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Abstract

A biological (aerobic incubation for 3 and 6 weeks) and a chemical method [successive extractions with cold 0.1 (H1-N) and 0.5 M HCl (H2-N)] were applied to 21 soils to determine: a) the potentially mineralizable-N; b) the most useful soil variables for predicting soil N availability; and c) their usefulness for predicting N uptake by a greenhouse wheat crop. At t=3, both net N mineralized (NNM) and net N mineralization rate (NNMR) were correlated: a) positively with SOM- and CEC-related variables; and b) positively with soil $\delta\ ^{15}N$ and negatively with soil pH, suggesting that N-mineralization, dominated by nitrification, is associated with NO_3^--N losses and soil acidification. At t=6, all previously discussed variables were important for NNM, but not for NNMR, mainly controlled by the available-P content. The importance of H1-N increased with N_2-inputs and decreased with NO_3^- losses and soil-N. Relationships of H1-N and H2-N with soil CEC and texture showed the strong relations among nutrients content, biological activity and N mineralization, as well as the recalcitrance of clay-bounded SOM. Soil total-N correlations with wheat-N in absolute amount (positive) and as percentage of soil-N (negative) showed an important supply of available-N by N-rich soils, despite their slow N turnover. The best regression models for wheat-N always included 1-2 main available nutrients. The percentage of soil N exported to plant biomass was negatively correlated with non-crystalline Al compounds and soil $\delta\ ^{15}N$. Mineralized-N and wheat-N pools did not share many correlations with soil properties and seemed to come from different sources; consequently, the former pool, which only explained a quarter of wheat-N variance, was not more useful than soil total-N for predicting it. Only a positive correlation with soil total-N was shared by wheat-N and hydrolysable-N, highlighting that the latter N pools are mainly unrelated. Nevertheless, half of wheat-N variation was explained by its negative relationship with the percentage of soil-N as (H1+H2)-N; a possible explanation is that chemically labile N is also biologically labile, being cumulated because of a limiting factor for microbial N mineralization or plant growth and emerging as a good predictor for wheat-N uptake.

Introduction

Although N reserves in the earth are very important, most of them are unavailable for living organisms: only 0.02% of the earth's N is within the biosphere and most of it is relatively inert (94% in soil organic matter) and only gradually made available to plants by microbial degradation (Porter 1975; Haynes 1986). Consequently, N fertility is so closely associated with organic N dynamics that soil organic matter (SOM) and SOM-dependent properties are considered the key to the long-term sustainability and productivity of soils (Peoples et al. 1995). Moreover, several variables related to soil N status and dynamics, such as N content, potentially mineralizable N and nitrification, have been proposed as basic soil quality indicators (Karlen et al. 1997; McCarty and Meisinger 1997; Knoepp et al. 2000).

Predicting *in situ* N mineralization has been one of the greatest challenges to improving N management in agriculture (Mikha et al. 2006). Field and greenhouse measurements of N mineralization capacity and crop production are necessary as reference data, but they are cost and time consuming. Consequently, many laboratory biological methods have been proposed during the last half century for assessing soil N availability. Most of them are based on short- or medium-term incubation of soils under controlled conditions that promote N mineralization, which is subsequently measured, but these methods are subjected to several criticisms. As (Clarholm 1985) indicates, during the incubation the microbial activity can decline due to the accumulation of inorganic-N and the shortage of an adequate source of energy, that would be consumed and supplied by plant roots, respectively, under natural conditions. (Wallwork 1983) highlighted that soil microorganisms can only act efficiently within the limits imposed by soil fauna; therefore, laboratory incubations, which exclude soil fauna can lead to unrealistic results. While some authors (Nordmeyer and Richter 1985; Kristensen et al. 2000) reported that N mineralization was stimulated by soil sieving and manipulation, others did not find significant effects (Ross et al. 1985; Franzluebbers 1999); these controversial results suggest that the effects of soil manipulation on organic N mineralization can be related to type, depth and management of soils. Nevertheless, despite these criticisms, short- or medium-term incubations are still considered an adequate method for studying the soil N mineralization kinetics (Connell et al. 1995; Curtin et al. 2006).

Although "it is manifestly impossible to devise a chemical extraction procedure that simulates the action of microorganisms in releasing plant-available forms of soil N" (Stanford 1982), a rapid and reliable chemical index of N availability to estimate soil N mineralization to improve N fertilizer recommendations has long been sought. Unfortunately, no one of the numerous chemical methods proposed over the years has received broad acceptance across a wide range of soils (Jalil et al. 1996; Landgraf 2001; Curtin et al. 2006; Bushong et al. 2008; Schomberg et al. 2009; Stanford 1982). Chemical

extractions with strong reagents solubilize amounts of N much higher than those supposed to be easily mineralizable and were rapidly discarded (see Stanford 1982). The studies with extraction methods using extractants of intermediate intensity have also shown inconsistent relations with biological indexes of N availabilty (Stanford 1982). As a result, it is now well known that only the extractions with mild reagents can provide an acceptable estimation of the soil net N mineralization (Gianello and Bremner 1986; González-Prieto et al. 1992; González-Prieto et al. 1997; Landgraf and Klose 2002; Curtin et al. 2006; Bushong et al. 2008; Stanford 1982).

Accordingly, the aim of the present study was to test a biological (aerobic incubation) and a chemical method (extractions with cold 0.1 and 0.5 M HCl) so that: a) to estimate the potentially mineralizable soil organic N; b) to evaluate wether these methods can predict the uptake of N by a greenhouse wheat crop; c) to study the influence of main soil properties on N availability; and d) to identify the most useful soil variables for predicting soil N availability.

Material and methods

Study site

The study was performed with 21 soils from the *Páramo* de Gavidia (Northern Andes, Mérida, Venezuela) characterized in a previous work (Abadín et al. 2002). All soils are acidic, stony and sandy Inceptisols, but the ranges of variation of the main soil properties were tight for pH (1 unit) and sand and silt contents (1.5x), moderate for C, N and clay (2.5-3x) and wide for the exchangeable macronutrients (17-48x), especially (134x) for P (see Table 1). Soil samples were taken from the A horizon (0-15 cm depth) with a stainless steel probe (3.5 cm internal diameter). Fifteen sub-samples were taken at random from the whole area of each plot, mixed and thoroughly homogenized after sieving at 4 mm.

Greenhouse experiment

Soil samples (500 g; two replicates) were placed in plastic pots (600 cm^3), seeded with 20 wheat seeds and daily watered with deionized water to keep the soils at 75% of water holding capacity. Six weeks after germination, roots and shoots from each pot were jointly harvested, washed with tap and deionized water, oven-dried at 60 °C, finely ground (< 100 μm) and analysed for total N content with a Carlo Erba CHNS 1108 elemental analyser. The same was done with wheat seeds (n= 50) to determine their contribution to the plant-N content and to evaluate accurately the amount of soil N taken up by the plants. Wheat was chosen by two reasons: a) worlwide it is one of the most important cultures; and b) in the crop-fallow chronosequence usually employed in the study area, after cropping potatoes for 1-2 years and before abandoning the plot, a cereal (barley or wheat) is usually cropped without

application of fertiliser; therefore, our wheat crop perfectly reflects the final part of the cultivation phase in this rotation.

Laboratory aerobic incubation

The N mineralization capacity was determined by aerobic incubation in an hermetic glass system with intermittent aeration with a flow of air previously humidified by bubbling it through a water column. Six replicate 45 g samples of each soil were placed in 500 ml Erlenmeyer flasks and incubated in a thermostated bath at 28 °C and 75% of water holding capacity for 42 d. After both 3 and 6 weeks of incubation three replicates were withdrawn, the inorganic N (NH_4^+ and NO_3^-) was extracted by shaking them with 250 ml of 2 M KCl for 1 h and the extracts were filtered through glass microfibre filters (Whatman GF/A, 125 mm diameter). The inorganic-N forms were converted to NH_3 by adding MgO (NH_4^+) or MgO and Devarda alloy (NO_3^-) and the resulting ammonia was steam distilled, collected into 10 ml of 0.005 M H_2SO_4 and measured by back titration of the excess of H_2SO_4 with 0.01 M NaOH. For each form of N two distillates were performed and titrated. The net N ammonification, nitrification and mineralization for a given period were calculated as the difference between the final and the initial concentrations of NH_4^+, NO_3^- and total inorganic N, respectively.

Chemical extraction of the labile soil N

After preliminary assays with a ^{15}N-labelled Cambisol from Galicia (NW Spain) developed over granites and with an Inceptisol from the study area mixed with ^{15}N-labelled *Lolium perenne* shoots, a step-wise method for the chemical extraction of the labile soil N was developed. Two replicates (5 g) of each dried and ground soil were shaken for 1 h at room temperature with 50 ml of 0.1 M HCl. By centrifugation at 16000 g for 30 minutes, the first extract (H1-N) was separated from the soil, which was shaken again for 1 h at room temperature with 50 ml of 0.5 M HCl. By centrifugation at 16000 g for 30 minutes, the second extract (H2-N) and the final residue were obtained. The residues were oven-dried at 105 °C and ground (< 100 μm), while the extracts were freeze-dried after adjusting them to pH 5.5 with $Ca(OH)_2$ (suspension with 180 g/l and saturated dissolution); after that, the total N content of residues and freeze-dried extracts was determined with an elemental analyser.

Statistical analysis

Correlation and simple and stepwise multiple linear regression analyses were performed using the SPSS 15.0 statistical package to study the relationships of: 1) the main soil properties with the soil organic-N aerobically mineralized, the chemically labile soil organic-N and the soil-N taken up by the wheat culture; and 2) the labile soil N chemically extracted with the available N evaluated by the biological methods (aerobic incubation and crop uptake). After checking the fulfilment of the

assumptions of linear regressions (linearity, independence, homocedasticity, normality and no multicollinearity), the best models were selected using the least number of variables, maximizing the adjusted R^2 and minimizing the standard error of the estimated residues.

Results

Table 2 shows the amount of available soil N obtained with the biological and chemical methods employed, as well as the N concentration in wheat plants. Compared to the amount of soil N taken up by the wheat culture, the inorganic-N content after 6 weeks of aerobic incubation was slightly lower (-25 to 0 mg kg^{-1}) in 3 soils, slightly higher (0 to 25 mg kg^{-1}) in 6 soils and clearly higher in the rest (>25 mg kg^{-1}), especially in 6 of them (difference 50-160 mg kg^{-1}). Also using as reference the amount of soil N taken up by the wheat culture and the same intervals for the differences: a) the soil N solubilized with cold HCl 0.1 M was slightly lower only in 3 soils, slightly higher in 3 soils and clearly higher in the rest; and b) the soil N solubilized with cold HCl 0.5 M was slightly higher in 2 soils and clearly higher in the other 19 soils.

Relationships among main soil properties and aerobically mineralized N

Either after 3 (t1) or 6 weeks (t2) of incubation, the soil NH_4^+-N content was negatively correlated with soil $pH_{p\text{-nitrophenol}}$ and positively with soil total C and N, water holding capacity (WHC), buffering index (BI), total CEC (T_{CEC}), H^+_{CEC}, free Al oxi-hydroxides and soil $\delta^{15}N$ (Table 3). Moreover, the soil NH_4^+-N_{t1} was also positively correlated with the sum of CEC-bases (S_{CEC}) and Ca^{2+}_{CEC}, while NH_4^+-N_{t2} was also positively correlated with free Fe oxi-hydroxides and negatively with pH_{H2O}. More than two thirds of the NH_4^+-N_{t1} variance was explained by single regression models with soil C content and WHC, and by multiple regression models with either C or WHC and Al^{3+}_{CEC} (Table 4). The same was true for NH_4^+-N_{t2} and single regression models with soil C, WHC and, to a lesser extent, soil total-N as independent variables (Table 4).

In the case of net N ammonification rates, $NNAR_{t1}$ was positively correlated with H^+_{CEC} and $\delta^{15}N$ and negatively with pH_{H2O} and $pH_{p\text{-nitrophenol}}$ (Table 3), while no significant correlations were found for $NNAR_{t2}$. Even the best regression models explained no more than 40 % of NNAR's variance (Table 4) and included as independent variables: a) the H^+_{CEC} alone or with the silt fraction for $NNAR_{t1}$; and b) Mg^{2+}_{CEC} and free Al oxi-hydroxides or WHC and Al^{3+}_{CEC} for $NNAR_{t2}$.

In all studied soils and sampling dates, NO_3^--N was, by far, the most important fraction of the soil inorganic N pool (82-98 %); as a consequence, similar correlations and regression models with the

main soil properties were obtained for NO_3^--N and total inorganic-N, and therefore only those with the latter are reported.

The soil total inorganic-N_{t1} was positively correlated with soil C, N, WHC, BI, CEC variables (T_{CEC}, S_{CEC}, H^+_{CEC}, Ca^{2+}_{CEC}, K^+_{CEC}), free Fe and Al oxi-hydroxides, and $\delta^{15}N$, while negative correlations were established with pH_{H2O} and $pH_{p\text{-nitrophenol}}$ (Table 3). With the only exception of Al oxides, similar correlations were found for inorganic-N_{t2}, although the correlations were weaker except for K^+_{CEC} and Fe oxides. More than half the variance of soil inorganic-N_{t1} was explained by simple linear regressions with T_{CEC} or WHC as independent variable, but the best results were obtained with multiple linear regressions models that included the latter variable and either K^+_{CEC} or $P_{available}$ (Table 4). A model with WHC and $P_{available}$ as independent variables also explained almost two thirds of the variance of the inorganic-N_{t2}, and the model was even improved by including Na^+_{CEC} as a third independent variable (Table 4),

In the case of the net N mineralization rate, $NNMR_{t1}$ was positively correlated with soil WHC, T_{CEC}, H^+_{CEC}, K^+_{CEC}, C and $\delta^{15}N$, while the only negative correlations were with pH measured by the three methods employed (Table 3). Conversely, $NNMR_{t2}$ was only correlated, positively, with $P_{available}$ (Table 3). Nearly half of the $NNMR_{t1}$'s variance was explained by a multiple regression model that, as for the soil inorganic-N_{t1}, included WHC and $P_{available}$ as independent variables (Table 4); the latter variable was the only one included in a regression model for $NNMR_{t2}$ (Table 4).

Relationships among main soil properties and chemically extractable soil-N

The amount of soil organic-N solubilized with HCl 0.1 M (H1-N) was only negatively correlated with CEC variables (Ca^{2+}_{CEC}, Mg^{2+}_{CEC}, S_{CEC} and BS_{CEC}; Table 3), while that solubilized with HCl 0.5 M (H2-N) showed both positive (C, N, WHC, BI, H^+_{CEC}, Na^+_{CEC}, free Al oxi-hydroxides and $\delta^{15}N$) and negative correlations (pH_{H2O}, $pH_{p\text{-nitrophenol}}$, Mg^{2+}_{CEC} and BS_{CEC}). The amount of non-extractable N was correlated with the same soil variables as H2-N (except pH_{H2O}, Na^+_{CEC}, Mg^{2+}_{CEC} and BS_{CEC}) and also with CEC variables (T_{CEC}, Ca^{2+}_{CEC}, K^+_{CEC} and S_{CEC}), sand, clay and free Fe oxi-hydroxides contents. No valid multiple regression models were found for the soil organic-N solubilized as H1-N, but 27-36% of its variance was explained by single regressions with either S_{CEC} or BS_{CEC} (Table 5). Conversely, for the organic-N solubilized as H2-N several multiple linear regression models explained most of its variance. These models included the BS_{CEC} and, as second independent variable, either H^+_{CEC}, C or WHC; in all cases, the results were improved when Na^+_{CEC} was also incorporated to the model. A good model was also obtained with $P_{available}$, Mg^{2+}_{CEC} and $pH_{p\text{-nitrophenol}}$. Nearly all the variance of non-extractable N was

explained by a single regression with soil N content, while multiple regression models with BI and one CEC variable (Ca^{2+}_{CEC}, Al^{3+}_{CEC} or S_{CEC}) or with $P_{available}$, Ca^{2+}_{CEC} and $pH_{p\text{-nitrophenol}}$ also explained more than 79 % of the variance.

The percentage of soil organic-N extracted as H1-N was negatively correlated with free Fe and Al oxi-hydroxides, fine particle size fractions (silt and clay), BI, WHC, SOM content (C and N), CEC variables (T_{CEC}, H^+_{CEC}, Ca^{2+}_{CEC}, K^+_{CEC}, S_{CEC} and BS_{CEC}) and soil $\delta^{15}N$, while the only positive correlations were with $pH_{p\text{-nitrophenol}}$ and sand (Table 3). Only negative correlations were found among the percentage of soil organic-N solubilized as H2-N and soil properties: CEC variables (T_{CEC}, Ca^{2+}_{CEC}, Mg^{2+}_{CEC}, K^+_{CEC}, S_{CEC} and BS_{CEC}), N and clay contents (Table 3). The portion of non-extractable soil N had similar correlations to those for H1-N (except with H^+_{CEC}, silt, free Al oxi-hydroxides and $\delta^{15}N$) and, moreover, it was positively correlated with Mg^{2+}_{CEC} and negatively with Al^{3+}_{CEC} (Table 3). A single regression model with T_{CEC} explained more than two thirds of the variance of the percentage of soil organic-N solubilized as H1-N (Table 5), however three multiple regression models were even better: a) with BS_{CEC} and either WHC or total N; and b) the soil contents in N, $P_{available}$ and silt. For the percentage of organic-N extracted as H2-N, most of its variance was explained by a model with BS_{CEC} and clay, which was improved by including the silt fraction as a third independent variable; a good alternative model (74 % of variance explained) was built with $P_{available}$, Mg^{2+}_{CEC} and clay (Table 5). In the case of the percentage of non-extractable soil N, the best model with two independent variables explained 81% of the variance and included again the BS_{CEC} and clay fraction; this model was improved by the inclusion of BI. Other good models with three independent variables (>73 % of variance explained) included Mg^{2+}_{CEC}, $P_{available}$ and N, or $P_{available}$, T_{CEC} and pH_{H2O} (Table 5).

Relationships among main soil properties and soil N taken up by the wheat crop

The absolute amount of soil N assimilated by the wheat plants during a 6 weeks growing period was positively correlated with the soil N content and several soil CEC variables (T_{CEC}, S_{CEC}, BS_{CEC}, Ca^{2+}_{CEC}, Mg^{2+}_{CEC} and K^+_{CEC}; Table 3). Half the variation of the soil N taken up by the wheat was explained by regression models with one (S_{CEC}) or two independent variable (Mg^{2+}_{CEC} and T_{CEC} or total N); the latter model was improved by including $P_{available}$ as a third independent variable (Table 4).

The percentage of soil N exported with the wheat crop was positively correlated only with $pH_{p\text{-nitrophenol}}$ and $P_{available}$, while negative correlations were found with BI, WHC, C, N, H^+_{CEC}, Al oxides and soil $\delta^{15}N$ (Table 3). As shown in Table 4, all regression models for the percentage of soil N taken up by the wheat included the $P_{available}$ and either one or two of the following variables: Mg^{2+}_{CEC}, $\delta^{15}N$ and $pH_{p\text{-}}$

_{nitrophenol} (50-70 % of variance explained).

Relationships among the N availability indices

Either expressed in absolute or relative amount, the different N availability indices obtained from the aerobic incubation usually have strong positive correlations among them, while those from the chemical fractionation (N solubilized as H1-N and H2-N) were only correlated when expressed as percentages of soil N (Table 6).

The soil inorganic-N content after 3 and 6 weeks of incubation was positively correlated with the absolute amount of soil N taken up by the wheat, although they can only explain a quarter of the wheat-N variance (Table 6; Fig. 1A). Conversely, the percentages of soil N solubilized as H1-N and H2-N were negatively correlated with the wheat N uptake (in mg kg^{-1}) and jointly considered they explained half the variance of the N taken up by the wheat (Table 6; Fig. 1B).

Discussion

Relationships among main soil properties and aerobically mineralized N

The strong positive correlations of the SOM and SOM-related variables (C, N, BI, WHC, H$^+_{CEC}$ and T$_{CEC}$) with both the absolute amount of N mineralized and the net N mineralization rate (NNMR) at the short-term (t= 3 weeks) highlighted the importance of the availability of SOM for the N mineralization processes, which became limited when the biologically labile SOM pool decrease (Herrmann and Witter 2008; González-Prieto et al. 1995; Monreal et al. 1981) .

Similarly, both the absolute (mg kg^{-1} soil) and relative (% total soil N) values of the short-term N mineralization were positively correlated with the availability of several macro-nutrients (Ca$^{2+}_{CEC,}$ K$^+_{CEC,}$ S$_{CEC}$). This result, which agreed with those of González-Prieto et al. (1992) and González-Prieto and Villar (2003), suggested that nutrients' availabilities are interdependent or that they share a common controlling factor. If the latter explanation is the correct one, this controlling factor could be the microbial biomass because Acea and Carballas (1990) reported a positive relationships among exchangeable nutrients and both the size and activity of microbial populations involved in the soil N cycle.

Considering that N losses usually discriminate against the heavy isotope (Högberg et al. 1995) and that nitrification was, by far, the predominant N mineralization process in the studied soils, the positive correlation between soil δ ^{15}N and short-term N mineralization (expressed as mg kg^{-1} soil and as percentage of total soil-N) suggests that, under field conditions, N mineralization is closely

associated with NO_3^- losses and, then, with open N cycles. This result agrees with those of (Abadín et al. 2002) who found, for the same soils, that a change from 'open' to 'closed' N cycling, showed by a decrease in soil δ ^{15}N, is the characteristic that better discriminates the soils along the crop-fallow chrono-sequence. In the same way, (Jussy et al. 2002) have proposed the soil δ ^{15}N as an index of previous agricultural management and of potential nitrification in forest soils as well.

Contrasting results have been found for the relationships between pH and N mineralization (Curtin et al. 1998): the reported correlations ranged from negative (González-Prieto et al. 1996; Côté et al. 2000) to positive (Cookson et al. 2007) ones, and other works showed no significant relationships (González-Prieto et al. 1992; Tietema et al. 1992). The same was true considering the ammonification and the nitrification processes separately (Bramley and White 1990; Curtin et al. 1998; Booth et al. 2005; Kemmitt et al. 2006; Cookson et al. 2007). This is due, at least in part, to the bidirectional effects between pH and N mineralization processes, specially nitrification. In this way, the negative correlations that we have found between soil pH and short-term N mineralization (expressed as mg kg^{-1} soil and as percentage of total soil-N) coincide with the positive relationship among soil δ ^{15}N, N mineralization and NO_3^- losses, suggesting that the latter are an important cause of acidification in these soils.

At the end of the incubation, the previously discussed variables (SOM-related, CEC, pH) were also correlated with the absolute amount of N mineralized, but not with the NNMR, suggesting that other factors became more important for this rate in the long term. By the contrary, the available P content, which was not correlated with the short-term N mineralization (expressed as mg N kg^{-1} soil and as percentage of total soil-N), showed the only significant correlation with NNMR in the long term, emerging as its main controlling factor in these soils. In the same way, (Côté et al. 2000) also reported that NNMR increased in the soils rich in available P. For plant litter, (Gusewell and Verhoeven 2006) reported that, within species, mass loss correlated mainly with N for litter with low N: P ratio, and with P for litter with high N:P ratio. If this is also true for the mineralizable SOM, we can hypothesize that during the first half of the incubation there was a preferential mineralization of SOM with low N:P ratio and that, after the progressive exhaustion of this pool, the contribution of SOM with high N:P ratio to N mineralization became more important, leading to the positive correlation of NNMR at t=6 with the available P.

No significant relationships between NH_4^+-N or NO_3^--N and Al^{3+}_{CEC} content were found, although (Kraal et al. 2009) reported that the former increased and the latter decreased in soils added with $AlCl_3$. Although many authors have reported a negative correlation between Al oxihydroxides and inorganic N content (Carballas et al. 1979; González-Prieto et al. 1992; Nannipieri and Eldor 2009; González-Prieto et al. 1991; González-Prieto and Carballas 1991) the reverse was true at the short-term in our soils. This result could be due to the existence of two N pools in soils rich in Al, as (González-

Prieto and Carballas 1991) have suggested: one pool of recalcitrant N, stabilized by interactions with Al compounds, and the other pool of more biologically labile N, made up by "fresh" organic N once the capacity of the extractable Al to sequester organic matter has been saturated; this latter fraction could be large enough to supply inorganic N in the short-term.

Despite the frequently reported relationships between N mineralization and soil texture (Côté et al. 2000; Bechtold and Naiman 2006; McLauchlan 2006; Kadono et al. 2008; Nannipieri and Eldor 2009), in the present study no significant correlations were found between N mineralization and any soil particle size fraction, which, moreover, were never included in the best regression models for N mineralization. Several explanations are possible for this result: a) the clay content in the studied soils, although spreading along a 3:1 range, did not surpass 30 % and, perhaps, was below a threshold for detecting the effect of fine textures on N mineralization; b) the net rate of decomposition of soil organic matter could depend not simply on soil texture but on the degree to which the protective capacity of the soil is already occupied, as in the model proposed by (Hassink and Whitmore 1997); and c) the effects of soil texture could be masked by other factors with stronger influence on N mineralization in these soils.

Relationships among main soil properties and chemically extractable soil-N

The contrasting relationships of the SOM variables and H1-N expressed in absolute amount or as a percentage of soil N (not significant and strongly negative, respectively), suggest that H1-N is a chemically labile N pool with decreasing relative importance as soil N content increases. For the same reasoning, the lability of the soil organic-N extracted as H2-N is less clear: the absolute amount was positively correlated with SOM variables, while the relative amount was negatively correlated with the soil N content and unrelated with the other SOM variables. As expected, either expressed in absolute or relative amount, the widely majority residue-N has strong positive correlations with SOM variables.

Either in absolute amount or as a percentage of soil N, both H1-N and H2-N decreased as the soil content in exchangeable bases (Ca, Mg, K, S_{CEC} and BS_{CEC}) increased, although some correlations did not reach significant levels. Conversely, the reverse was true for the residue-N. Taking into account the strong positive relationships among nutrients content, biological activity and N mineralization (González-Prieto et al. 1992; González-Prieto and Villar 2003; Andersson 2005; Acea and Carballas 1990; Côté et al. 2000), these results suggested that H1-N and H2-N are biologically labile and did not cumulate in soils unless biological activity is limited by other(s) nutrient(s); as an indirect consequence, in soils rich in nutrients with reduced amounts of H1-N and H2-N the relative importance of the residue-N increased.

In absolute amount, neither H1-N nor H2-N were correlated with any soil physical fraction; however, when expressed as percentage of soil N, H1-N has strong correlations with all particle size

fractions (positive with sand; negative with silt and clay), and H2-N a negative one with clay content. By the contrary, expressed either in absolute or relative amount, the residue-N has strong correlations with sand (negative) and clay (positive) contents. As chemical lability decreases from H1-N to H2-N and to residue-N, all these results agree with the well known physical protection of SOM by the clay particle size fraction, to which the oldest and most recalcitrant SOM is associated (Sorensen 1981; Tiessen and Stewart 1983; Anderson and Paul 1984; Hassink 1995; Bechtold and Naiman 2006; McLauchlan 2006; Nannipieri and Eldor 2009).

The non-crystalline Al compounds appeared negatively correlated with the percentage of soil N in the most chemically labile H1-N fraction and positively correlated with the absolute amount of the residue-N, thus agreeing with the frequently reported role of aluminium in SOM stabilization (Carballas et al. 1979; Stevenson 1982; González-Prieto et al. 1992; Abadín et al. 2002; Scheel et al. 2008a; Scheel et al. 2008b; Nannipieri and Eldor 2009). However, the positive correlation of non-crystalline Al compounds with the absolute amount of H2-N was by far the strongest one. A joint explanation for all these relationships could be a moderate chemical stabilization of SOM by aluminium and/or the previously discussed existence of (at least) two N pools of different biological lability in Al rich soils suggested by (González-Prieto and Carballas 1991). As the relationships of the soil N pools with the non-crystalline Fe compounds were similar to those with Al, it seems that these Fe compounds could also play an important role in SOM stabilization.

The strong negative correlation of soil δ ^{15}N with the percentage of soil N in the H1-N pool suggests that the importance of this N fraction is higher in soils with: a) low N losses, which discriminate against the heavier isotope (Högberg et al. 1995); and/or b) high N inputs by N_2 fixation, due to the negative δ ^{15}N value of the N_2 fixed by *Lupinus meridanus*, the most abundant N_2-fixing species in the study area (Abadín et al. 2002). The reverse must be true for H2-N and, especially, for the residue-N taking into account the positive correlations of their absolute amounts with the soil δ [15]N .

Relationships among main soil properties and soil N taken up by wheat crop

Compared to the values reported by (Andersson 2005) and (Kätterer et al. 1993) for wheat plants of similar age (1.35 and 1.8 % N, dw basis, respectively), the N concentration that we found was lower in most cases (76 and 100 % of plots, respectively). Moreover, in 12 out the 21 studied soils the N concentration in the wheat plants was below the 1.13 % found by in N zero plots with a production at harvest 27 % lower than plots that had received 90 kg N ha^{-1}. Therefore, it could be concluded that wheat growth was N limited in most soils of the present study.

Although a high soil N content does not necessarily imply a high soil N availability (González-Prieto and Villar 2003; Côté et al. 2000), in the studied soils, in line with (Bonito et al., 2003), the

absolute amount of N taken up by the wheat culture was positively correlated with the total soil N content. However, the percentage of soil N exported to the wheat culture was negatively correlated not only with the total soil N, but also with all SOM-related variables. These results suggested that the soils rich in N can supply important amounts of available N in spite of having a globally slow N turnover, as previously reported , likely due to the existence of a labile pool turning over fast and contributing significantly to the supply of available N.

The strong positive correlations between the absolute amount of N assimilated by the wheat and the soil exchangeable nutrients, as well as that between the relative amount of N taken up and the soil available P, agreed with the tight relationships among the main available plant nutrients . Consequently, the best regression models for wheat-N always included 1-2 major available nutrients as independent variables.

The percentage of soil N exported to the culture was negatively correlated with the non-crystalline Al compounds and soil $\delta^{15}N$. The former result was likely due to the SOM-stabilizing role of aluminium and the latter to the relationships of low soil $\delta^{15}N$ with reduced N losses and/or high N inputs by biological N_2 fixation, as previously discussed.

Relationships among the N availability indices

The positive correlation between the amount of soil N taken up by the wheat and the soil inorganic-N content after 3 and 6 weeks of incubation showed the relationship between the results from both biological experiments, without plants at the laboratory and with plants at the greenhouse; however, it should be highlighted that all N incubation indices are mediocre predictors of soil N taken up by wheat because they only explained a quarter of the wheat N uptake variance.

When expressed as absolute amounts, the soil N taken up by the wheat and those extracted as H1-N and H2-N were poorly correlated; an explanation for this result could be that chemically labile N, even with these mild and cold extractants, is not biologically labile N. However, the negative correlation of the amount of soil N taken up by the wheat with the percentage of soil N extracted as H1-N and H2-N suggested that the chemically labile N is also biologically labile and that it constitutes an important soil N fraction only in those plots where the existence of a limiting factor for microbial N mineralization or plant growth allowed its cumulation. Whatever explanation was correct, data on chemical lability of soil N provided an acceptable prediction of N uptake by wheat, explaining half of its variance (i.e. twice that explained by incubation data).

Conclusions

The aerobically mineralized N and the N taken up by the wheat, expressed in absolute

amounts, have in common: a) positive relationships with the soil exchangeable cations and total N; and b) lack of correlations with soil texture variables. This latter result and a positive correlation with available P were also shared by both N pools when expressed as percentages of soil N. However, many other correlations were not shared, suggesting that the wheat available N and the mineralized N are two noticeable different soil N pools, which agree with the fact that only a quarter of the former's variance was explained by the latter.

Except a positive one with soil total N, no correlation with the main soil properties was shared by the N taken up by the wheat and the chemically labile N (absolute amounts), suggesting that these two N pools could have different nature. Nevertheless, half the variation of the amount of soil N taken up by the wheat was explained by its negative relationship with the percentage of soil N in H1-N and H2-N; this result could be explained whether the chemically labile N is also biologically labile and it constitutes an important soil N fraction only in those soils where the existence of a limiting factor for microbial N mineralization or plant growth allowed its cumulation.

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Table 1 Main characteristics of the studied soils. Abbreviations: R, *rompedura* (recently ploughed soil after a fallow period); C-1 and C-2, soils after 1- and 2-year potato crop, respectively; F-1, F-4 and F-8, soils with a 1-, 4- or 5- and 8- year fallow period, respectively; VP, virgin *páramo* (never cultivated soil). Note: BI, buffering index; S_{CEC}, sum of CEC bases; T_{CEC}, total CEC; BS_{CEC}, base saturation of CEC; WHC, water holding capacity.

| | | pH | | | | Total C | Total N | Available P | CEC (cmol kg⁻¹) | | | | | | | | BS_{CEC} | Sand | Silt | Clay | WHC | Oxy-hydroxides | |
| | | | | | | | | | | | | | | | | | | | | | | Al | Fe |
Sector	Plot	H₂O	KCl	*p*-nitro	BI	(g kg⁻¹)	(g kg⁻¹)	(µg g⁻¹)	Na⁺	K⁺	Ca²⁺	Mg²⁺	H⁺	Al³⁺	S_{CEC}	T_{CEC}	(%)	(g kg⁻¹)	(g kg⁻¹)	(g kg⁻¹)	(g kg⁻¹)	(g kg⁻¹)	(g kg⁻¹)
	R	4.70	4.00	5.10	6.33	100.3	5.7	5.0	0.05	0.14	12.20	0.94	55.30	11.28	13.33	79.91	16.7	564	168	264	616	30.5	35.4
	C-1	4.62	3.96	5.19	5.95	84.9	4.2	18.5	0.02	0.19	6.66	0.58	47.93	10.80	7.45	66.18	11.3	638	152	210	542	30.6	26.1
	C-2	4.70	3.95	5.11	6.20	101.5	5.9	23.2	0.03	0.48	29.76	1.54	53.89	9.81	31.81	95.51	33.3	619	149	232	622	29.6	29.8
Ramón	F-1	4.60	4.04	5.20	6.08	89.2	4.3	12.8	0.08	0.11	14.02	0.20	48.76	11.01	14.41	74.18	19.4	648	161	191	574	31.4	19.7
	F-5	5.25	4.30	5.31	6.26	97.8	5.9	14.3	0.07	0.38	27.12	1.28	43.96	9.69	28.85	82.50	35.0	599	144	257	590	31.0	27.0
	F-8	5.00	4.12	5.21	6.22	113.9	5.5	5.5	0.32	0.13	5.48	0.38	52.34	11.79	6.31	70.44	9.0	627	166	208	572	40.5	20.8
	VP	5.25	4.26	5.22	6.50	118.0	6.3	0.4	0.06	0.32	28.46	2.54	48.43	9.00	31.38	88.81	35.3	666	135	200	602	27.5	26.1
	R	4.90	4.50	5.42	6.32	62.3	3.8	18.8	0.03	0.08	6.26	0.48	35.45	14.55	6.85	56.85	12.0	721	138	141	406	21.5	16.5
	C-1	5.00	4.26	5.70	4.74	46.1	2.6	53.8	0.03	0.36	10.04	1.38	20.13	10.77	11.81	42.71	27.7	741	123	136	349	17.2	21.3
	C-2	4.90	4.00	5.35	5.50	76.5	4.3	10.8	0.03	0.12	5.40	0.46	44.14	7.44	6.01	57.59	10.4	536	169	296	481	31.2	33.4
Volcanes	F-1	5.20	4.23	5.56	5.20	58.1	3.2	14.2	0.01	0.17	4.70	0.46	31.03	10.86	5.34	47.23	11.3	711	132	158	392	24.5	22.7
	F-4	5.25	4.22	5.57	5.14	45.6	2.7	21.1	0.03	0.13	8.90	0.76	22.15	10.02	9.82	41.99	23.4	736	125	140	397	17.2	20.2
	F-8	5.45	4.35	5.70	4.91	46.9	2.7	13.2	0.02	0.08	6.48	0.74	24.65	10.56	7.32	42.53	17.2	659	168	174	369	22.8	20.1
	VP	5.20	4.30	5.74	4.67	47.3	2.5	1.4	0.05	0.03	1.40	0.14	22.11	14.46	1.62	38.19	4.2	811	94	96	298	19.1	19.6
	R	5.45	3.80	5.30	5.31	82.9	4.4	18.1	0.01	0.01	4.36	0.20	36.95	5.61	4.58	47.14	9.7	607	140	255	459	34.5	26.1
	C-1	4.70	3.95	5.25	5.74	80.1	4.5	20.3	0.05	0.32	9.34	0.74	43.77	7.35	10.45	61.57	17.0	676	124	201	453	21.2	19.9
	C-2	4.90	3.90	5.23	5.71	79.3	4.9	18.1	0.04	0.25	15.76	0.84	45.68	7.74	16.89	70.31	24.0	572	147	280	477	22.6	25.4
Yaques	F-1	5.15	4.10	5.53	5.07	58.9	3.8	31.1	0.04	0.48	19.28	1.46	26.91	10.50	21.26	58.67	36.2	674	129	197	364	15.1	22.4
	F-4	5.55	4.55	5.70	5.31	69.5	4.5	6.8	0.07	0.25	27.52	1.76	26.92	9.87	29.60	66.39	44.6	635	140	225	459	19.8	25.5
	F-8	5.10	4.05	5.56	4.88	51.9	3.1	11.9	0.02	0.07	3.96	0.26	25.11	12.75	4.31	42.17	10.2	683	136	182	375	24.7	26.2
	VP	5.60	4.42	5.60	5.43	61.4	3.3	4.9	0.03	0.16	14.70	3.40	24.04	12.54	18.29	54.87	33.3	644	165	192	409	21.7	27.0

Table 2. Soil inorganic N content (NH_4-N and NO_3-N) at different times of the aerobic incubation (0, 3 and 6 weeks); soil N successively solubilized with cold HCl 0.1 and 0.5 M; and soil N assimilated by the wheat culture; all figures are in mg kg^{-1}, except wheat-N content (% dw). Abbreviations: R, *rompedura* (recently ploughed soil after a fallow period); C-1 and C-2, soils after 1- and 2-year potato crop, respectively; F-1, F-4 and F-8, soils with a 1-, 4- or 5- and 8- year fallow period, respectively; VP, virgin *páramo* (never cultivated soil)

Sector	Plot	NH₄-N			NO₃-N			N solubilized		Soil N taken up by wheat	Wheat N (% dw)
		t=0	t=3	t=6	t=0	t=3	t=6	HCl 0.1 M	HCl 0.5M		
	R	20.7	19.4	14.9	31.7	120.2	169.3	108.6	246.0	77.4	1.64
	C-1	16.9	13.4	9.5	25.4	167.6	182.6	87.8	238.1	68.3	1.10
	C-2	20.4	13.7	8.8	37.8	241.4	271.0	106.1	207.1	119.7	1.01
Ramón	F-1	18.0	12.4	8.0	6.1	98.1	141.0	112.5	275.8	42.2	1.34
	F-5	21.2	12.7	7.8	18.8	141.9	156.7	77.2	221.5	73.6	0.92
	F-8	19.5	13.8	9.3	5.8	89.1	66.2	140.6	335.3	54.3	1.10
	VP	26.8	21.3	14.8	4.6	94.8	126.9	85.9	187.6	108.6	1.44
	R	4.9	2.0	2.1	7.3	24.0	63.4	102.7	194.3	68.4	1.12
	C-1	7.2	3.8	4.6	18.9	64.9	129.0	96.4	115.0	91.7	1.01
	C-2	6.8	3.1	4.0	7.6	44.2	102.6	110.3	206.9	66.0	1.49
Volcanes	F-1	4.9	1.4	2.5	6.3	36.3	95.2	132.5	204.5	71.3	1.07
	F-4	6.5	1.8	2.7	5.5	46.7	118.5	98.2	146.2	44.0	1.01
	F-8	5.8	1.8	2.8	5.2	32.2	82.5	100.6	176.5	55.0	1.24
	VP	5.5	3.3	2.2	0.8	16.8	50.1	146.4	185.8	37.4	1.35
	R	4.1	1.7	3.2	1.0	44.7	60.0	106.8	217.1	65.8	1.12
	C-1	4.5	2.9	4.0	2.0	91.8	100.3	158.0	202.3	85.2	1.13
	C-2	6.1	2.2	4.7	0.7	76.4	104.6	108.1	172.6	78.6	1.10
Yaques	F-1	5.6	2.9	3.6	3.0	61.2	87.9	89.1	107.4	68.6	1.00
	F-4	8.0	1.9	1.5	3.0	64.5	102.3	97.7	116.4	103.5	1.30
	F-8	8.3	1.5	2.8	0.0	52.1	64.2	123.9	182.9	58.0	1.31
	VP	11.7	5.3	3.1	0.0	40.7	57.3	92.0	120.8	75.0	1.28

Table 3 Correlations among the indices of N availability studied and the main soil characteristics

	Inorganic-N (mg kg⁻¹ soil)		Mineralized N (mg kg⁻¹ soil)		Mineralized N (% total soil-N)		N solubilized (mg kg⁻¹ soil)		Residue-N (mg kg⁻¹ soil)	N solubilized (% total soil-N)		Residue-N (% total soil-N)	N taken up by wheat	
	t=3 weeks	t=6 weeks	t=3 weeks	t=6 weeks	t=3 weeks	t=6 weeks	HCl 0.1 M	HCl 0.5 M		HCl 0.1 M	HCl 0.5 M		(mg kg⁻¹ soil)	% total soil-N
pH$_{H20}$	-0.542*	-0.551**	-0.532*	-0.543*	-0.556**	-0.318	-0.257	-0.513*	-0.290	0.161	-0.145	0.012	-0.031	0.261
pH$_{KCl}$	-0.389	-0.319	-0.439*	-0.349	-0.453*	-0.108	-0.244	-0.421	-0.295	0.184	-0.053	-0.055	0.028	0.356
pH$_{p\text{-nitrophenol}}$	-0.714***	-0.583**	-0.715**	-0.541*	-0.550**	0.073	0.000	-0.684**	-0.808**	0.672**	0.187	-0.434*	-0.250	0.568**
Buffering index	0.637**	0.512*	0.605**	0.435*	0.376	-0.197	-0.180	0.593**	0.846***	-0.754**	-0.286	0.533*	0.339	-0.498*
Total C	0.696***	0.506*	0.682***	0.429	0.440*	-0.261	-0.071	0.657**	0.938***	-0.743**	-0.306	0.540*	0.394	-0.518*
Total N	0.707***	0.550**	0.700***	0.490*	0.423	-0.255	-0.168	0.479*	0.999***	-0.824**	-0.513*	0.704***	0.539*	-0.443*
WHC	0.807***	0.700***	0.776***	0.631**	0.576**	-0.014	-0.215	0.625**	0.901***	-0.793**	-0.299	0.559**	0.389	-0.486*
H$_{CEC}$	0.721***	0.593**	0.708***	0.537*	0.504*	-0.114	0.046	0.744***	0.848***	-0.659**	-0.151	0.406	0.281	-0.570**
Ca $_{CEC}$	0.572**	0.562**	0.581**	0.569**	0.417	0.110	-0.540*	-0.234	0.661**	-0.686**	-0.792**	0.809***	0.718***	0.074
Mg $_{CEC}$	0.174	0.157	0.163	0.140	0.103	-0.020	-0.527*	-0.517*	0.260	-0.406	-0.718**	0.631**	0.607**	0.403
K $_{CEC}$	0.562**	0.563**	0.587**	0.590**	0.546*	0.334	-0.331	-0.312	0.440*	-0.458*	-0.710**	0.650**	0.692***	0.325
Na $_{CEC}$	0.145	-0.093	0.139	-0.178	0.031	-0.377	0.307	0.585**	0.336	-0.139	0.127	-0.011	-0.143	-0.402
Al $_{CEC}$	-0.178	-0.198	-0.244	-0.277	-0.189	-0.070	0.098	-0.007	-0.386	0.412	0.390	-0.433*	-0.333	0.049
S	0.556**	0.544*	0.564**	0.548*	0.405	0.103	-0.550**	-0.262	0.645**	-0.681**	-0.808**	0.816***	0.729***	0.105
T	0.812***	0.717***	0.807***	0.680**	0.577**	-0.022	-0.275	0.371	0.951***	-0.839**	-0.557**	0.738***	0.603**	-0.338
V	0.214	0.272	0.224	0.302	0.204	0.213	-0.628**	-0.622**	0.233	-0.445*	-0.793**	0.695***	0.586**	0.417
Sand	-0.411	-0.337	-0.413	-0.315	-0.256	0.201	0.268	-0.330	-0.664**	0.740***	0.410	-0.602**	-0.279	0.358
Silt	0.281	0.231	0.242	0.173	0.163	-0.117	-0.269	0.417	0.373	-0.551**	-0.075	0.308	0.035	-0.285
Clay	0.413	0.336	0.431	0.331	0.263	-0.212	-0.240	0.259	0.699***	-0.731**	-0.490*	0.646**	0.340	-0.345
Al$_2$O$_3$	0.457*	0.269	0.429	0.176	0.269	-0.287	0.085	0.873***	0.585**	-0.447*	0.196	0.093	-0.041	-0.597**
Fe$_2$O$_3$	0.435*	0.457*	0.388	0.413	0.259	-0.009	-0.282	0.076	0.517*	-0.507*	-0.418	0.496*	0.402	-0.101
Available P	0.056	0.194	0.064	0.250	0.262	0.595**	-0.202	-0.379	-0.283	0.055	-0.166	0.075	0.182	0.604**
δ ^{15}N soil	0.588**	0.455*	0.611**	0.437*	0.503*	-0.099	-0.055	0.521*	0.640**	-0.639**	-0.211	0.432	0.134	-0.573**

Statistical significance: * $P<0.05$; ** $P<0.01$; ***$P<0.005$

Table 4 Best multiple linear regressions models for the biological N availability indices. Note: the name of variables in italic indicates that the Z scores of the variables were used. BI, Buffering index. BS, Base saturation of CEC. NNMR, net N mineralization rate. NNAR, net N ammonification rate

		Adjusted R²	Standard error estimate	Tolerance
Soil inorganic-N content at t=3 weeks	= -98.29 + 3.50*WHC	0.581	29.031	1.000
	= -54.29 + 0.28*T	0.642	34.998	1.000
	= -140.48 + 4.16*WHC + 15.54*$K^+_{exchangeable}$	0.754	29.026	0.927
	= -187.03 + 5.30*WHC + 6.98*$P_{available}$	0.716	31.170	0.914
Soil inorganic-N content at t=6 weeks	= -129.44 + 4.59*WHC + 8.99*$P_{available}$	0.627	33.604	0.914
	= -136.34 + 5.13*WHC + 7.79*$P_{available}$ - 27.16*$Na^+_{exchangeable}$	0.707	29.803	> 0.831
Soil NH₄-N content at t=3 weeks	= -19.17 + 0.56*WHC	0.706	3.522	1.000
	= -10.94 + 2.37*C_{total}	0.669	3.739	1.000
	= -22.07 + 0.62*WHC + 2.04*$Al^{3+}_{exchangeable}$	0.789	2.983	0.909
	= -13.30 + 2.68*C_{total} + 2.18*$Al^{3+}_{exchangeable}$	0.762	3.167	0.893
Soil NH₄-N content at t=6 weeks	= -10.09 + 0.34*WHC	0.679	2.261	1.000
	= -5.21 + 1.44*C_{total}	0.654	2.347	1.000
	= -5.15 + 25.50*N_{total}	0.540	2.709	1.000
NNMR at t=3 weeks	= -1.55 + 0.06*WHC + 0.13*$P_{available}$	0.483	0.545	0.914
NNMR at t=6 weeks	= 1.66 + 0.20*$P_{available}$	0.320	0.734	1.000
NNAR at t=3 weeks	= -0.19 + 0.01*H^+	0.231	0.043	1.000
	= -0.22 + 0.01*H^+ - 0.02*$Silt$	0.345	0.040	0.749
NNAR at t=6 weeks	= -0.13 - 0.04*WHC - 0.04*$Al^{3+}_{exchangeable}$	0.403	0.057	0.909
	= -0.08 - 0.01*$Mg^{2+}_{exchangeable}$ - 0.03*Al_{oxides}	0.244	0.064	0.931
Soil N (mg kg⁻¹) taken up by wheat	= 50.09 + 0.16*S	0.506	14.929	1.000
	= 32.19 + 1.18*$Mg^{2+}_{exchangeable}$ + 0.06*T	0.502	15.002	0.893
	= 26.83 + 1.32*$Mg^{2+}_{exchangeable}$ + 76.92*N_{total}	0.489	15.192	0.951
	= 59.26 + 1.31*$Mg^{2+}_{exchangeable}$ + 11.40*N_{total} + 7.84*$P_{available}$	0.605	13.362	> 0.868
Soil N (%) taken up by wheat	= 1.40 + 0.11*$P_{available}$ - 0.27*$\delta^{15}N_{soil}$	0.565	0.358	0.980
	= 1.40 + 0.11*$P_{available}$ + 0.26*$pH_{p\text{-}nitro}$	0.550	0.365	0.975
	= 1.05 + 0.13*$P_{available}$ + 0.03*$Mg^{2+}_{exchangeable}$	0.505	0.382	0.997
	= 1.14 + 0.11*$P_{available}$ +0.02*$Mg^{2+}_{exchangeable}$ - 0.24*$\delta^{15}N_{soil}$	0.697	0.299	> 0.959
	= 1.13 + 0.11*$P_{available}$ + 0.24*$pH_{p\text{-}nitro}$ + 0.03*$Mg^{2+}_{exchangeable}$	0.697	0.299	> 0.963

Table 5 Best multiple linear regressions models for the chemical N availability indices. Note: the name of variables in italic indicates that the Z scores of the variables were used. BI, Buffering index. BS, Base saturation of CEC

		Adjusted R^2	Standard error estimate	Tolerance
Soil N (%) solubilized with HCl 0.1M	= 5.46 - 0.01*T	0.688	0.598	1.000
	= 7.38 - 0.03*BS - 0.08*WHC	0.737	0.548	0.990
	= 6.37 - 0.02*BS - 6.99*N_{total}	0.741	0.545	0.965
	= 6.18 - 0.11*$P_{available}$ - 7.11*N_{total} - 0.35*$Silt$	0.772	0.511	> 0.812
Soil N (%) solubilized with HCl 0.5 M	= 9.01 - 0.08*BS - 0.11*Clay	0.784	0.636	0.992
	= 7.01 - 0.08*BS - 0.16*Clay + 0.20*Silt	0.831	0.563	> 0.614
	= 9.01 - 0.15*$P_{available}$ - 0.12*$Mg^{2+}_{exchangeable}$ - 0.13*Clay	0.742	0.695	> 0.963
Residual soil N (%)	= 84.47 + 0.11*BS + 0.26*Clay	0.806	0.991	0.992
	= 85.68 + 0.11*BS + 0.20*Clay + 0.69*BI	0.882	0.773	> 0.791
	= 84.65 + 0.26*$P_{available}$ + 13.13*N_{total} + 0.14*$Mg^{2+}_{exchangeable}$	0.786	1.040	> 0.868
	= 84.68 + 0.01*T + 0.31*$P_{available}$ + 1.08*pH_{H2O}	0.730	1.167	> 0.759
Soil N (mg kg^{-1}) solubilized with HCl 0.1 M	= 133.65 - 0.98*V	0.362	16.750	1.000
	= 124.97 - 0.12*S	0.265	17.973	1.000
Soil N (mg kg^{-1}) solubilized with HCl 0.5 M	= 85.25 - 2.87*V + 3.88*WHC	0.849	21.548	0.990
	= 137.95 - 2.65*V + 16.39*C_{total}	0.821	23.488	0.999
	= 134.92 - 2.07*V + 0.30*H^+	0.769	26.637	0.964
	= 96.90 - 2.68*V + 3.24*WHC + 26.51*$Na^+_{exchangeable}$	0.937	13.975	> 0.853
	= 139.23 - 2.01*V + 0.24*H^+ + 29.34*$Na^+_{exchangeable}$	0.876	19.540	> 0.846
	= 146.43 - 2.52*V + 13.36*C_{total} + 21.51*$Na^+_{exchangeable}$	0.865	20.340	> 0.745
	= 247.29 - 6.45*$P_{available}$ - 3.18*$Mg^{2+}_{exchangeable}$ - 32.58*$pH_{p-nitro}$	0.723	29.170	> 0.963
Residual soil N (mg kg^{-1})	= -219.65 + 9803.92*N_{total}	0.997	63.699	1.000
	= 3321.09 + 793.90*BI + 4.62*$Ca^{2+}_{exchangeable}$	0.800	515.009	0.810
	= 3325.44 + 804.24*BI + 4.17*S	0.795	521.688	0.821
	= 5577.11 + 942.39*BI - 16.15*$Al^{3+}_{exchangeable}$	0.790	527.750	0.992
	= 3366.13 - 73.06*$P_{available}$ + 6.33*$Ca^{2+}_{exchangeable}$ - 759.33*$pH_{p-nitro}$	0.890	382.808	> 0.915

Table 6 Correlations among the indices of N availability studied. Note: w, weeks of aerobic incubation

	Inorganic-N (mg kg⁻¹ soil)		Mineralized N (mg kg⁻¹ soil)		Mineralized N (% total soil-N)		N solubilized (mg kg⁻¹)		Residue-N (mg kg⁻¹)	N solubilized (% total soil-N)		Residue-N (% total soil-N)	N taken up by wheat	
	t=3 w	t=6 w	t=3 w	t=6 w	t=3 w	t=6 w	HCl 0.1 M	HCl 0.5 M		HCl 0.1 M	HCl 0.5 M		(mg kg⁻¹)	(% total soil-N)
Inorganic-N t=3 w (mg kg⁻¹ soil)	1.000													
Inorganic-N t=6 w (mg kg⁻¹ soil)	0.917***	1.000												
Mineralized N t=3 w (mg kg⁻¹ soil)	0.986***	0.877***	1.000											
Mineralized N t=6 w (mg kg⁻¹ soil)	0.875***	0.984***	0.856***	1.000										
Mineralized N t=3 w (% total soil-N)	0.901***	0.827***	0.925***	0.829***	1.000									
Mineralized N t=6 w (% total soil-N)	0.359	0.621**	0.331	0.678**	0.541*	1.000								
N solubilized HCl 0.1 M (mg kg⁻¹ soil)	-0.229	-0.327	-0.181	-0.308	-0.193	-0.254	1.000							
N solubilized HCl 0.5 M (mg kg⁻¹ soil)	0.384	0.210	0.358	0.124	0.233	-0.230	0.374	1.000						
Residue-N (mg kg⁻¹ soil)	0.706***	0.556**	0.699***	0.499*	0.423	-0.244	-0.207	0.433	1.000					
N solubilized HCl 0.1 M (% total soil-N)	-0.614**	-0.529*	-0.603**	-0.489*	-0.433*	0.091	0.617**	-0.185	-0.842	1.000				
N solubilized HCl 0.5 M (% total soil-N)	-0.308	-0.297	-0.332	-0.318	-0.213	0.051	0.536*	0.472*	-0.555**	0.695***	1.000			
Residue-N (% total soil-N)	0.480*	0.433	0.489*	0.427	0.336	-0.075	-0.620**	-0.199	0.738***	-0.899**	-0.940**	1.000		
N taken up by wheat (mg kg⁻¹ soil)	0.508*	0.520*	0.510*	0.527*	0.378	0.132	-0.293	-0.275	0.567**	-0.544*	-0.771**	0.728***	1.000	
N taken up by wheat (% N)	-0.182	-0.015	-0.195	0.026	-0.038	0.417	-0.168	-0.707**	-0.414	0.246	-0.259	0.041	0.475*	1.000

Statistical significance: * $P<0.05$; ** $P<0.01$; ***$P<0.005$