other losses to occur, for example, in the form of nitrous oxide (N_2O). The results from this study might also be applicable to other volcanic agricultural soils, such as those found in Japan and New Zealand.

MATERIALS AND METHODS

Soil sampling and preconditioning

Soils were collected in April 2007 and July 2008 from three grassland locations (each with three field replicates) in the south of Chile from the 0–10 cm surface layer. The soils were: Osorno (Osorno series, Andisol, Typic Hapludands; 40°31′ S 73°03′ W); Chiloé (Chonchi series, Andisol, Acrudoxic Durudands; 40°15′ S 73°39′ W) and Cudico (Cudico series, Ultisol, Typic Hapludults; 40°39′ S 73°21′ W). The soil OM contents were: Chiloé, 27%; Osorno, 17% and Cudico, 14% (see other properties in Table 1). The soils were dried at ambient temperature, sieved through a 2-mm mesh and mixed to be used for further analysis. The soils were subsampled for analysis of NH_4^+ and NO_3^- by extraction in 2M potassium chloride (KCl) for 1 h on an orbital shaker. The extracts were filtered through Whatman No. 5 filter paper before determination by automated colorimetry (Kamphake *et al.* 1967; Searle 1984). Total N, total C, water pH and Soil Moisture Content (SMC) were assessed according to Sadzawka *et al.* (2006) and Rowell (1997), respectively. In order to determine potential for soil processes associated with N cycling, all experimental work was carried out under near-optimum standardized conditions.

Measurements of mineralization and nitrification in the air-dried soils were done in Chile. Additional soil was collected at depths of 0–10, 10–30 and 30–60 cm, sieved through a 2-mm mesh, air dried and stored for the measurement of NH_4^+ and NO_3^- adsorption in the soil profile. The remaining air-dried soils (0–10 cm surface layers) were exported to the UK and kept under strict quarantine conditions at ambient temperature for more detailed microbial analyses, and all samples and materials were autoclaved before disposal.

Mineralization

An aerobic incubation following the procedure by Stanford and Smith (1972) was used. Briefly, three samples of each of the dry soils (25 g) were placed in 200-mL bottles and incubated for up to 30 weeks at 70% water holding capacity (WHC) at 25°C. There were nine sampling times during the incubation so enough replicates were available for destructive sampling on each occasion. Every week the bottles were weighed and water losses compensated by the addition of the correct amount of deionized water. Extractions were carried out on days 0, 7, 14, 28, 55, 85, 115, 145 and 226 after the start of the incubation. On each sampling

Table 1 Characteristics of the topsoil layer (0–10 cm)

Parameter	Osorno	Chiloé	Cudico
Land use	Permanent pasture (10 years)	Permanent pasture (8 years)	Permanent pasture (10 years)
Main species	*Lolium multiflorum* L., *Holcus lanatus* L.	*Holcus lanatus* L., *Agrostis tenuis* S., *Lotus uliginosus* S.	*Holcus lanatus* L., *Agrostis tenuis* S., *Anthosatum odoratum* L.
Soil texture	Loamy	Loamy sand	Loamy clay
Annual rainfall, mm	1260	2184	950
Soil type[1]	Andisol	Andisol	Ultisol
Humins, g $100g^{-1}$ OM	97 ± 0.2	90 ± 0.06	90 ± 0.4
Fulvic acids, g $100g^{-1}$ OM	1.2 ± 0.12	1.8 ± 0.11	1.9 ± 0.41
Humic acids, g $100g^{-1}$ OM	1.1 ± 0.04	2.5 ± 0.16	4.2 ± 0.03
Mineralogy[2]	Allophane	Allophane	Haloisite-7A
Aluminum (Al) oxides[1], %	2.67	2.90	0.72
Iron oxides[1], %	0.85	0.85	0.89
pH, water	5.8 ± 0.03	5.6 ± 0.01	5.2 ± 0.01
Organic matter, g kg^{-1}	170 ± 7.0	270 ± 11.8	140 ± 21.9
Olsen phosphorus, mg kg^{-1}	12 ± 0.80	4 ± 0.10	2 ± 0.08
Calcium, cmol (+) kg^{-1}	2 ± 0.05	4 ± 0.05	1.4 ± 0.05
Magnesium, cmol (+) kg^{-1}	0.5 ± 0.01	1.5 ± 0.03	1.0 ± 0.01
Al[3], cmol (+) kg^{-1}	0.3 ± 0.02	0.3 ± 0.01	1.7 ± 0.01
Cation exchange capacity[2], cmol (+) kg^{-1}	23.1– 50.7	46.9–77.5	29.2–49.9
Sulphur, mg kg^{-1}	8 ± 0.10	2 ± 0.04	2 ± 0.08
Al saturation, %	2.0 ± 0.05	4.9 ± 0.05	1.4 ± 0.05
Nitrate, mg N kg^{-1}	2 ± 0.1	4 ± 0.2	9 ± 0.0
Ammonium, mg N kg^{-1}	14 ± 1.3	7 ± 1.4	36 ± 1.6
Total nitrogen, g $100g^{-1}$	1.00 ± 0.03	1.13 ± 0.04	0.54 ± 0.02
Total carbon, g $100g^{-1}$	11.2 ± 0.59	14.8 ± 1.15	7.85 ± 0.51
Bulk density, g cm^{-3}	0.64	0.64	1.21
Organic carbon[4], %	9.86	15.66	8.12

[1]According to CIREN (2003); [2]According to Tosso (1985); [3]Al saturation index, proportion of available Al found in soil solution in relation to the total cation content (calcium + magnesium + potassium + sodium + aluminum); [4]Organic carbon calculated as OM/1.724 according to Walkley (1947).

occasion, three replicates of each soil were taken and mineral N extracted using the KCl extraction methodology.

An assessment of the trend in mineralization was made following Stanford and Smith (1972) by plotting cumulative net N mineralization versus the square root of time. Estimates of potential mineralization capacity were obtained using the expression:

$$1/\mathrm{Nt} = 1/\mathrm{No} + \mathrm{b}/\mathrm{t} \tag{1}$$

where Nt is mg N kg^{-1} mineralized (cumulative) during time t (weeks); b is the slope (week mg^{-1} N kg^{-1}); and No (mg N kg^{-1}) is the mineralization potential.

We also fitted exponential functions using Genstat version 11.0 through the data:

$$\mathrm{Y} = \mathrm{A} + \mathrm{B} * \mathrm{R}^{\mathrm{X}} \tag{2}$$

where Y = Nt; A = No; B = response value (upper asymptote – A); if A = B = No (curve fitted through zero); $R = e^{-k}$; t = time.

If fitted through zero the curve is described by:

$$\mathrm{N_t} = \mathrm{No}\left(1 - \mathrm{e}^{-\mathrm{kt}}\right) \tag{3}$$

where Nt is the mineralization (in mg N kg^{-1}) at time t (in days), No is the potential mineralization (mg kg^{-1} dry soil) and k is the rate of the process (day^{-1}).

Nitrogen and ammonium adsorption in the soil profile

For NO_3^- adsorption (i.e., soil retention), we followed the methodology developed by Wild (1972). The soil used was collected at different depths as described in Section 2.1. Centrifuge tubes (50 mL) were weighed and 5 g of dry soil added with 25 mL of 0.01M potassium nitrate (KNO_3) (for NO_3^- retention analysis). The tubes were centrifuged at 4000 rpm (26,000 *g*) for 10 min, after which the supernatant was discarded. This step was repeated three more times to ensure that the soil was well mixed with KNO_3 every time. At the end of this process the tubes with soil were weighed (including the entrained water). The retained NO_3^- was then

displaced by washing the soil four times with 25 mL of 1M sodium chloride (NaCl). The washings were combined and analysed for NO_3^-. The amount of NO_3^- adsorbed was calculated from the concentration of NO_3^- found in the washings (total volume of washings) multiplied by the original volume (total washings + entrained water) divided by 5 g dry soil. The results were expressed as positive charges in mequiv 100 g^{-1} dry soil. The amount of NO_3^- was converted to mequiv by the formula:

$$(\text{moles} * 1000/\text{valency}) * 100/5 \quad (4)$$

For NH_4^+ adsorption analysis, ammonium chloride (NH_4Cl) was used in place of KNO_3 after Thompson and Blackmer (1992) and Lumbanraja and Evangelou (1994). The solution was shaken at ambient temperature (25°C) for 4 h and centrifuged at 4000 rpm for 10 min. The supernatant was removed and kept and the soil washed four times with 25 mL of 1M NaCl (removing the supernatant every time). The supernatants from each sample were filtered every time through Whatman No. 1 and combined, then frozen until analysis for NH_4^+ and NO_3^-. The data was further transformed to mg kg^{-1} dry soil of nitrate-nitrogen (NO_3^--N) or ammonium-nitrogen (NH_4^+-N).

Microbial analysis

Quantitative polymerase chain reaction (qPCR) was used to estimate the relative abundance of bacterial and archaeal 16S rRNA, nitrification *amoA* genes and the bacterial denitrification genes, *nirK, nirS and nosZ*, in the three Chilean grassland soils. DNA extractions were performed using the MO BIO Laboratories, Inc. PowerSoil® DNA Isolation Kit as described previously (Clark *et al.* 2012) and DNA purity and concentrations checked using a combination of NanoDrop™ spectrophotometer (Thermo Scientific, Waltham, MA, USA) scan between 200–300 nm and PicoGreen® dsDNA Quantification kit (Molecular Probes). DNA was extracted from each of the three replicate soil samples and each of these was analysed in duplicate using qPCR. Extracted DNA concentrations were consistent within treatments and of a similar range to those obtained in previous studies. To ensure there were no detrimental effects of PCR inhibitors, various DNA dilutions were tested. Prior to qPCR, environment-specific standards for each primer set were generated. Serial dilutions of an aliquot of amplified product (generated from DNA extracted from pooled soil samples) were used with the relevant primer set to generate the standard curves for estimating gene copy number; the gel was purified and quantified using a PicoGreen® dsDNA Quantification kit (Molecular Probes). This was to minimize the bias inherent in the usual approach, where one or only a few genes are used to standardize complex communities (Töwe *et al.* 2010). The qPCR assays were run on an Applied Biosystems 7900HT Fast Real-Time PCR System in a 384-well format with 10 µL final volume per well. The reaction consisted of 10 ng template DNA (quantified by PicoGreen fluorescence), 1X QuantiTect® SYBR® Green Master Mix (Qiagen) with optimized primer concentrations and conditions as shown in Table 2. Results and

Table 2 Primers and conditions used in the microbial determinations

	Primer				Primer	
Target gene	Name	Sequence	Degeneracy	Size (bp)	Conc.	Reference
Bacterial16S rRNA	bac331F	tcc tac ggg agg cag cag t	0	194	0.5 µM	Nadkarni *et al.* (2002)
	muyzer 534rev	att acc gcg gct gct gg	0			Muyzer *et al.* (1993)
Archeal16S rRNA	arch349F	gyg cas cag kcg mga aw	32	458	10 µM	Takai and Horikoshi (2000)
	arch806R	gga cta cvs ggg tat cta at	4			
Bacterial *amoA*	amoA-1F	ggg gtt tct act ggt ggt	0	491	10 µM	Rotthauwe *et al.* (1997)
	amoA-2R	ccc ctc kgs aaa gcc ttc ttc	4			
Archeal *amoA*	arch-amoAF	sta atg gtc tgg ctt aga cg	2	635	0.25 µM	Francis *et al.* (2005)
	arch-amoAR	gcg gcc atc cat ctg tat gt	0			
nirK	nirK876	aty ggc ggv cay ggc ga	12	165	5 µM	Henry *et al.* 2004
	nirK1040	gcc tcg atc agr ttr tgg tt	4			
nirS	nirS_cd3a-F	gts aac gts aag gar acs gg	16	410	2.5 µM	Michotey *et al.* (2000)
	nirS_R3cd-R	gas ttc ggr tgs gtc ttg a	8			Throbäck *et al.* (2004)
nosZ	nosZ2FHenry	cgc rac ggc aas aag gts mss gt	64	268	5 µM/2.5 µM	Henry *et al.* 2006
	nosZ2RHenry	cak rtg cak sgc rtg gca gaa	32			

Polymerase chain reaction (PCR) cycling conditions: annealing temperature 58°C (15 sec), extension temperature 72°C (30 sec, Archaeal *amoA* 45 sec), denaturing 95°C (15 sec) for forty cycles. bp, base pairs.

efficiency of reactions (R^2 values 0.9675–0.9939) were analysed using LinRegPCR program version 11.1 (Ramakers *et al.* 2003; Ruijter *et al.* 2009). Although the presence of genes does not prove that they are active, we have observed a strong correlation between nitrification rates in agricultural soils and the number of bacterial ammonia oxidizers (Mendum *et al.* 1999) and the abundance of *nirK* with N_2O fluxes (Clark *et al.* 2012).

RESULTS

Mineralization

The rates of potential mineralization obtained showed similar trends for the Andisols (Fig. 1a and b). The initial rapid increase in NH_4^+ observed in our study in the Andisols (about 60 mg N kg^{-1} dry soil in the first 7 d) coincided with a smaller change in NO_3^- (1.2 and 6.4 mg N kg^{-1} dry soil increase in Osorno and Chiloé, respectively). The Cudico soil showed a decrease in both NH_4^+ and NO_3^- (NH_4^+ decreased by 23.4 and NO_3^- by 6.8 mg N kg^{-1} dry soil) for the same period. Between days 14 and 28, both NO_3^- and NH_4^+ were correlated in the Andisols with a decrease in NH_4^+ of 49.8 and 39.0 mg N kg^{-1} dry soil for Osorno and Chiloé respectively, corresponding to an increase in NO_3^- of 67.4 mg N kg^{-1} dry soil for Osorno and 64.3 mg N kg^{-1} dry soil for Chiloé. In both soils, NO_3^- subsequently continued to increase, whereas NH_4^+ changed only marginally. The Cudico soil showed a smaller increase in NO_3^- for the rest of the incubation but there was no further change in NH_4^+.

A net mineralization in the 17 weeks of the incubation was observed in all three soils (Fig. 1a and b), with a total for both Andisols of 215 and 254 mg kg^{-1} dry soil for Osorno and Chiloé, respectively, and considerably higher than the Ultisol (viz., 127 mg kg^{-1} dry soil).

Stanford and Smith (1972) provide two methods to calculate mineralization potential (i.e., the first and second estimates). The method to calculate the first estimate of mineralization potential (No) when applied to our study resulted in values of total mineralization (NO_3^- + NH_4^+ up to 226 d) of 256.4, 285.7 and 400.0 mg kg^{-1} N for Osorno, Chiloé and Cudico soils, respectively. The corresponding coefficients of determination (r^2) were 92, 96 and 99%, respectively. Applying the second estimate

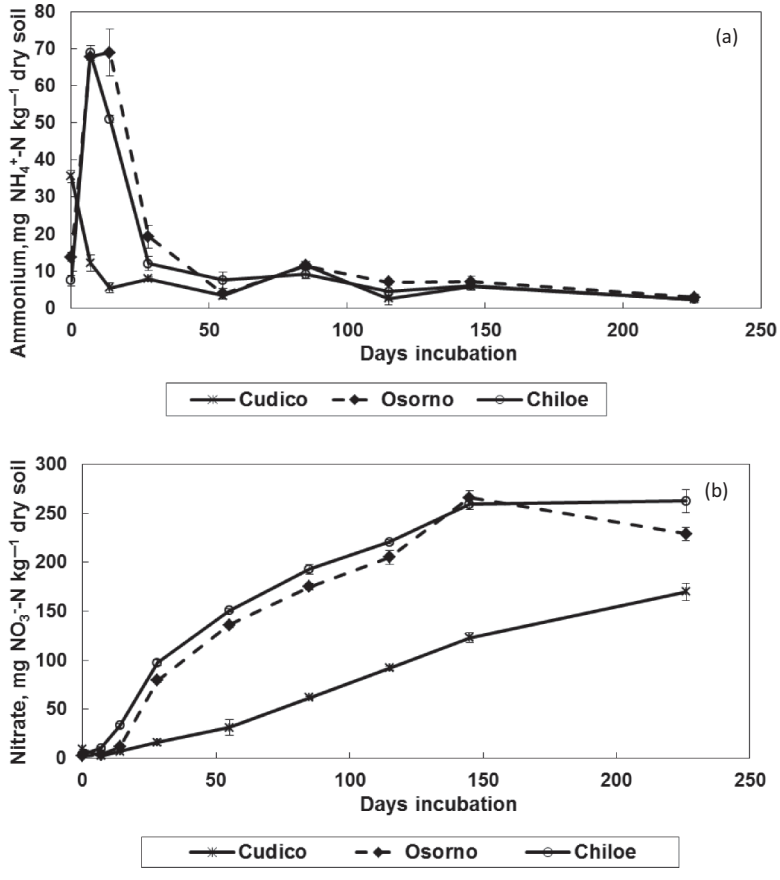

Figure 1 (a) ammonium-nitrogen (NH_4^+-N) and (b) nitrate-nitrogen (NO_3^--N) contents in the aerobic mineralization incubation experiment. Error bars are the standard error of three replicates.

methodology using curve fitting in Genstat for Chiloé and Osorno to the total mineralization data (NO_3^- + NH_4^+ up to 226 d) resulted in functions of the type: $Y = A + B * R^x$, where A = No and k = –log R (see Table 3; Fig. 2a and b). This function did not fit the data for the Cudico soil and as the curve showed a non-exponential growth, S-shaped functions were used, including logistic, generalized logistic and Gompertz functions. These three gave very similar results with 93.3, 94.0 and 92.5%, respectively, of the variance accounted for. We report the results of the logistic function in Table 3, where

$$Y = A + C/\{1 + EXP[-B * (X - M)]\} \quad (5)$$

B = reaction rate; M = inflexion point; C = higher asymptote; A = lower asymptote (see Fig. 2c). The relative rates of mineralization for Osorno and Chiloé obtained from –log (R) (Table 3) were 0.0057 and 0.0060 mg N kg^{-1} day^{-1}, respectively. The results from the S-shaped curve fitted through the data from the Cudico soil gave a rate of 0.0194 mg N kg^{-1} day^{-1}.

If there was an effect of the preparation of the soils, (drying, mixing, sieving) on the processes investigated, this might be reflected in the initial samplings of the incubation period. We fitted curves for the three soils after removing the first three data points in each case (which is when steady state was established; see Fig. 1a). The resulting parameters for both Osorno and Chiloé before and after removing the first three data points were similar, suggesting there was no marked effect of disturbance. We also calculated the intersection with the x axis (time) and obtained a negative value for both soils to see if there was a lag in the process (a positive value is expected if there is a lag).

In the case of Cudico, when removing the first three points the function resulted in almost no curvature so a linear regression was fitted, resulting in Y = 1.16 + 0.7594 X, with an R^2 = 0.99. As in the two Andisols, the intersection with the x axis did not give a positive value (no evidence of a lag).

Nitrogen adsorption in the soil profile

Adsorption of N in the topsoil was greater for NH_4^+ compared with NO_3^- in the Andisols by 1.8:1 and 1.6:1 in Osorno and Chiloé, respectively. In Cudico the ratio was 2:1. Adsorption of NH_4^+ ranged between 38.2 and 76.6 mg NH_4^+-N kg^{-1} dry soil with the lowest value from Osorno and the highest from Cudico, equivalent to from 38 to 216 kg N ha^{-1}, respectively. The results also showed NO_3^- adsorption at all depths in all three Chilean soils of between 14.8 and 46.2 mg NO_3^--N kg^{-1} dry soil. The lowest value was from Osorno and the highest from Chiloé (Fig. 3), equivalent to from 19 to 85 kg N ha^{-1}. The highest adsorption of inorganic N (expressed as mg N kg^{-1} soil) was observed in the 0–30 cm layer in all three soils. Because of differences in soil bulk density the highest potential for N adsorption in the soil profile was found in the Cudico soil (583 kg N ha^{-1} in the 0–60 cm soil layer), whilst Osorno and Chiloé soil had a similar potential for N adsorption (390 and 345 kg N ha^{-1}, respectively) in the 0–60 cm soil layer.

Table 3 Values of function parameters from curves fitted for the soils

	Osorno		Chiloé	
Parameter	all points	except first 3 points	all points	except first 3 points
A	265.1	262.4	278.4	283.4
B	–232.4	–253.1	–244.9	–251.1
R	0.98709	0.98568	0.98633	0.98718
F probability	< 0.001	< 0.001	< 0.001	< 0.001
% variance accounted for	94.4	86.6	97.4	98.7
		Cudico		
		all points		
B		0.0194		
M		129.9		
C		187.9		
A		6.7		
F probability		< 0.001		
% variance accounted for		93.3		

In Osorno and Chiloé: A = No and k = –log R. In Cudico (logistic function): B = reaction rate; M = inflexion; C = higher asymptote; A = lower asymptote.

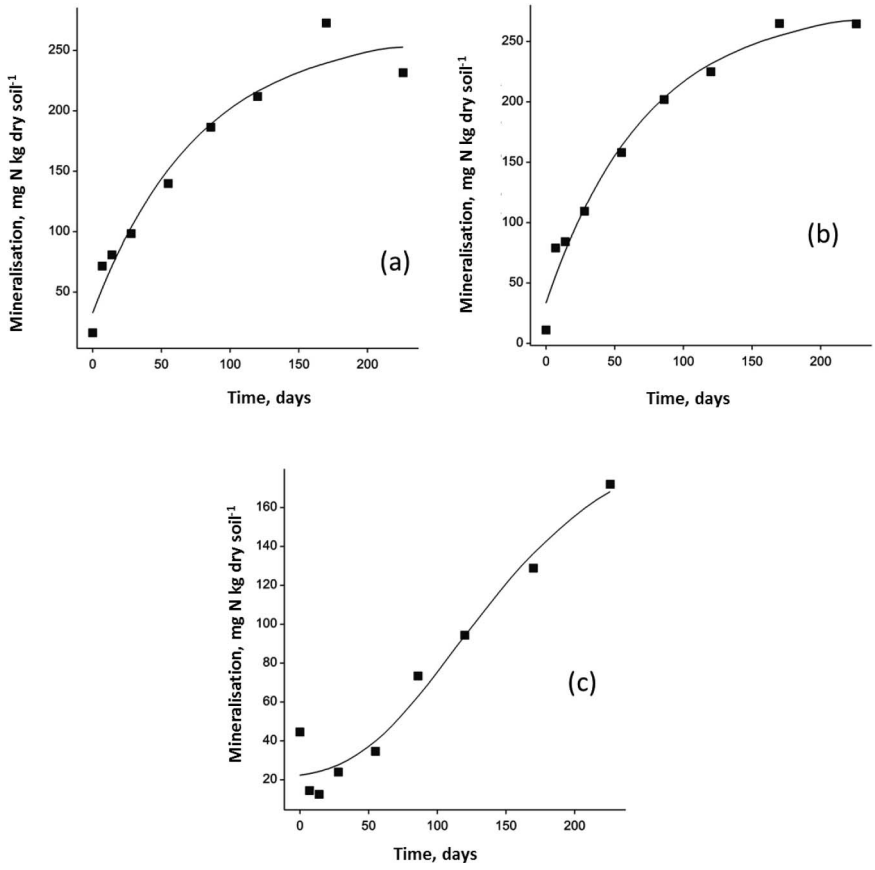

Figure 2 Exponential curves fitted to mineralization measurements in the Andisols: (a) Osorno, (b) Chiloé, (c) non-exponential curve fitted to mineralization measurements in the Ultisol Cudico soil. N, nitrogen.

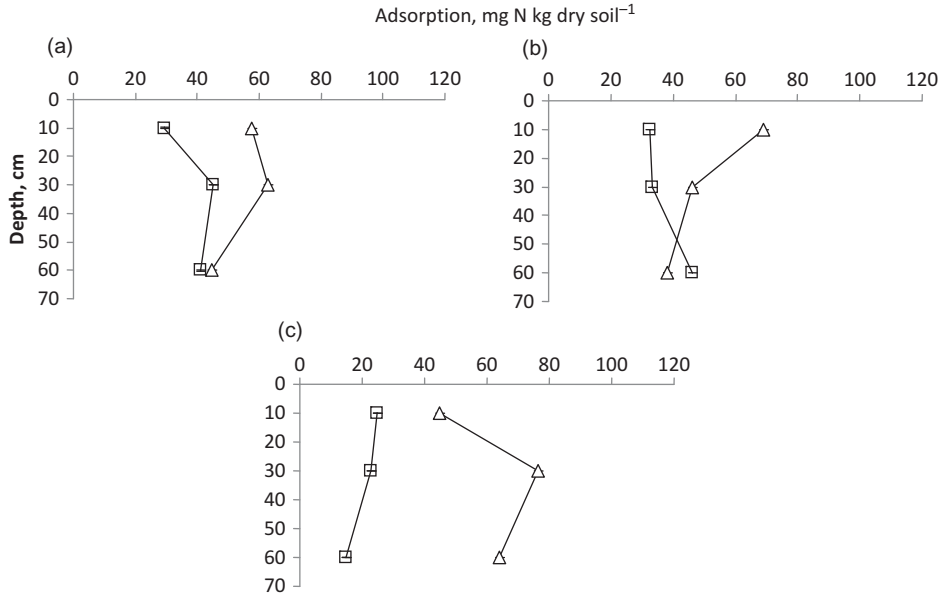

Figure 3 Ammonium-nitrogen (NH_4^+-N; Δ) and nitrate-nitrogen (NO_3^--N; □) adsorption in the 0–60 cm soil profile in (a) Osorno, (b) Chiloé, (c) Cudico. N, nitrogen.

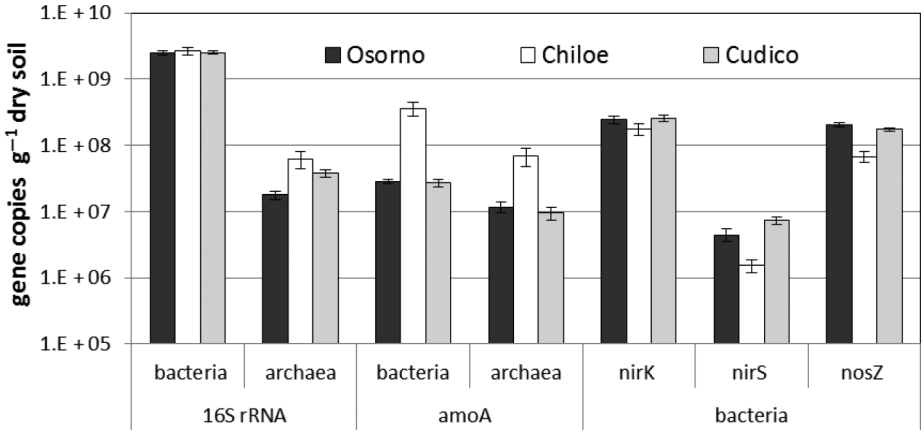

Figure 4 Quantitative real-time polymerase chain reaction (PCR) estimates of gene abundance.

Microbial community structure

In all three soils the abundance of bacteria based on the number of 16S rRNA gene copies was similar, c. 2×10^9 when expressed per g dry soil, with Archaea 50-fold less abundant in the Chiloé and Cudico soils and c. 150-fold less in the Osorno soil (Fig. 4). A similar trend was seen with NH_3 oxidizers estimated from *amoA,* with bacteria more abundant than archaea, but all NH_3 oxidizers were 5- to 10-fold more abundant in Chiloé than in Cudico and Osorno soils (Fig. 4). The denitrifier communities in all soils appeared to be dominated by *nirK* which was 50- to 100-fold more abundant than *nirS*. Osorno and Cudico soils contained similar numbers of *nirK* and *nosZ* and more than Chiloé, whereas Cudico had the most and Chiloé the fewest copies of *nirS* g^{-1} dry soil (Fig. 4). The ratio of nitrite reductase to N_2O gene copies (*nirK* + *nirS : nosZ*) was greatest in the Chiloé soil (2.6:1) and least in the Osorno soil (1.2:1), Cudico soil having an intermediate ratio (1.5:1). Expressing results as gene copies per cm^3 soil rather than per g dry soil makes little difference, although results for Cudico soil are slightly increased as a consequence of the greater bulk density (results not shown).

DISCUSSION

The results from the potential mineralization by aerobic incubation agree with the trend observed previously when measuring mineralization potential in the same soils from an anaerobic incubation (Dixon *et al*. 2011). They found potential rates about four times larger in Osorno and Chiloé with respect to Cudico, whereas our study showed values two times larger. Gross mineralization rates measured in the same study with a ^{15}N labelling technique provided similar values in the two Andisols which were about twice the value from the Ultisol and in agreement with our results.

Our results showed that these soils have the capacity to nitrify, but this potential was only observed after 7–14 d from the start of the incubation. Cartes *et al*. (2009) found a delay in nitrification of 20–25 d in their soil with a higher OM content. This is noteworthy, as during this time, NO_3^- leaching would be expected to be limited and NH_4^+ could be either free in the soil solution or retained in the soil. The Cudico soil did not show a net increase in NO_3^- in the first 28 d, but NH_4^+ decreased, suggesting that in this soil there was a delay before nitrifying activity developed and some of the NH_4^+ could have been immobilized during that time. Stanford and Smith (1972) quote low rates of mineralization in the first 4 weeks in some of the soils they studied, possibly reflecting a lag in microbial activity and/or assimilation by microorganisms due to decomposition of small amounts of low-N plant material. Dixon *et al*. (2011) observed low gross nitrification rates in the same soils at time 0, with proportions of mineralized N which were nitrified of 0.01, 0.03 and 0.003% for Osorno, Chiloé and Cudico, respectively. It seems the observed delay in nitrification in our incubation could be a contributing factor in the low N leaching in these soils.

The "first estimate" of potential mineralization (Stanford and Smith 1972) suggested a similarity between the two Andisols, but they differed from the Ultisol. The latter did not reach a plateau during the incubation period (unlike the Andisols that reached a maximum on day 145; Fig. 1), suggesting that the estimate obtained using data from the aerobic incubation cannot be taken as a true potential for this soil. Values obtained by Stanford and Smith (1972) ranged between 21 and 354 mg N kg^{-1} for a total of 39 soils, with the Ultisols generally giving the lower values.

The calculation of a potential mineralization following Stanford and Smith (1972) has been used previously for Chilean soils (Oyanedel and Rodriguez 1977; Rodriguez and Silva 1983). The various methods used for estimating mineralization potential confirm that NO_3^- is produced in these soils, but it would appear that a period is required before an increase in NO_3^- is observed. The delay in NO_3^- production suggests that other processes might be removing mineralized N (as NH_4^+) during this period, before it can be transformed to NO_3^-. In this short period (up to two weeks) there could be adsorption of NH_4^+ in the soil. Adsorption of N in the form of NH_4^+ was indeed larger than for NO_3^- in all the soil profiles which may retard nitrification and reduce the potential for leaching. The low nitrification rates measured by Dixon *et al.* (2011) in the same soils would support this, as these authors did not introduce a period for adaptation before their soil assays. On the other hand, the increase in NO_3^- observed in all soils after day 28 in our study, coincident with no change in NH_4^+, might suggest that NH_4^+ was produced at the same rate as it was being converted to NO_3^- (steady state).

In the longer term (more than 2 weeks) there could be adsorption of either NO_3^- or NH_4^+ throughout the soil profile. NO_3^- adsorption was larger in the Andisols than the Ultisol in the deeper layers. Andisols were also found to have lower leaching compared with the Ultisol (Alfaro *et al.* 2009). The greatest adsorption per kg of soil was found in Cudico at 30–60 cm depth. Our values compare closely with those of Wild (1972) who found NO_3^- retention of between 10–30 mg NO_3^--N kg^{-1} dry soil in a bare fallow soil in Nigeria.

The adsorption of N can be related to the OM content of these soils. According to other data obtained for similar soils (Tosso 1985), the organic C concentration in the topsoil for each soil was 5.6, 13.3 and 4.3% for Osorno, Chiloé and Cudico, respectively. The middle layer [depth average for the soils of 30 cm (10–40 cm depth)] had organic C concentrations of 5.3, 12.4 and 1.9% for Osorno, Chiloé and Cudico, respectively. The values for the deepest layer were 3.0, 6.6 and 1.5% for Osorno, Chiloé and Cudico, respectively. Comparing the adsorption potentials for NO_3^- and NH_4^+ for all three layers with organic C gave high positive correlations between NH_4^+ and organic C for Chiloé and Osorno (97%) and a negative correlation for Cudico (–86%). Correlations between NO_3^- and organic C were –97, –74 and 84% for Chiloé, Osorno and Cudico, respectively. These values suggest similar retention properties in both Andisols but different in the Ultisol. The results from Dixon *et al.* (2011) showed that there was a large proportion of the mineralized N that was not accounted for in the processes they studied (viz., nitrification and immobilization). This suggests that the potential for NH_4^+ retention found in our study could explain the fate of the mineralized N which was previously unaccounted for. These results could explain the smaller and similar leaching rates observed in the Andisols compared to the Ultisol (Salazar *et al.* 2012).

These findings agree with recent studies carried out on Chilean forest soils (Huygens *et al.* 2007). According to our results, NO_3^- retention in the first 10 cm of the soil would not contribute significantly to low NO_3^- leaching losses in these soils, but retention seems to occur in deeper layers for the Andisols. Total N retention (sum of NO_3^- and NH_4^+) was greatest in the middle layer in Osorno and Cudico, whereas for Chiloé it was in the top layer.

The results from the microbial analyses showed that ammonia oxidizing bacteria and Archaea were relatively more abundant in the Chiloé soil; conditions in this soil are conducive to their survival, benefiting from a steady supply of NH_3/NH_4^+ to provide energy for growth. Other studies have shown bacterial ammonia oxidizer abundance to increase with increasing N supply, which is in agreement with the observation that total N was highest in the Chiloé soil, even though it contained the least NH_4^+. It is likely that N cycling and slow release of bound forms of N provide a regular flow of substrate for nitrifiers, and that there are microsites that are less acid so that NH_3 is available and more suitable for nitrification. Sieving during the preparation of soil for nitrification potential assays may have exposed the microorganisms to a lowered pH and thus impaired nitrification. Fungi are sometimes responsible for nitrification in acid soils, but it is unlikely in this case because sieving disrupts fungal hyphae. Archaeal ammonia oxidizers are reported to thrive at a lower pH than bacterial ammonia oxidizers, because their ammonia monooxygenase has a higher affinity for NH_3 and so is active at lower available concentrations than that of bacteria (Lehtovirta-Morley *et al.* 2011; Tourna *et al.* 2011) and they are more abundant in many soils (Leininger *et al.* 2006). Despite the acid pH of the Chilean soils, ammonia oxidizing Archaea appeared to be less abundant and so unlikely to be responsible for nitrification in this case. Some ammonia oxidizing bacterial groups are abundant and active in low-pH soils (Nugroho *et al.* 2005). The higher relative abundance of the bacterial *amoA* in these Chilean soils implicates bacteria as the most important group of nitrifiers.

The fact that *nirK* numbers are larger than *nirS* in all soils would suggest that the nitrite reductase encoded by this gene is more important for denitrification than that encoded by *nirS*, but as their functions were not measured

in this study, we cannot be certain of this. In the Chiloé soil, *nirK* was slightly less abundant than in the other soils; this difference was more marked for *nirS* and *nosZ*, indicating fewer denitrifying bacteria and thus a lower potential for N_2O losses in this soil, in an inverse relationship to the nitrifiers. However, the abundance of genes involved in denitrification may be uncoupled from N_2O emissions and N fluxes in acidic soils (Čuhel *et al.* 2010).

In Osorno and Cudico soils, the ratios of denitrifying: nitrifying enzyme gene copy numbers (nirS + nirK)/amoA) were 6.2:1 and 7.2:1, respectively. This was greater than the ratio in the high-OM Chiloé soil (0.4:1), which was similar to that reported in two forest soils (0.4:1 and 0.6:1) by Wallenstein and Vilgalys (2005). This could indicate differences in the potential for removal of NO_3^- by denitrification compared to its formation via nitrification, subject to the caveat that the presence of genes does not necessarily indicate that they are active. The denitrifying bacteria were least abundant in the Chiloé soil, which is surprising from the reports of other studies that indicate increasing numbers and activity to be correlated with soil OM and total N. However, the rapid immobilization of NO_3^- in this soil may mean that the availability of NO_3^- for microorganisms, plant uptake or losses to the wider environment is limited.

We found that although mineralization rates were higher and similar in the Andisols compared with Cudico (Ultisol), there were also similarities between the Osorno and Cudico soils, suggesting that even with similar characteristics, soils still show differences in some functions. For example, total N retention was higher and similar in Osorno and Cudico compared with Chiloé, but nitrifier abundance was lower. Nitrification rates, on the other hand, were lowest in the Cudico soil. Denitrifier abundance was lower in Chiloé, which could suggest that the higher N retention potential could be related to a higher potential for N_2O losses in Osorno and Cudico. Chiloé, on the other hand, has a lower nitrification potential due to low nitrifier abundance, and this potential developed after a short period. The results from Dixon *et al.* (2011) showed that a large proportion of the mineralized N (gross rate of release) results in plant available N (net release). The difference between these two parameters (5.6, 11.2 and 6.3 μg g^{-1} day^{-1} for Osorno, Chiloé and Cudico respectively) was attributed to NH_4^+ immobilization in Dixon *et al.* (2011). Our study suggests physico-chemical retention as an alternative fate for N which can be up to one order of magnitude larger than microbial immobilization in these soils.

The findings from this study strongly suggest that the low leaching rates in these soils are likely to be the result of a combination of factors. Results suggest that the larger potential for NH_4^+ adsorption could have caused the observed delay in nitrification, and so there would be less NO_3^- available to be lost by leaching and denitrification, as previously reported in field conditions (Salazar *et al.* 2011; Vistoso *et al.* 2012). The Andisols were also shown to have a greater potential to retain NO_3^- compared with the Ultisol, which agrees with the lower leaching rates found in the former. Further work, including analysis of the enzymes' functions, fractionation of the organic matter and field measurements of leaching and gaseous emissions will help to determine the overall fate of N and the particular properties of these soils that inhibit N losses via leaching, and to develop, define and refine suitable mitigation strategies.

The results from this study show the potential of these volcanic Chilean soils to retain nutrients, especially N, reducing the possible losses to water and the atmosphere. Further investigations into the relevant properties of these soils should follow to help develop mitigation strategies that can be applied more widely.

CONCLUSIONS

This study has provided information on the potential for production and removal of NO_3^--N and NH_4^+-N in high OM volcanic soils. We found that Chilean volcanic soils have a large potential to mineralize N, greater in the Andisols compared with an Ultisol. Nitrification occurred after a short period from the start of the incubation. Model fitting suggested a single pool for mineralization and gave potential estimates of 265.1 mg N kg^{-1} for Osorno, 278.4 mg N kg^{-1} for Chiloé and 187.9 mg N kg^{-1} for Cudico, respectively. N retention was larger for NH_4^+ compared with NO_3^- in all the soils, and the total N retained was higher and similar in Osorno and Cudico in the middle soil layer compared with Chiloé. Gene abundance indicated that nitrifiers were most abundant and denitrifiers least abundant in the Chiloé soil, indicating the relative availability of NH_3 but not NO_3^-.

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