Biol Fertil Soils (2000) 32:177–185

ORIGINAL PAPER

Ángeles Prieto-Fernández · Tarsy Carballas

Soil organic nitrogen composition in *Pinus* forest acid soils: variability and bioavailability

Received: 13 July 1999

Abstract The total N content in the acid forest soils studied ranged between 0.41% and 1.43%, and in more than 98% was composed of organic N. Total hydrolysable organic N, hydrolysable unknown N (HUN) and α-aminoacidic N represented around 70%, 34% and 20% of the organic N, respectively, and varied in wide ranges. The percentages of amidic N and of the organic N compounds solubilised to NH_4^+ were approximately 6% and 5%, respectively, and ranged in narrow intervals. Aminoglucidic N reached a maximum of 3.8% of the organic N and was undetectable in some of the samples analysed. Most of the hydrolysable N, HUN and α-aminoacidic N was solubilised with 1 N and 3 N HCl, while a high amount of the compounds recovered as NH_4^+ (60%) was obtained with 6 N HCl. The distribution of aminoglucidic N in the four fractions of increasing hydrolytic intensity was very irregular. The organic N composition in the 0 to 5-cm and 5 to 10-cm layers was not significantly different. The variation among samples was determined mainly by the organic N compounds less resistant to acid hydrolysis (hydrolysable N and HUN less resistant to acid hydrolysis, amidic N and labile ammoniacal N) and by all α-aminoacidic N fractions. Aminoacidic N was positively correlated with electrical conductivity and negatively correlated with exchangeable Al. The net N mineralisation over 10 weeks of incubation was positive in all the soil samples analysed. The inorganic N content after the incubation and the microbial N content were positively correlated with other variables – mainly with amidic N and α-aminoacidic N, as well as with HUN and the hydrolysable N less resistant to hydrolysis.

Á. Prieto-Fernández (✉) · T. Carballas
Soil Biochemistry, Instituto de Investigaciones Agrobiológicas de Galicia (CSIC), Apartado 122,
15780 Santiago de Compostela, Spain
e-mail: apf@iiag.cesga.es
Fax: +34-981-592504

Key words Organic nitrogen composition · Organic nitrogen bioavailability · Nitrogen mineralisation · Hydrolysable nitrogen compounds · Microbial nitrogen

Introduction

Nitrogen, despite being one of the most abundant elements in surface soils, frequently limits the development of ecosystems because in the majority of the soil surface layers it is mainly present in unavailable organic forms (Bremner 1965a; Stevenson 1982; Schnitzer 1991; Nilsson et al. 1995; Schulten and Schnitzer 1998). The accumulation of organic N compounds is more evident in forest soils (Pritchett and Fisher 1987), and N mineralisation is markedly retarded in soils like those developed over granite due to the abundance of organo-aluminium compounds, which present a high resistance to microbial mineralisation (González-Prieto et al. 1991, 1996). It is therefore important to know the composition, variability and bioavailability of the nitrogenated substrate of soils.

Most studies on the organic N composition of soils have been carried out in soils with different edaphogenesis (Bremner 1965a, 1965b, 1965c; Stevenson 1982; González-Prieto and Carballas 1991; Schnitzer 1991; Schulten and Schnitzer 1998). However, available information on the variability of the organic N composition within the same type of soil or within the same soil, as well as on the bioavailability of the organic N compounds, is scarce, and most studies refer to agricultural soils (Kelley and Stevenson 1987; Pal et al. 1987; González-Prieto et al. 1991, 1997). A wider knowledge of these topics can be very useful for the management of ecosystems, can serve as reference data for evaluating soil chemical, biological or biochemical disturbance by natural or human processes (fire, deforestation, contamination, climatic change, etc.), and can provide new, useful information on the organic N compartments of

soils for the development of predictive mathematical models for the complex biogeochemical N cycle.

Acid soils are common in temperate humid zones and are frequently dedicated to forestry exploitations. Pine stands are also widespread in Atlantic and Mediterranean areas due to the high number of *Pinus* species adapted to varied soil and climatic conditions and to their ecological and economic importance.

The aim of this work is on the one hand, to improve knowledge of the composition and variability of the organic N of forest soils of the same type and, on the other hand, to analyse the relationships between organic nitrogenated forms and N mineralisation. To carry out this study, the acid step-wise hydrolytic procedure was selected from among the different methods available for studying soil organic N composition. In comparison with other procedures, this method has been shown to avoid the destruction and neoformation of organic N molecules and to allow a distinction to be made between organic N pools of different chemical resistance (González-Prieto and Carballas 1988).

Materials and methods

Seven soils classified as Humic Cambisols (FAO 1988) and developed over granite were selected for this study. All soils were under 30 to 40-year-old *Pinus* stands: *P. pinaster* Aithon on soils Req, Sal, Lag, Arm and Xia, *P radiata* D. on soil Rio and *P. silvestris* on soil Mnz. The undergrowth was mainly composed of *Ulex* spp. and *Pteridium aquilinum* L., except on soil Mnz where the undergrowth comprised *Chamaespartium tridentatum* L. and *Erica arborea* L.

The soils studied were located in a temperate, humid Atlantic European zone (Galicia, N.W. Spain). During the period of study the mean daily temperature oscillated from −1 °C to 23 °C and the annual precipitation from 1200 mm to 2000 mm. Around 40%, 30%, 20% and 10% of the rainfall occurred in winter, autumn, spring and summer, respectively.

Soil samples were composed of 3–5 subsamples of 2 kg each, taken at random from the 0 to 5-cm layer (all soils) and the 5 to 10-cm layer (only soils Mnz and Req) of the A horizon. Soils Mnz and Req were sampled in September in 3 consecutive years; the rest of the soils were sampled only once, in September (Arm and Sal), in May (Lag and Rio) and in February (Xia). Fresh soil samples were sieved and the fraction <4 mm thoroughly homogenised. For physical and chemical determinations, 500 g soil were air dried. The rest of the soil sample was stored at 4 °C and at field moisture for organic N fractionation and N mineralisation studies. The soils studied were acidic, unsaturated, with a coarse sandy loam texture and a deep A horizon rich in organic matter with a high C:N ratio (Table 1).

The methods of Guitian-Ojea and Carballas (1976) were used for measuring the following soil variables: texture (international mechanical analysis), pH in H_2O (ratio soil:water, 1:2.5), electrical conductivity (ratio soil:water 1:5), field capacity (at 10 kPa in a Richard's membrane plate extractor), Fe and Al content (extraction with ammonium oxalate and $Na_2S_2O_4$ and exchangeable cations (extraction with NH_4OAc at pH 7). Organic C was determined by combustion in a Carmhograph 12 apparatus (Wösthoff OHG, Bochum, Germany). Total N was measured by Kjeldahl digestion (Bremner 1965c) and inorganic N (NH_4^+-N and NO_3^--N) was measured as proposed by Bremner (1965a), but MgO was replaced by $Na_2B_4O_7$ (Stevenson et al. 1967). Organic N was calculated as the difference between total N and inorganic N.

Table 1 Selected characteristics of the soils studied. *EC* Electrical conductivity, *FC* field capacity, *SB* base saturation, *S* 0 to 5-cm layer, *D* 5 to 10-cm layer, *nd* not determined

Sample	pH	EC ($S\ m^{-1}$)	FC ($kg\ kg^{-1}$)	Total C %	C:N	Fe_2O_3	Al_2O_3	Ca^{2+}	Mg^{2+}	K^+	Na^+	Al^{3+}	SB (%)
						($g\ kg^{-1}$)		($mEq\ 100\ g^{-1}$)					
Mnz S[a]	4.4±0.2	7.1±3.4	647±99	12.5±3.4	19±2	5.4±0.8	6.3±1.1	1.47±0.83	0.43±0.20	0.22±0.08	0.13±0.07	2.71±0.81	4.4±1.3
Mnz D[a]	4.4±0.2	6.0±2.2	574±87	10.4±2.2	18±2	6.0±1.1	7.3±1.4	0.77±0.10	0.18±0.07	0.15±0.05	0.10±0.08	3.31±0.91	2.6±0.9
Req S[a]	4.6±0.3	5.8±3.3	488±96	9.5±1.6	16±1	8.5±1.8	8.6±2.3	0.48±0.10	0.31±0.07	0.14±0.04	0.11±0.04	3.21±0.66	2.4±0.4
Req D[a]	4.6±0.3	4.8±2.6	491±55	9.0±1.9	15±1	8.9±1.6	8.7±3.0	0.24±0.13	0.12±0.05	0.08±0.02	0.09±0.02	3.26±0.57	1.2±0.4
Sal S	4.7±0.0	6.8±0.2	455±2	9.7±0.1	22±1	9.6±0.5	7.6±0.2	0.26±0.03	0.54±0.04	0.11±0.01	0.25±0.01	1.73±0.29	3.0±0.2
Arm S	4.4±0.0	5.4±0.2	686±2	13.7±0.1	15±0	14.0±0.1	17.9±0.5	0.46±0.03	0.19±0.01	0.08±0.01	0.16±0.02	2.96±0.14	1.4±0.0
Lag S	4.9±0.0	8.3±0.3	473±2	10.0±0.3	20±1	6.5±0.1	8.6±0.2	0.71±0.03	0.70±0.03	0.16±0.01	0.25±0.01	2.30±0.10	4.3±0.2
Rio S	4.1±0.0	4.6±0.2	725±7	14.3±0.2	15±0	8.0±0.4	6.6±0.2	0.76±0.01	0.42±0.00	0.15±0.01	0.13±0.01	2.53±0.12	2.3±0.1
Xia S	4.4±0.2	nd	nd	18.1±0.5	14±1	1.3±0.1	1.8±0.2	nd	nd	nd	nd	nd	nd

[a] Mean ± SD of the values obtained in the three samples collected

Organic N composition was analysed by step-wise acid hydrolysis fractionation (González-Prieto and Carballas 1988). Soil subsamples with 50 mg N were successively hydrolysed with 1 N HCl for 3 h, 3 N HCl for 3 h, 6 N HCl for 4 h and 6 N HCl for 20 h. The four hydrolysates obtained are hereinafter referred to as HI, HII, HIII and HIV. Both the neutralisation of the hydrolysates and the measurement of the different forms of organic N were carried out as described by González-Prieto and Carballas (1988). Amidic N was calculated as the difference between the total amount of NH_4^+-N obtained in HI and the NH_4^+-N pre-existing in the soil (NH_4^+-N extractable with 2 N KCl) (Yonebayashi and Hattori 1980). NH_4^+-N derived from the decomposition of other organic compounds was recovered in the other three hydrolysates (González-Prieto and Carballas 1988). Repetition of the hydrolysis on replicates of one soil showed that the coefficient of variation for the different organic N forms was less than 10%. Taking this into account, the hydrolysis was performed on one single replicate of each soil. Nevertheless, the different organic N forms were measured in triplicate in each hydrolysate.

For N mineralisation studies, four 50 g subsamples of each soil were placed in polypropylene jars; the moisture content was adjusted to 75% of the field capacity; the jars were covered with polyethylene film and incubated for 10 weeks in the laboratory in the dark at 28 °C. Net N mineralisation was calculated as the difference between the inorganic N content at the beginning and at the end of the incubation.

ANOVA, *t*-test and principal component analysis (PCA) with varimax rotation were applied to the data obtained from the fractionation of N. The honestly significant difference (HSD) was calculated by applying an ANOVA analysis and a Tukey's test to the data obtained from the mineralisation studies. Pearson correlation coefficients between all the pairs of variables measured were also calculated. All statistical analyses were carried out with the computer program SPSS 5.0.1.

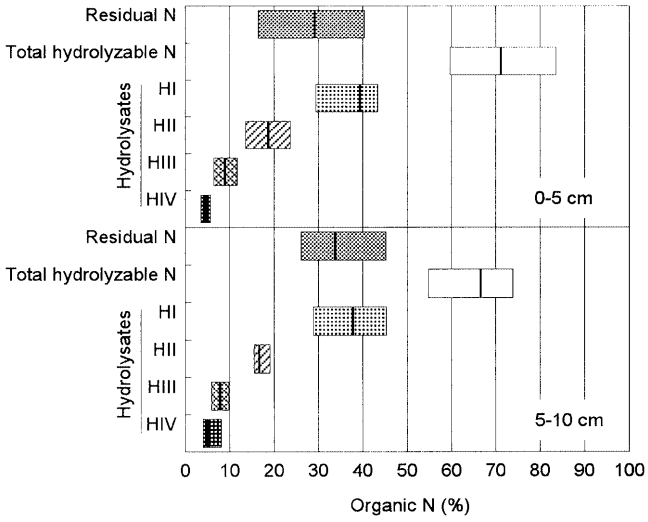

Fig. 1 Ranges of variation and means (*black line*) of the residual N and the hydrolysable N, as percentages of the organic N

Results and discussion

The total N content in the soil samples studied varied in a wide range, between 1.43% and 0.41% (mean 0.73 ± 0.30%). The inorganic N extractable with 2 N KCl represented < 0.2% of the total N. Therefore, in soils like those studied, with a very low content of clay (mainly kaolinite), it can be assumed that most of the N is in organic forms.

The distributions of organic N forms and fractions are summarised in Table 2 and Figs. 1 and 2. In both the 0 to 5-cm and the 5 to 10-cm layers, the total percentages of hydrolysable N and of the two major organic N forms, hydrolysable unknown N (HUN) and α-aminoacidic N, varied in wide ranges (Figs. 1, 2). In contrast, amidic N and organic N compounds recovered as NH_4^+, which represented around 6% and 5% of the organic N, respectively, ranged in narrow intervals (Fig. 2). The amount of aminoglucidic N (Fig. 2) was very low, reaching maxima of 3.8% and 2.3% of the or-

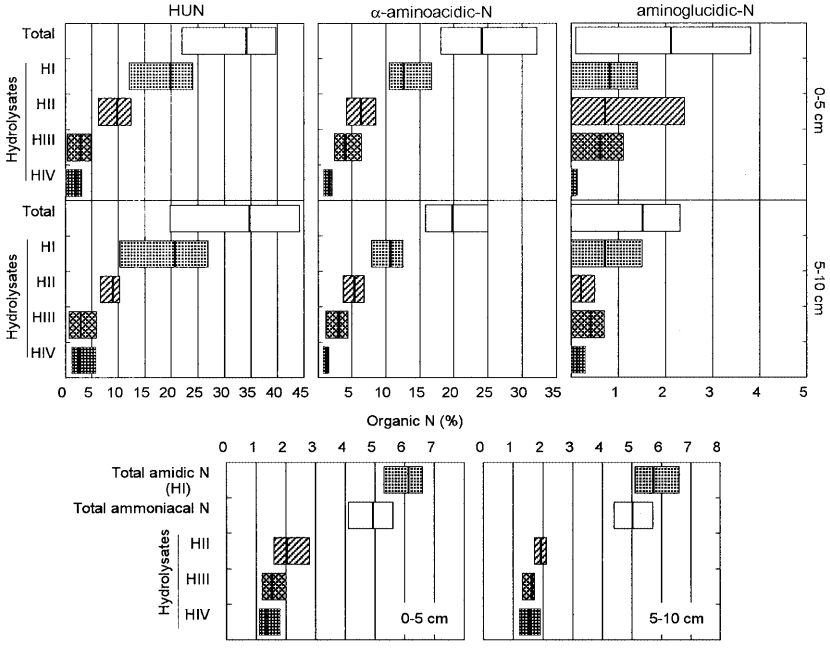

Fig. 2 Ranges of variation and means (*black line*) of the different organic N compounds, as percentages of the organic N

Table 2 Amount of residual N and distribution in the four hydolysates of hydrolysable unknown N (*HUN*), α-aminoacidic N, amidic N, ammoniacal N and aminoglucidic N, in mg N kg^{-1} soil or aminoglucidic N (mean ± SD). *HI* Hydrolysis with 1 N HCl for 3 h, *HII* hydrolysis with 3 N HCl for 3 h, *HIII* hydrolysis with 6 N HCl for 4 h, *HIV* hydrolysis with 6 N HCl for 20 h, *1* first sampling, *2* second sampling *3* third sampling; for other abbreviations, see Table 1

	HUN				α-Aminoacidic N			
	HI	HII	HIII	HIV	HI	HII	HIII	HIV
	(mg N kg^{-1} soil)							
Mnz S1	1878 ± 22	722 ± 25	103 ± 10	253 ± 20	963 ± 89	406 ± 39	232 ± 13	61 ± 21
Mnz S2	1499 ± 8	565 ± 3	198 ± 21	73 ± 1	915 ± 24	518 ± 16	239 ± 32	114 ± 9
Mnz S3	580 ± 41	337 ± 24	30 ± 38	114 ± 33	542 ± 64	298 ± 26	237 ± 31	44 ± 8
Mnz D1	1486 ± 23	570 ± 19	87 ± 10	137 ± 4	471 ± 18	263 ± 36	153 ± 12	57 ± 8
Mnz D2	1362 ± 8	401 ± 1	136 ± 2	89 ± 10	487 ± 4	397 ± 52	178 ± 10	55 ± 6
Mnz D3	485 ± 13	349 ± 14	32 ± 25	68 ± 1	568 ± 82	277 ± 22	160 ± 22	31 ± 20
Req S1	1398 ± 4	585 ± 3	243 ± 13	80 ± 9	728 ± 6	252 ± 8	138 ± 9	69 ± 3
Req S2	1128 ± 2	637 ± 15	228 ± 18	131 ± 7	574 ± 35	230 ± 13	134 ± 7	51 ± 10
Req S3	1444 ± 33	761 ± 6	250 ± 9	97 ± 12	889 ± 21	402 ± 13	323 ± 24	118 ± 9
Req D1	1809 ± 13	687 ± 40	394 ± 8	78 ± 10	790 ± 28	270 ± 7	75 ± 9	103 ± 12
Req D2	936 ± 12	475 ± 1	160 ± 1	271 ± 16	528 ± 42	179 ± 26	128 ± 4	42 ± 3
Req D3	1283 ± 16	659 ± 16	212 ± 3	88 ± 19	825 ± 39	448 ± 47	291 ± 45	86 ± 10
Sal S	907 ± 1	538 ± 11	218 ± 20	120 ± 16	584 ± 64	390 ± 21	183 ± 14	79 ± 2
Arm S	2115 ± 32	701 ± 31	303 ± 1	22 ± 6	1062 ± 46	544 ± 22	290 ± 26	164 ± 21
Lag S	948 ± 2	577 ± 5	206 ± 0	105 ± 2	668 ± 4	390 ± 2	219 ± 1	94 ± 1
Rio S	1709 ± 6	594 ± 31	180 ± 5	103 ± 10	1028 ± 38	442 ± 13	354 ± 28	143 ± 15
Xia S	2010 ± 258	1640 ± 309	52 ± 12	123 ± 32	2315 ± 172	1030 ± 108	883 ± 66	210 ± 1

	Amidic N	Ammoniacal N			Aminoglucidic N				Residual N
	HI	HII	HIII	HIV	HI	HII	HIII	HIV	
	(mg N kg^{-1} soil)								
Mnz S1	517 ± 5	123 ± 6	92 ± 12	106 ± 5	12 ± 11	62 ± 13	82 ± 4	7 ± 5	2287 ± 39
Mnz S2	409 ± 12	139 ± 3	104 ± 7	107 ± 6	103 ± 8	18 ± 7	57 ± 7	1 ± 2	2272 ± 18
Mnz S3	256 ± 4	102 ± 6	77 ± 2	71 ± 9	44 ± 7	115 ± 6	23 ± 2	3 ± 3	1939 ± 54
Mnz D1	336 ± 3	115 ± 5	77 ± 4	87 ± 5	29 ± 11	24 ± 2	35 ± 2	18 ± 6	2031 ± 40
Mnz D2	328 ± 11	118 ± 4	101 ± 6	95 ± 2	89 ± 8	24 ± 2	29 ± 2	1 ± 2	2165 ± 5
Mnz D3	239 ± 5	99 ± 1	81 ± 1	88 ± 8	71 ± 10	3 ± 6	33 ± 0	2 ± 3	2128 ± 17
Req S1	384 ± 8	91 ± 6	91 ± 4	68 ± 10	26 ± 7	14 ± 7	3 ± 5	3 ± 4	1666 ± 10
Req S2	307 ± 13	111 ± 6	91 ± 1	71 ± 1	0 ± 0	0 ± 0	0 ± 0	5 ± 8	1778 ± 2
Req S3	479 ± 2	126 ± 8	115 ± 3	100 ± 4	69 ± 12	30 ± 5	37 ± 7	9 ± 2	1942 ± 30
Req D1	440 ± 4	115 ± 6	101 ± 2	79 ± 8	5 ± 6	8 ± 4	5 ± 5	6 ± 4	1753 ± 15
Req D2	260 ± 6	96 ± 3	82 ± 2	75 ± 4	0 ± 0	0 ± 0	0 ± 0	2 ± 3	1561 ± 40
Req D3	393 ± 10	123 ± 8	100 ± 5	96 ± 6	63 ± 6	32 ± 8	41 ± 2	1 ± 2	1853 ± 32
Sal S	295 ± 8	111 ± 9	59 ± 1	62 ± 3	24 ± 3	22 ± 8	37 ± 1	0 ± 0	944 ± 3
Arm S	564 ± 11	169 ± 6	126 ± 5	164 ± 5	83 ± 13	71 ± 10	76 ± 16	0 ± 0	2611 ± 20
Lag S	304 ± 0	103 ± 1	65 ± 0	59 ± 0	45 ± 1	25 ± 1	49 ± 0	6 ± 1	759 ± 5
Rio S	565 ± 12	174 ± 12	180 ± 1	100 ± 6	100 ± 19	64 ± 12	34 ± 15	12 ± 10	3587 ± 4
Xia S	823 ± 23	385 ± 15	222 ± 16	156 ± 17	192 ± 11	117 ± 11	45 ± 4	9 ± 8	3653 ± 66

ganic N in the 0 to 5-cm and 5 to 10-cm layers, respectively, and in both layers at least one sample presented an undetectable amount of hexosamines.

The percentages of total hydrolysable N and α-aminoacidic N were similar to those most frequently found in soils by using continuous (Stevenson 1982; Schulten and Schnitzer 1998) or step-wise (González-Prieto and Carballas 1991) acid hydrolysis. The percentages of HUN and ammoniacal N were higher and lower, respectively, than those obtained in soils by continuous acid hydrolysis (Stevenson 1982; Schulten and Schnitzer 1998), but similar to the values observed in Humic Cambisols by using step-wise hydrolysis (González-Prieto and Carballas 1991). This discrepancy can be explained in part because the amidic N was determined separately from ammoniacal N, but it also confirms that the method used prevents the breakdown of organic compounds, mainly HUN compounds, to NH_4^+ (González-Prieto and Carballas 1991). In agreement with this, the amounts of NH_4^+ were also lower than those calculated by correcting the values obtained by continuous hydrolysis to discount amidic N and NH_4^+ fixed in clays (Schnitzer 1991). The percentages of amidic N fell in the middle part of the ranges cited for different types of soils (Yonebayashi and Hattori 1980; González-Prieto and Carballas 1991). Most of the percentages of aminoglucidic N were included in the range of values found in soils from the same region, but they were low-

er than those obtained in soils developed over granite (González-Prieto and Carballas 1991) and also lower than the percentages considered normal in soils (Bremner 1965b, 1965c; Stevenson 1982; Schulten and Schnitzer 1998). The soils studied present a high content of microbial C and N (Prieto-Fernández et al. 1998) and an abundant and diverse microbial population (Acea and Carballas 1990; Vázquez et al. 1993). Thus, the microbial synthesis of aminosugars and the degradation of substrates, like quitine, which comprise a high proportion of aminosugars, is probably not inhibited. The low content of hexosamines observed could be attributed to the stabilisation and transformation in the soil (Parsons 1981; Kögel-Knabner 1993; Schulten and Schnitzer 1998) and/or to a rapid mineralisation.

In both the surface and the subsurface layers the percentage of total hydrolysable N, HUN and α-aminoacidic N hydrolysed at each step of the hydrolytic fractionation decreased from HI to HIV (Figs. 1, 2), these compounds being mainly solubilised in the hydrolysis with 1 N and 3 N HCl (on average, 81%, 86% and 79%, respectively, of the total amount of these compounds). NH_4^+-N was distributed quite uniformly in the three last hydrolysates, since the percentage of total NH_4^+ obtained after hydrolysis with 3 N HCl (around 40%) was only slightly higher than that obtained in HIII and HIV (around 30% for both hydrolysates). Aminoglucidic N was not distributed uniformly amongst the first three hydrolysates, and the amount present in the last hydrolysate was very low or undetectable in all the samples. The soils studied presented percentages of labile hydrolysable N higher than those of other soils of different type developed over granite (González-Prieto and Carballas 1991), which could be related to the accumulation of unhumified organic matter, especially in the surface layers of these soils (Fernández et al. 1997), which is characterised by a low resistance to acid hydrolysis (González-Prieto et al. 1995).

The organic N composition was similar in the two layers studied. The ranges of values of all organic N forms corresponding to both layers overlapped (Figs. 1, 2). Nevertheless, the percentage of amidic N in the 0 to 5-cm layer was higher than that in the 5 to 10-cm layer sampled on the same day. In contrast, at every sampling the percentage of ammoniacal N was lower in the surface layer than in the subsurface one. In horizons from different types of soils an increase (González-Prieto and Carballas 1991), decrease or no variation (Abadín et al. 1998) in amidic N has been cited. The increase in NH_4^+ with depth could be a consequence of an increase in the proportion of humified organic matter, given that the amount of ammoniacal N in some tree leaves was found to be lower than in the soil organic matter (González-Prieto et al. 1995). In soil Mnz, but not in soil Req, a reduction in α-aminoacidic and hydrolysable N and an increase in residual N with depth was observed; these differences could be related to the lower and more contrasting temperatures of the Mnz site throughout the year. The ANOVA applied to all the organic N variables of the soils which were collected 3 times (Mnz and Req) indicated that, except in the case of HUN obtained in HII and HIII, there were no significant differences between the two soils sampled.

It is noteworthy that the organic N composition of the surface and subsurface samples studied presented high variability, and that despite the fact that the samples analysed were from the same type of soil, the ranges of variation for the percentages of hydrolysable N, HUN and α-aminoacidic N were as wide as those exhibited by soils of very different type (Stevenson 1982; González-Prieto and Carballas 1991; Schulten and Schnitzer 1998).

A PCA was applied to the data to simplify the interpretation of the results and to elucidate relationships among the variables of the organic N fractionation. The PCA data assigned a similar percentage of the total variance among samples to the first and the second axis obtained from the analysis (30% and 29%, respectively) (Table 3). According to this, the main sources of variation among samples were the labile organic N forms (hydrolysable N and HUN obtained in HI and amidic N), which defined the positive extreme of axis I, and the α-aminoacidic N and other organic N compounds of medium resistance to hydrolysis, located on the positive extreme of axis II (Table 3). The total percentages of hydrolysable N and HUN were also associated with axis I, probably because they were positively correlated with the labile fractions of these compounds. HUN obtained in HII and α-aminoacids of higher resistance to hydrolysis presented loading factors on both positive extremes of the first two axes. In comparison with the data obtained for soils of different types (González-Prieto and Carballas 1995), in the samples studied the variation of the labile organic N forms was bigger than the variation of the more resistant organic N compounds. Therefore, it seems that the variability of labile organic N forms is related to variations among samples from the same soil or the same type of soils, while the variation of organic N compounds more resistant to hydrolysis is related to variations in the edaphogenesis of soil and so allows differentiation among different types of soil. The third axis of the analysis explained 12% of the variance and was defined by variables that represented a minor proportion of the organic N; the positive extreme was defined by the aminoglucidic N fractions and the negative extreme by the hydrolysable N and HUN highly resistant to hydrolysis, which were negatively correlated with labile aminosugars (Table 3).

In agreement with González-Prieto and Carballas (1991), the PCA divided the organic N compounds recovered as NH_4^+ into two pools: the ammoniacal N obtained with 3 N HCl (HII), which was abundant in samples with a high percentage of hydrolysable N, was positively correlated with α-aminoacidic N and associated with the positive extreme of axis II, and the recalcitrant

Table 3 Variance explained and loading factors for the first three axis of the principal components analysis. *T* Total hydrolysable N, *AM* amidic N, *A* ammoniacal N, *AC* aminoacidic N, *AG* aminoglucidic N, *S* total percentage of each organic N form, I, II, III and IV hydrolysates

Variable	Factor[a]			Variable	Factor[a]		
	I	II	III		I	II	III
	Loading factors				Loading factors		
T-I	0.96			AC-S		0.93	0.31
AM	0.89			T-II		0.91	
HUN-S	0.84		−0.47	AC-III		0.82	0.31
T-S	0.83	0.52		AC-II		0.81	0.39
HUN-I	0.81	−0.47		A-II	−0.35	0.80	
A-S	−0.75	0.46		AC-I		0.77	
HUN-III	0.73		−0.37	T-III	0.49	0.72	
AC-IV	0.61	0.52		HUN-II	0.53	0.54	−0.52
A-III	−0.54			AG-IV			
A-IV	−0.48			HUN-IV			−0.82
				T-IV			−0.77
				AG-S	−0.32	0.40	0.76
				AG-I	−0.41	0.33	0.72
				AG-III		0.34	0.53
				AG-II			0.41

[a] Variance explained by factors I, II and III: 30%, 29% and 12%, respectively

ammoniacal N obtained with 6 N HCl (HIII and HIV), associated with the negative extreme of axis I. The positive correlation above cited suggests that a large amount of the NH_4^+ originated from the deamination of α-aminoacids (Bremner 1965c; Stevenson 1982) is obtained after hydrolysis with 3 N HCl.

With respect to the distribution of samples along axes I, II and III (Fig. 3), all the subsurface samples, except one sample from soil Req (Req D1), presented projections on the axis I lower than the corresponding surface samples; this probably reflects the slight decrease in amidic N with depth. Along axis II, the soils Xia, Lag and Sal were separated from the rest of the soils, which could be attributed to the differences in the composition of organic N related to the season of sampling (Xia was sampled in winter, Lag and Sal in spring and the rest of the samples at the beginning of autumn), although the separation observed could also be due to small differences in the edaphogenesis of these soils (González-Prieto and Carballas 1995). Along axis III, the samples Req S2 and Req D2, with extremely low percentages of hexosamines, were separated from the others.

The percentages of the different organic N forms and fractions were not significantly correlated with most of the physical, chemical or physico-chemical variables determined. This is not surprising because all the samples were from soils of the same type and, therefore, presented very similar characteristics. Electrical conductivity was positively correlated with α-aminoacidic N from HII and HIII and with the total percentages of these compounds ($r=0.60$, $r=0.69$ and $r=0.66$, respectively, $P\leq 0.01$). Exchangeable Al, which was negatively correlated with electrical conductivity ($r=-0.49$, $P\leq 0.06$), showed correlation coefficients with α-aminoacidic N fractions similar but negative to those indicated above for electrical conductivity ($r=-0.65$, $r=-0.65$ and $r=-0.74$, respectively, $P\leq 0.01$). The other exchangeable cations measured were not significantly correlated with electrical conductivity or with α-aminoacidic N, except Mg and Na that presented weak positive correlations with these compounds ($r\geq 0.42$, $P\leq 0.11$). Therefore, ionic strength and/or exchangeable Al content seem to be factors related to the processes of accumulation, destruction or transformation of aminoacids in these soils.

After 10 weeks of incubation a positive net N mineralisation was observed in all of the ten surface and the six subsurface samples studied (Fig. 4). The range of the amounts of N mineralised (14.2–110.9 mg N kg^{-1} dry soil) had lower and upper values higher than those found in other forest soils (Connell et al. 1995; Carlyle et al. 1998); nevertheless the percentage of organic N mineralised (0.26%–1.74% of organic N) fell within the lower part of the range found by Connell et al. (1995). Amounts of inorganic N produced and percentages of organic N mineralised after incubation were included within the range of variation found by González-Prieto et al. (1996) for soils from the same area that were incubated for only 6 weeks. In most cases, net ammonification was the main N mineralisation process (Fig. 4), which is common in acid forest soils (Prieto-Fernández et al. 1993; Connell et al. 1995; González-Prieto et al. 1996).

The inorganic N content in the soil after 10 weeks of incubation was positively correlated with the total organic N content. The organic N fractionation performed allowed to evidence that there were forms and pools of the total organic N related to N mineralisation, while other forms and pools did not show any correlation with N mineralisation (Table 4). Inorganic N content after incubation presented a strong positive correlation with the amount of total hydrolysable N, but was not significantly correlated with residual N; moreover, N mineralisation was positively correlated with the hydrolysable N less resistant to chemical attack, but was not significantly correlated with the hydrolysable N highly resistant to HCl (HIV). Although it has been shown

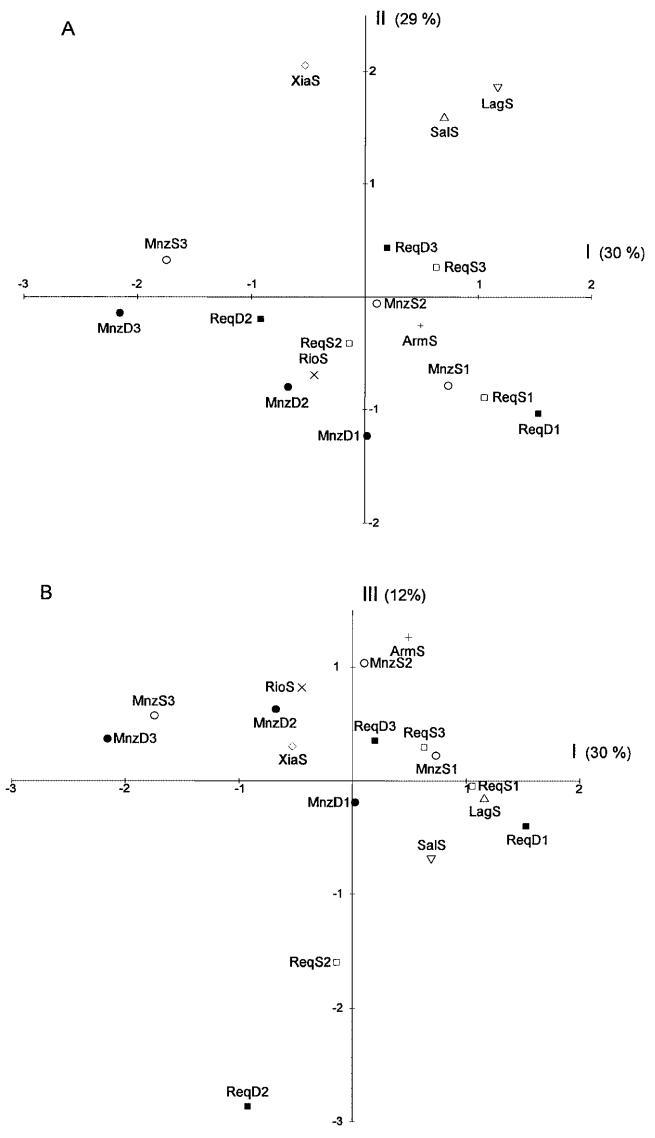

Fig. 3 Distribution of samples on the planes defined by axes I and II (**A**) and axes I and III (**B**) obtained by principal components analysis. *Values in parentheses* show variance explained by each component. *S* 0 to 5-cm layer; *D* 5 to 10-cm layer; *1, 2, 3* first, second and third sampling of soils Mnz and Req

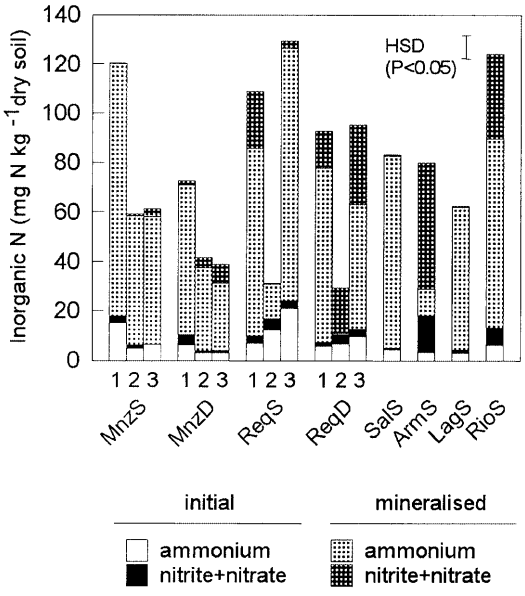

Fig. 4 Inorganic N (NH_4^+ and NO_2^- plus NO_3^-) content before and after 10 weeks of soil incubation. For abbreviations, see Fig. 3

Table 4 Coefficients of correlation between the organic N fractions and forms (mg N kg^{-1} dry soil) and the inorganic N content after 10 weeks of incubation and the microbial N. For abbreviations, see Table 2

	Total	HI	HII	HIII	HIV
Inorganic N after 10 weeks of incubation					
Organic N	0.60*				
Residual N	0.30				
Hydrolysable N	0.68**	0.66**	0.66**	0.66**	0.36
HUN	0.60*	0.57*	0.65**	0.32	−0.08
α-Aminoacidic N	0.63**	0.69**	0.38	0.52*	0.54*
Amidic N	0.75**	0.75**			
Ammoniacal N	0.40		0.40	0.47	0.23
Aminoglucidic N	0.35	0.12	0.34	0.32	0.37
Microbial N					
Organic N	0.72**				
Residual N	0.21				
Hydrolysable N	0.81**	0.79**	0.79**	0.61*	0.36
HUN	0.67**	0.70**	0.62*	0.24	−0.04
α-Aminoacidic N	0.73**	0.73**	0.55*	0.45	0.60*
Amidic N	0.85**	0.85**			
Ammoniacal N	0.49		0.54*	0.33	0.46
Aminoglucidic N	0.49	0.10	0.37	0.60*	0.33

* $P \leq 0.05$, ** $P \leq 0.01$

that residual N is not inert to microbial attack (Schulten and Schnitzer 1998), the bioavailability of the hydrolysable N seemed to be much higher.

Among the organic N forms, amidic N presented the highest positive correlation with the inorganic N accumulated after incubation (Table 4), and this was even observed when samples of soils Req and Mnz were analysed separately ($r=0.92$, $P \leq 0.01$ for soil Req and $r=0.82$, $P \leq 0.05$ for soil Mnz). This correlation agrees with the wide distribution of amidase and asparaginase in soils, whose activity is also positively correlated with total N (Frankenberger and Tabatabai 1981a, 1991); these enzymes are able to hydrolyse glutamine and asparagine, thought to be the main constituents of amidic N in soils (Bremner 1965c; Stevenson 1982). A rapid mineralisation of the amidic N could be the reason for the observed diminution of amidic N with depth and would explain, at least in part, the observed slight decrease in N mineralisation with depth. The high correlation between amidic N and N mineralisation supports Frankenberger and Tabatabai (1981b), who proposed the potential use of amides as N fertilisers. The inorganic N content was also positively correlated with α-aminoacidic N and with HUN; as in the case of the total

hydrolysable N, the labile fractions of these organic N forms presented higher correlation coefficients than the resistant fractions (Table 4). When samples from soils Mnz and Req were analysed separately, the positive and significant correlation with labile and total α-aminoacidic N was maintained in the case of soil Req ($r=0.94$, $P\leq0.01$ and $r=0.83$, $P\leq0.05$, respectively); in Mnz, the correlation was also positive but not significant at the 95% probability level ($r=0.63$ and $r=0.50$, respectively). The correlations found with α-aminoacidic N are probably related to the well known fact that α-aminoacids, mainly polymerised to form proteins, represent one of the major pools of organic N in soils (Stevenson 1982; Schulten and Schnitzer 1998); free α-aminoacids are continuously released from proteins by the action of proteases, and are quickly absorbed or mineralised by microorganisms (Barraclough 1997; Jones 1999). The results obtained agreed with those of González-Prieto et al. (1997) who found that, in agricultural soils, easily hydrolysable α-aminoacidic N and amidic N are important sources of N potentially available to plants. The inorganic N content after incubation was also positively correlated with the amidic N expressed as a percentage of organic N ($r=0.75$, $P\leq0.01$) and negatively correlated with the percentage of NH_4^+ ($r=-0.69$, $P\leq0.01$), but it did not show any significant correlation with the percentages of other organic N forms or fractions. Correlation coefficients between the net N mineralised and the organic N forms and fractions (data not shown) were similar to the ones described for the inorganic N content after incubation.

The inorganic N present in the soils after incubation represented only a small proportion of the labile organic N forms (10–28% of the amidic N, 5–15% of α-aminoacidic N and 3–11% of HUN obtained in HI), which are probably more easily degraded by microorganisms. Therefore, the organic N forms solubilised after hydrolysis with 1 N HCl, especially the amidic N, could represent important pools of labile organic N available for microorganisms.

Regression analyses including the variables of the organic N fractionation and the microbial N (previously published by Prieto-Fernández et al. 1998) of samples from soils Mnz (samples I-1d, I-1y and I-2y; Prieto-Fernández et al. 1998), Req (II-1m, II-1y II-2y), Sal (VIII) and Lag (VI) were carried out. Correlation coefficients between microbial N and the different organic N forms were similar to those discussed above for N mineralisation (Table 4), but the relationships between the labile organic N forms and the microbial N were clearly limited to the labile N and were higher than in the case of the N mineralisation parameters. It is likely that the microbial N was solubilised in the first hydrolysate, obtained under conditions (boiling with 1 N HCl for 3 h) that should have destroyed most of the microbial cells; however, it represented only 3.4–8.4% of the total N in HI. Therefore, the presence in HI of microbial N does not explain completely the high correlations observed, and seems to confirm the high lability of the compounds less resistant to hydrolysis with HCl to microbial degradation. The positive correlation between the microbial N and the aminosugars obtained in HIII, a correlation that increased when sample Req D1 was discarded in the analysis ($r=0.71$, $P\leq0.01$) and that became significant for the total content of aminosugars ($r=0.62$, $P\leq0.05$), suggests that part of the aminoglucidic N stabilised in the soil has a microbial origin or is related to microbial activity.

All the correlations cited above indicated that the resistance of organic N compounds to acid chemical hydrolysis seems to be strongly related to its bioavailability, and reinforce the usefulness of the information obtained by the hydrolytic method used for the study of organic N composition.

Acknowledgements The authors thank Mr. J. Salmonte and Ms B. Arnaiz for their technical assistance and Mr. B. Putt for reviewing the English text. This research was supported financially by the Conselleria de Educacion e Ordenacion Universitaria of the Xunta de Galicia and by the Comision Interministerial de Ciencia y Tecnologia (CICYT) of the Ministerio de Educacion y Ciencia, Spain.

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