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How the chemical composition and heterogeneity of crop residue mixtures decomposing at the soil surface affects C and N mineralization

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Abstract

The effects of plant litter characteristics on its decomposition in soil or at the soil surface is of primary importance for adequate management of nutrients and carbon (C) in agro-ecosystems. However, understanding the influence of mixtures of residues, which is actually the most common situation encountered in agriculture, is still poorly known in cultivated soils. Therefore we analyzed the effect of mixing leaf and stem litters from 25 species of plants (main crops and cover crops), representative of agricultural systems in subtropical conditions, on subsequent C and nitrogen (N) mineralization. We characterized the chemistry of leaves, stems and mixtures and determined the heterogeneity of the mixtures using Gower's similarity coefficient. We incubated crop residues at the surface of a sandy loam soil at 25°C over 120 days and continuously measured the mineralization of C and N. The 25 mixtures represented a wide range of chemical qualities and heterogeneity. Significant differences in C mineralization and soil N accumulation clearly differentiated crop families (notably Poaceae species vs. Fabaceae species), and plant parts (stems vs. leaves). The differences between observed and expected values for C mineralization were low or nil, indicating mostly an additive effect of mixing. Significant synergetic effects existed for only 7 species and resulted in an average additional 9% C mineralized. For N, an antagonistic effect was observed only with Fabaceae mixtures having high average N content and high chemical heterogeneity. We concluded that the decomposition of mixtures appeared mainly controlled by their average chemical composition and to a less degree by their chemical heterogeneity. In these cases, the availability of N in the mixtures appeared to increase the microbial N immobilization, reducing the net accumulation of mineral N in the soil.

Keywords: decomposition; chemical composition; functional trait; leaf; stem; litter mixture

1. Introduction

No-till systems use a large diversity of crop plant species that directly influence the cycles of carbon (C) and nitrogen (N) in soil (Jensen et al., 2005). In these cropping systems, the crop residues form mulch at the soil surface composed of a mixture of different plant parts (Lal et al., 2007; Thippayarugs et al., 2008). Physical factors (of soil or residue) and biological processes either individually or in combination can drive decomposition, but the intrinsic characteristics, or functional traits, of crop residues are important in controlling their decomposition rate in soils (Trinsoutrot et al., 2000; Jensen et al., 2005; Aulen et al., 2012). In general, leaves have higher rates of C and N mineralization than stems (Cobo et al., 2002; Abiven et al., 2005; Thippayarugs et al., 2008)

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due to their more readily decomposable tissue composition, lower lignin content and higher total N content (Quemada and Cabrera, 1995; Bertrand et al., 2006).

Many studies have been performed to better understand the effect of plant residue quality on C or N mineralization using plant leaves, stems or mixtures (shoots), with residues incorporated into the soil (e.g., Abiven et al., 2005; Bertrand et al., 2006; Thippayarugs et al., 2008) or left on the soil surface (Quemada and Cabrera, 1995; Cobo et al., 2002, Li et al., 2013a,b). However, in field conditions, different types of plant residues often decompose together in a mixture (Hättenschwiler et al., 2005). Indeed, this question is attracting much attention, particularly in the field of ecology, in investigations of the role of functional diversity (in this case, diversity of plant traits) on ecosystem functioning (Hättenschwiler et al., 2005). Trait similarity measures are used to characterize differences in chemical and physical characteristics of plant litters and to analyze non-additive effects (Gessner et al., 2010; De Bello et al., 2013). The C and N mineralization of litter mixtures can differ from that expected based on the decomposition of single components because the composition of the residue can modify the processes involved in decomposition (Hoorens et al., 2002; Gartner and Cardon, 2004; Berglund et al., 2013). The decomposition of residue mixtures has been reported to exhibit synergistic effects (i.e., higher rates of decomposition than expected) (Quemada and Cabrera, 1995; Zeng et al., 2010), negative effects (i.e., lower rates than expected) or additive effects (i.e., rates equal to those expected) (Liu et al., 2007; Li et al., 2013a; Li et al., 2013b). However, high variations in the range of responses of decomposition rates to mixing have been observed (Gartner and Gardon, 2004) depending on the type of residue, time scale and process considered (e.g., mass loss, C mineralization or N dynamics). Non-additive effects of mixing residues were assumed to result from the chemical heterogeneity of the mixtures (Harguindeguy et al., 2008) and particularly to the transfer of N between N-rich and N-poor litters (Berglund et al., 2013). Despite its acknowledged importance, this issue has been rarely addressed in agricultural systems, (Garnier and Navas, 2012). In this context, the published studies about residue mixtures usually involved a small number of species, which makes it difficult to generalize the findings (Gartner and Gardon, 2004). In addition, these studies have analyzed most often C mineralization or that of N, but rarely both together (e.g., Quemada and Cabrera, 1995; Shi and Marschner, 2014), which is needed to understand the interactions between decomposition and N dynamics in soil.

Thus, the primary objective of the present study was to quantify, in an agricultural context, the effect of mixing crop residues (leaf and stem) left at the soil surface on their decomposition (C mineralization) and the associated soil N dynamics. We had the aim of exploring a wide variety of quality of mixtures in order to be better able to generalize the results for arable crops. To do so, we used crop residues (leaves, stems and mixtures of leaves and stems) obtained from 25 different crop species grown in field, from five botanical families, mainly Fabaceae and Poaceae, and incubated them under controlled conditions. We tested the proposed hypothesis from Harguindegy et al. (2008) that the effects of mixing crop residues will depend on both the average chemical quality of mixtures, and of the heterogeneity of the components of these mixtures and that these factors would affect C and N dynamics differently.

2. Materials and methods

2.1 Collection of plant material

Twenty-five representative species of plants (main crops or cover crops) of agricultural systems in Brazil were studied (Table 1). The plants selected included eleven Fabaceae (legumes), ten Poaceae (Gramineae), two Brassicaceae, one Euphorbiaceae and one

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Asteraceae. The plants were cultivated during the spring/summer (14 species) and autumn/winter (11 species) in a Typic Hapludalf soil under a no-till system in the experimental area of the Soil Department (29°41′ S, 53°48′ W; approximately 90 m elevation) of the Federal University of Santa Maria in the state of Rio Grande do Sul, Brazil. The region has a subtropical climate with a mean annual precipitation of 1,686 mm and a mean air temperature of 19.3°C. For the previous 12 years, the experimental site had been cultivated using a no-till system. All the crops were managed according to the technical recommendations for the area. Plant shoots with 3 field replicates were collected at flowering and harvest for species of cover crops and main crops, respectively. The leaves senescing before harvest were collected gradually until harvest and stored. Subsequently, the plant shoots were separated into leaves and stems to determine their proportion in each plant species (Table 1). First, the residues were dried at 40°C and the leaves and stems were cut into pieces 1 cm long. Then, the residues were cut again along the length into pieces with a thickness of approximately 0.5 to 0.3 cm for leaves and stems, respectively. Mixtures of leaves and stems were prepared with a leaf:stem ratio for each species similar to the ratio in dry biomass determined in the field (Table 1). One subsample of each residue (leaf, stem and mixture) per species was dried at 40°C and ground to 1 mm particles, and a second subsample of each type of residue was dried at 65°C and finely ground (<1 mm) for chemical analyses.

2.2 Chemical characterization of plant residues

The total organic C and total N contents of the leaves, stems and mixtures of leaves + stems were determined in a finely ground subsample dried at 65°C using an elemental autoanalyzer (FlashEA 1112, Thermo Finnigan, Milan, Italy). A proximate analysis using the Van Soest method was performed using subsamples of ground residues pre-dried at 40°C. The soluble (SOL), cellulose (CEL), hemicellulose (HEM) and lignin (LIG) fractions of the residues were determined by proximate analysis (Van Soest, 1963). Neutral digestion was performed with 0.3 g of residues and 30 ml of neutral detergent solution. For acid digestion, 0.6 g of residues and 60 ml of detergent solution acid were used. The digestions were performed by boiling the material in the digester block at 150°C for 1 hour. After digestion, the samples were filtered by vacuum suction using a filter crucible and then washed with hot distilled water (90°C) and acetone (30-40 ml). The fibers were dried at 105°C for 12 h. The SOL content was determined based on the difference in the weight before and after neutral digestion. The HEM content was determined based on the difference between the percentage of total neutral fiber and the percentage of acid detergent fiber. The CEL content of the acid fiber was obtained after digestion with 12M H_2SO_4 for 3 h, and the material remaining after quantification of the CEL content was burned in a muffle furnace at 500°C for 3 h to determine the LIG content.

The water-soluble organic C (Csw) and water-soluble total N (Nsw) were extracted using 0.5 g of finely ground residues pre-dried at 40°C. The residues were placed in a 60-ml snap cap with distilled water (20°C) and mechanically stirred for 30 min. After mixing, the material was filtered (Whatman n°5), and the contents of Csw and Nsw in the filtrate were determined at 65°C. The quantification of soluble polyphenols (POL) was conducted with 0.75 g of finely ground residues pre-dried at 40°C. POL extraction was performed with aqueous methanol (1:1) in a water bath at 80°C for 1 hour (Tian et al., 1995). After extraction, the aqueous solution was filtered (Whatman n°2). After addition of the Follin-Denis reagent, the POL content was determined by colorimetry (absorbance at 760 nm) (King and Health, 1967). All the analyses were performed with three replicates, and the summarized results are shown in Table 2.

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Table 1: Description, agricultural use of crops used and proportion of their leaves and stems determined in field (% total of DM).

Latin name	English name	Family	Plant use	% Leaves[a]	% Stems	REF[b]
Phaseolus vulgaris	Bean	Fabaceae	Main crop	43 ± 3.1	57 ± 3.7	1
Glycine max	Soybean	Fabaceae	Main crop	38 ± 3.2	62 ± 2.1	2
Zea mays	Maize	Poaceae	Main crop	26 ± 3.6	74 ± 3.9	3
Helianthus annuus	Sunflower	Asteraceae	Main crop	39 ± 4.2	61 ± 5.1	4
Crotalaria juncea	Sunn hemp	Fabaceae	Cover crop	19 ± 2.3	81 ± 4.6	5
Canavalia ensiformis	Jack bean	Fabaceae	Cover crop	72 ± 4.9	28 ± 3.8	6
Stizolobium niveum	Gray mucuna	Fabaceae	Cover crop	42 ± 3.6	58 ± 2.9	7
Pennisetum glaucum	Millet	Poaceae	Cover crop	32 ± 4.1	68 ± 2.5	8
Sorghum bicolor	Sorghum	Poaceae	Main crop	55 ± 3.5	45 ± 4.3	9
Crotalaria spectabilis	Showy rattlebox	Fabaceae	Cover crop	30 ± 3.1	70 ± 3.1	10
Avena strigosa	Black oat	Poaceae	Cover crop	48 ± 2.0	52 ± 2.7	11
Vicia sativa	Vetch	Fabaceae	Cover crop	62 ± 1.8	38 ± 3.5	12
Triticum aestivum	Wheat	Poaceae	Main crop	42 ± 4.1	58 ± 3.3	13
Raphanus sativus oleiferus	Oilseed radish	Brassicaceae	Cover crop	38 ± 2.2	62 ± 3.3	14
Secale cereale	Rye	Poaceae	Main crop	29 ± 3.1	71 ± 4.2	15
Pisum arvensis	Pea	Fabaceae	Cover crop	68 ± 2.4	32 ± 2.4	16
Triticosecale rimpaui	Triticale	Poaceae	Main crop	44 ± 2.8	56 ± 3.9	17
Brassica napus oleifera	Oilseed rape	Brassicaceae	Main crop	28 ± 3.6	72 ± 2.8	18
Hordeum vulgare	Barley	Poaceae	Main crop	50 ± 3.3	50 ± 2.0	19
Lolium multiflorum	Ryegrass	Poaceae	Cover crop	37 ± 3.2	63 ± 2.9	20
Ricinus communis	Castor bean	Euphorbiaceae	Main crop	52 ± 5.1	48 ± 4.4	21
Cajanus cajan	Dwarf pigeonpea	Fabaceae	Cover crop	34 ± 3.2	66 ± 3.7	22
Lupinus angustifolius	Blue lupine	Fabaceae	Cover crop	57 ± 2.7	43 ± 4.3	23
Lupinus albescens	Native lupine	Fabaceae	Cover crop	51 ± 3.7	49 ± 4.1	24
Oryza sativa	Rice	Poaceae	Main crop	50 ± 2.9	50 ± 3.4	25

[a] Proportion of leaves and stems in the total dry matter (DM) of shoots determined at flowering for cover crops and harvest for main crops. Means (n=3) ± standard deviation (S.D.).
[b] Reference.

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2.3 Soil, treatments and experimental conditions

The soil used in the incubation experiment was a Typic Hapludalf (USDA classification) collected from the 0-10 cm layer in the same area as the plants. This soil contained 120 g kg^{-1} clay, 280 g kg^{-1} silt, 600 g kg^{-1} sand, 8.7 g kg^{-1} organic C and 0.9 g kg^{-1} total N, with a pH (H_2O) of 5.4. The soil initially contained 9.4 mg mineral N kg^{-1} dry soil. After visible organic residues had been removed by hand, the soil was sieved to 4 mm. Distilled water was applied to the soil to achieve a soil moisture content of 80% of field capacity. The soil was pre-incubated in plastic bags at 25°C for 5 days. The experiment consisted of incubations conducted for 120 days in the dark at 25±1°C to measure the C and N mineralization of leaves, stems and mixtures of leaves + stems. The experimental design consisted of two sets of jars prepared and monitored in parallel, with one jar used to evaluate C dynamics and one to evaluate N dynamics. The 76 treatments consisted of a non-amended soil (as a control), soil + leaves, soil + stems and soil + mixture (leaves + stems) for the 25 plant species listed in Table 1 with three replicates per treatment and per sampling time. The treatments were arranged in a completely randomized design. For each replicate, a sample of 134 g of soil at 13.8% moisture (equivalent to 80% of field capacity) was used. First, a moist soil subsample (67 g) was placed in a 110-ml cylindrical acrylic pot (5.0 cm in diameter and 5.0 cm in height) and compressed to a height of 2.5 cm. A second soil subsample (67 g) was placed in the same acrylic pot and compressed to a total height of 5 cm. Thus, the soil in each pot reached a final bulk density of 1.2 g cm^{-3}. The residues were applied on the surface of the soil at a rate of 0.56 g dry matter (DM)/pot (equivalent to a basis of 4.74 g DM per kg of dry soil or a rate of 3.06 Mg DM ha^{-1}), which was equivalent to the addition of 1790 (rice stem) to 2210 (dwarf pigeonpea leaf) mg C kg^{-1} of dry soil and 13 (sunflower stem) to 220 (gray mucuna leaf) mg N kg^{-1} of dry soil. Each acrylic pot was placed in a 1000-ml glass jar prior to incubation.

2.4 Analytical procedures

C mineralization of the crop residue mixtures was assessed by quantifying the CO_2 release. The CO_2 produced in the soil was trapped in 10 ml of 1 M NaOH in a beaker placed inside each glass jar. The NaOH beakers were changed at each sampling, i.e., at 2, 4, 7, 10, 14, 21, 28, 35, 50, 70, 90 and 120 days. The carbonate trapped in the NaOH was precipitated with a 2 M $BaCl_2$ solution, and the remaining NaOH was back-titrated with 1 M HCl. At all sampling times, the jars were aerated for 10 min. to renew the internal atmosphere and the soil water content was checked by weighing and adjusted as necessary. The apparent C mineralization of the crop residue mixtures was calculated by subtracting the amounts of CO_2-C evolved from the control treatment from the amounts of CO_2-C evolved from the amended treatments. Apparent mineralization assumes that there is no effect of crop residue addition on soil C mineralization (no priming effect) or that this effect is similar regardless of the type of crop residue added.

The soil mineral N (NH_4^+ + NO_2^- + NO_3^-) was measured in all treatments at time 0 and at 7, 14, 21, 35, 63, 90 and 120 days of incubation. At each sampling time, the visible residue particles were removed from the soil surface. The mineral N was extracted from fresh soil samples with 1 M KCl (30 min. shaking) and a soil:solution ratio of 1:4. The soil KCl suspension was settled for 30 min. until the supernatant liquid was clear, and the mineral N in a soil extract aliquot was measured by steam distillation (Keeney and Nelson, 1982). The jars were opened periodically and aerated and the humidity was adjusted when necessary. The net N mineralization was calculated by subtracting mineral N in the control from the amount of mineral N accumulated in each amended treatment.

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Table 2: Parameters of chemical composition, C mineralization and N mineralization of different types of residues used in this study (minimum, maximum and mean values).

Parameter	Leaves			Stems			Mixture		
	Min.	Max.	Mean	Min.	Max.	Mean	Min.	Max.	Mean
Chemical fraction (g kg^{-1} DM)									
SOL[a]	167	817	546	111	442	249	141	585	373
HEM	3	406	143	60	331	197	60	323	170
CEL	139	443	258	351	614	452	243	511	375
LIG	16	137	53	30	198	102	33	143	82
C	391	486	432	387	458	442	394	466	441
N	5.6	51.8	26.1	2.6	24.7	9.7	4.3	40.3	17.0
Csw	33.1	326.3	141.6	28.3	227.8	83.3	40.8	252.5	110.3
Nsw	3.4	35.8	12.6	1.4	29.3	6.8	2.9	34.0	9.5
POL	4.3	78.2	30.2	2.8	17.3	9.6	6.4	49.8	20.1
C and N mineralization									
C_0[b] (%)	46.9	64.3	56.0	25.8	56.9	46.1	41.1	60.7	51.0
k[c] (day^{-1})	0.022	0.128	0.063	0.012	0.109	0.039	0.021	0.104	0.050
C min 120 d (% added C)	43.9	67.1	58.0	26.0	61.5	46.7	39.4	63.4	52.6
Net N min 120 d (mg N kg^{-1} soil)	-20.0	140.8	45.3	-38.0	52.7	-7.7	-26.3	79.1	13.1

[a] SOL: Soluble fraction (Van Soest); HEM: Hemicellulose; CEL: Cellulose; LIG: Lignin; C: Total organic carbon; N: Total nitrogen; Csw: Water-soluble carbon; Nsw: Water-soluble nitrogen; POL: Soluble polyphenols; DM: Dry matter.

[b] Potentially mineralizable C pool.

[c] Mineralization constant rate.

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2.5 Analytical procedures

Data of N mineralization and of cumulative mineralized C measured over 120 days were analyzed by one-way analysis of variance. Least significant difference tests (P<0.05) were used to determine whether significant differences occurred.

To obtain a quantitative measure of the relative importance of the initial chemical characteristics of residues in determining residue mineralization, we first calculated C mineralization using an exponential equation according to Jung et al. (2011) as follows:

$$Cmin = C_0 (1 - e^{-k\,\mathrm{day}}) \qquad (1)$$

where *Cmin* is the amount of mineralized C, C_0 is the potentially mineralizable C pool, *k* is a mineralization constant and day is the incubation period. Stepwise multiple regression analysis was then used to determine which combinations of chemical variables best explained the variations in C_0 and *k*. Only those variables that were found to be significant at P<0.05 were retained in the regressions. Regressions were performed with all available chemical variables of residues.

A principal component analysis (PCA) was also performed to investigate the chemical traits that differentiate plant organs and plant families according to Aulen et al. (2012). PCA was performed on the correlation matrix obtained from the proximate analysis (i.e., SOL, CEL, HEM and LIG), C, N, water-soluble fractions (i.e., Csw and Nsw) and POL. The potentially mineralizable C pool (C_0) and the mineralization constant (*k*) were added as supplementary variables (i.e., not included in the ordination of species) to analyze their relationships with chemical characteristics. The PCA produced an ordination of the species and the chemical composition of the leaves, stems and mixtures, which were plotted in one and two dimensions, respectively, based on the scores of the first two principal components (PCs). A correlation circle was computed to assess the importance of each chemical component in the PC axes. We considered *r* values (i.e., the correlation coefficient) as significant contributors when r≥0.5 (Guénon et al., 2013).

To address the question of the non-additivity of mineralization in residue mixtures, we compared the evolved CO_2, soil mineral N content and net N mineralization in residue mixture treatments ('observed') to the 'expected' values for each crop species. The expected C-CO_2 mineralized from crop residue mixtures at days 14 and 120 was calculated as the weighted mean of the evolved leaf-derived C-CO_2 and stem-derived C-CO_2 according to their respective proportions in the mixture (Table 1). This procedure assumes an additive effect of mixing the two types of residues. A similar calculation was performed for the net mineralization of N. The calculated values were compared to the observed values, and differences were analyzed using Tukey's test. Based on the results of the statistical analysis, it was possible to classify residue interactions for C and N mineralization into 3 types as follows (Robinson et al., 1999): (1) measured and expected values do not significantly differ, and the contribution of each residue type to the mixture is additive; (2) measured values are significantly greater than expected values, indicating that mixing the residues has a positive (or synergistic) effect; and (3) measured values are significantly lower than expected values, indicating that mixing the residues has a negative effect (or antagonistic) on mineralization.

Deviation from additivity (Da) was calculated using the signed non-additivity calculation according to Makkonen et al. (2013) as follows:

$$\mathrm{Da} = \left(\frac{\text{Observed CO2 (or Nmin)}}{\text{Expected CO2 (or Nmin)}}\right) - 1 \qquad (2)$$

We used a multiple-trait-similarity measure to characterize differences in the chemical traits (composition) of the leaf and stem residues of each mixture for each species (De Bello et al., 2013) and to assess the effect

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of similarity in functional traits on non-additive effects. According to Gower and Legendre (1986) and Pavoine et al. (2009), we used Gower's (1971) general coefficient of similarity applied to quantitative variables with only positive and no missing values. The similarity between leaf residue *i* and stem residue *j* for a variable *k* is measured as the difference between different traits, and this difference is standardized between 0 and 1 for each trait as follows:

$$S_{ijk} = 1 - |X_{ik} - X_{jk}| / R_k \quad (3)$$

where S_{ijk} is the similarity between residue *i* and residue *j* calculated for the *k* the variable; X_k is the measured variable; and X_i and X_j are the values taken by the X_k variable of residues *i* and *j*, respectively; and R_k is the range of X_k variables measured. In this work, R_k was taken for each variable as the maximal difference between all values of the variable in the entire population (25 species) as follows:

$$R_k = \text{Max}\ (X_{ik} - X_{jk}) \quad (4)$$

As the leaf:stem ratio in the mixtures were not 50:50 but, rather, depended on the leaf:stem ratios in the field, S_{ijk} was weighted according to the proportion of the two plant parts in the mixture. The values of S_{ijk} and S_{ij} lie in the interval of 0 to 1. In the present study, the so-called 'species' of the original method were leaf and stem, and the data set included 25 pairs of leaves and stems (corresponding to the 25 plant species studied) and 9 variables corresponding to the chemical characteristics (or traits) measured. The relationships between the average additivity value and the single-trait similarity (S_{ijk}) were analyzed by linear regression. The traits that showed significant regressions with additivity for C or for N were selected to build the multiple trait similarity coefficients (S_{ij}).

3. Results

3.1 Chemical quality of leaves, stems and mixtures

The parameters of the chemical composition (minimum, maximum and mean values) of leaves, stems and mixtures (leaf + stem) of the 25 plant species are summarized in Table 2. The residue C and N contents ranged from 387 to 486 g kg^{-1} and from 2.6 to 51.8 g kg^{-1}, respectively, resulting in a C:N ratio ranging from 8.6 to 178. The measured chemical compositions of the residue mixtures were close ($r^2 \geq 0.96$) to the expected chemical composition (data not shown). The chemical characteristics were analyzed by a PCA to extract the most significant differences between organs and species (Fig. 1). The PCA showed that the first two PCs accounted for 70% of the total variance of the data. PC 1 explained 55% of the variance of the data and separated leaves of Fabaceae and Poaceae species as follows: in the positive part of the PCA, leaves from Poaceae species were most strongly correlated with CEL (r=+0.91) and HEM (r=+0.76) contents; and in the negative part of the PCA, leaves from Fabaceae species were most strongly correlated with SOL, total N, POL and water-soluble fractions (in the range of r=-0.65 to -0.97) (Fig. 1a, c). PC 2 explained 15% of the variance of the data and separated stems of Poaceae from those of other families (Fabaceae, Asteraceae, Brassicaceae and Euphorbiaceae) as follows: in the negative part of the PCA, stems from Poaceae species were slightly correlated with HEM content (r=-0.32) and stems from other families were correlated with LIG content (r=+0.78) (Fig. 1a, b, c). The mixture (leaf + stem) (Fig. 1a) showed an intermediate distribution between leaves and stems for PC 1 and PC 2, as expected. When analyzed only according to botanical families (Fig. 1b, c), PC 1 clearly showed that all residues from Poaceae species (stems, leaves and mixtures) were close to each other and were strongly correlated with CEL (r=+0.91) and HEM (r=+0.76) contents. Conversely, the distribution of residues from

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Fabaceae species was much wider along the two axes.

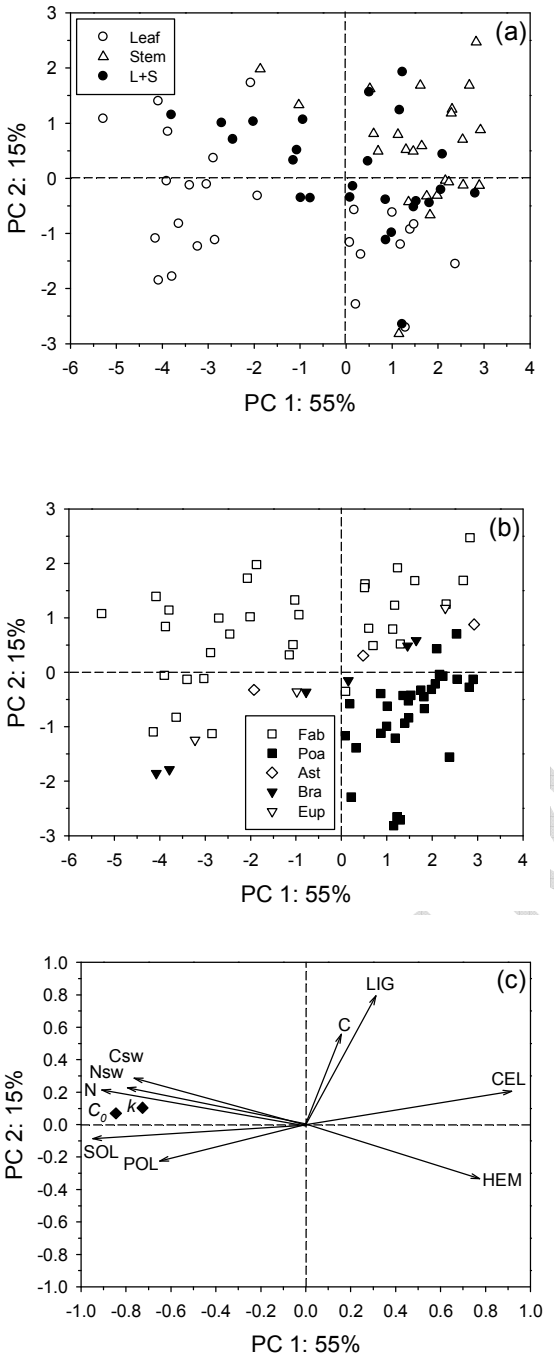

Fig. 1: Factorial map (PC 1 x PC 2) (a, b) and correlation circle (c) obtained from principal component analysis (PCA) performed on biochemical properties (i.e., Van Soest fractions, total N, total C, total N, water-soluble fractions and polyphenols) of leaves, stems and mixtures of leaves + stems (L+S). Mineralization constant (*k*) and mineralizable C pool (C_0) were added as supplementary variables, i.e., not included in the ordination of species. Fab: Fabaceae; Poa: Poaceae; Ast: Asteraceae; Bra: Brassicaceae; Eup: Euphorbiaceae.

Similarity coefficients for each individual chemical characteristic and aggregated similarity coefficients expressed the extent of differences among the chemical traits of leaves and stems for each species. Varied conditions occurred in this study. For example, sunn hemp and oilseed radish exhibited the widest variation in chemical composition between leaves and stems (S_{ij}=0.28), and rice exhibited a close composition in leaves and stems for all criteria (S_{ij}=0.93) (Supplementary data A). As expected from the PCA, the similarity coefficients also effectively expressed the differences between families, with S_{ij}=0.46±0.12 for Fabaceae and S_{ij}=0.81±0.20 for Poaceae. Other families (represented by one or two species; i.e., Brassicaceae, Euphorbiaceae and Asteraceae) had a low S_{ij} value, indicating a large difference in the chemical traits between stems and leaves. The 'weighted' similarity coefficients reflected the actual contribution of leaves and stems to chemical traits by taking into account their actual proportion in mixtures. The range of (weighted) S_{ij} values was narrowed and varied from 0.44 (pea) to 0.96 (rice) (Supplementary data B). The average weighted S_{ij} changed to 0.60±0.09 for Fabaceae species, but it remained unchanged for Poaceae species (0.81±0.16) because of the similarity of the chemical composition of the leaves and stems of Poaceae mixtures.

3.2 C and N mineralization

The cumulative C and N mineralization in soils amended with leaves, stems and mixtures varied widely among species (Fig. 2). The cumulative apparent C mineralization at day 120 ranged from 44±1.0 to 67±2.2% added C for leaves (Fig. 2a), 26±2.8 to 62±2.7% added C for stems (Fig. 2c) and 39±1.8 to 63±1.7%

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added C for the mixtures (Fig. 2e). The net N mineralization in soil showed a contrasting pattern among residue types in different species. Most of the leaf treatments induced a net increase in soil mineral N (+141 to -20 mg N kg^{-1} soil at day 120) (Fig. 2b), but stem decomposition induced stronger N immobilization, with net mineralization in the range of +53 to -38 mg N kg^{-1} soil at day 120 (Fig. 2d). As expected, the mixtures generated intermediate results (+79 to -26 mg N kg^{-1} soil) (Fig. 2f, Table 2). In several conditions, the mineral N content in soil was almost depleted during the first 14 days and remained depleted until the end of incubation (day 120), as observed for residues of maize (3), rye (15) and barley (19) species (Fig. 2b, d, f). The net N mineralization at day 120 was positively and linearly related to the residue N concentrations, with residue with low N content (<12.4 g N kg^{-1} residue DM or C:N ratio >35.2) inducing a net immobilization (Fig. 3).

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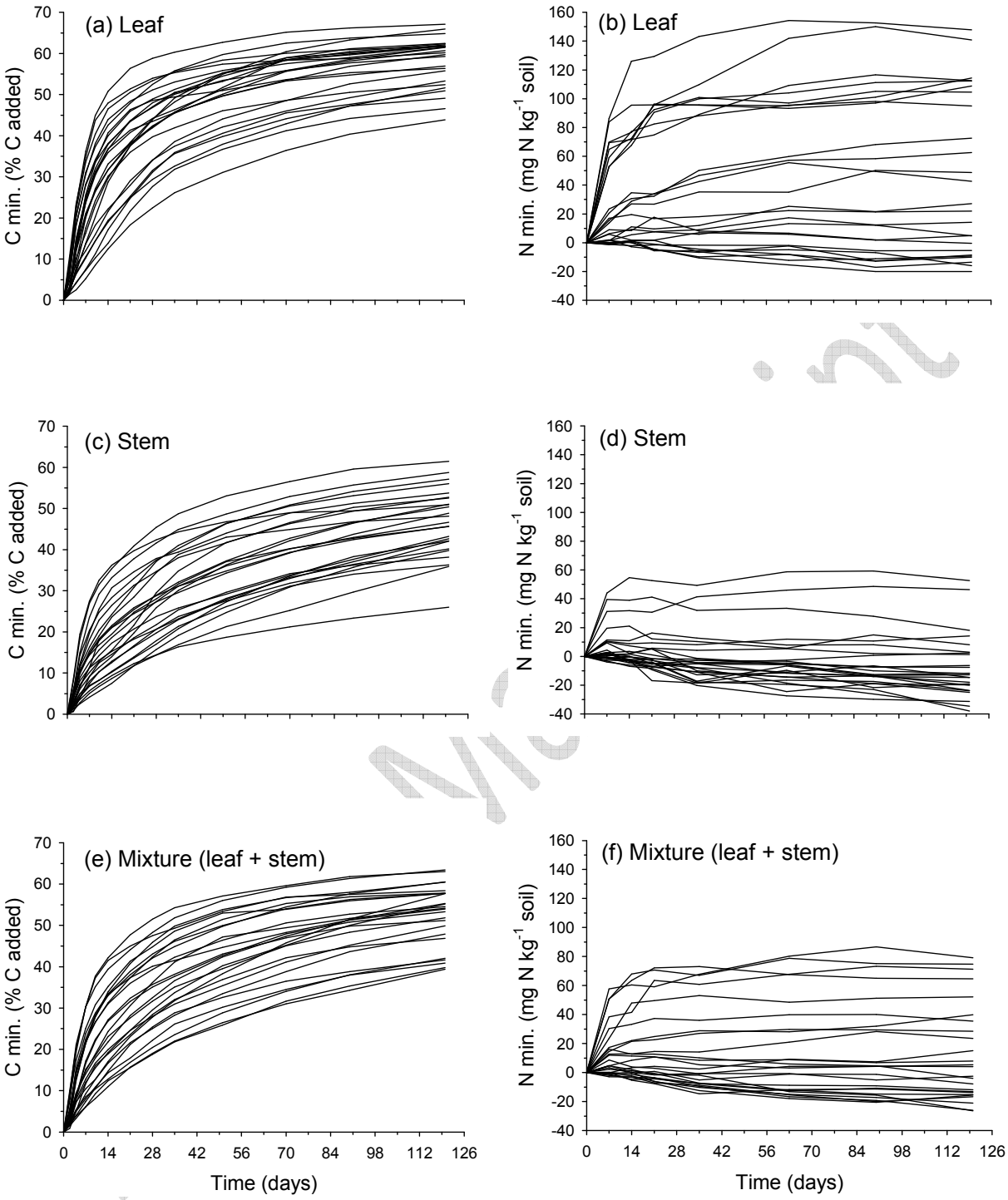

Fig. 2: Cumulative CO_2 mineralization (a, c, e) and net changes in soil mineral N contents (b, d, f) for leaves, stems and mixtures (leaf + stem) during decomposition in soil at 25°C for 120 days. Net mineralization was calculated by the difference between amended and non-amended control treatments. Values are the mean of 3 replicates.

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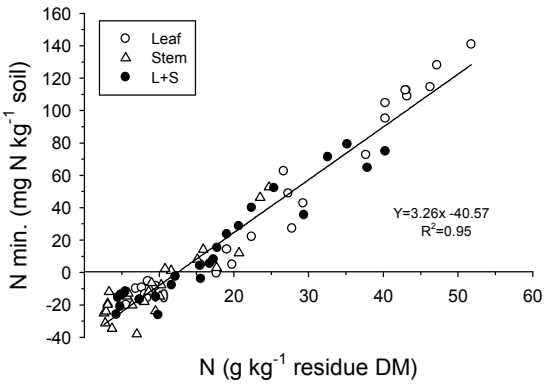

Fig. 3: Relationships between net N mineralized in soil after 120 days of incubation and initial total N content in the dry matter (DM) of the leaves, stems and mixtures of leaves + stems (L+S) for 25 crop species. Line indicate the 95% confidence intervals for the difference of means (P<0.001).

The C_0 of residues represented, on average, 46.1% added C for stems and 56.0% added C for leaves, and these values were close to the observed cumulative C mineralized at day 120 (Table 2). As expected, the mineralization constant (k) of the C_0 differed among residues and ranged from 0.012 (sunflower stem) to 0.128 (oilseed radish leaf) (Table 2; Supplementary data C). The PCA analyses indicated that total N, total soluble polyphenols and soluble fractions (Van Soest; water-soluble N and C) were correlated with a high C_0 and with k when considering all residues (stems, leaves and mixtures) (Fig. 1c). But the relationships between k or C_0 and the initial residue chemical composition varied greatly according to plant organ (leaf vs. stem vs. mixture) and to the range of species considered (Table 3). N content was the greatest explanatory chemical trait for leaves ($r^2 \geq 0.78$), which was not the case for stems, for which HEM and/or CEL were more significant ($r^2 \geq 0.42$). The LIG content was never selected, except for k in mixtures and Poaceae stems ($r^2 \geq 0.66$). All of the equations were highly significant (P<0.001).

3.3 Interactions between leaves and stems in mixtures

The relationship between observed and expected mineralization was analyzed using residue C mineralization and soil N content at days 14 (early decomposition) and 120 (end of incubation) and also expressed by signed no-additivity calculation (Da). Most of the statistically significant differences observed at day 120 had been previously obtained at day 14 for C and N (Supplementary data D), and the results discussed here are values obtained after 120 days of incubation. Overall, the effect of mixing residues was low for C mineralization (Fig. 4a; Supplementary data D) with the following significant effects: additive for 15 mixtures, positive for 7 mixtures and negative for 3 mixtures. The positive or synergistic effect (mean +9% added C mineralized) occurred with mixtures of sunflower (4), vetch (12), wheat (13), oilseed radish (14), oilseed rape (18), ryegrass (20) and dwarf pigeonpea (22). The negative or antagonistic effect (mean -5% added C mineralized) occurred with mixtures of bean (1), showy rattlebox (10) and rye (15). Da varied in the range -0.1 to +0.2.

The effects of mixing on soil mineral N content differed from the effect on C mineralization with the following significant effects: additive for 18 species; positive for 2 mixtures (mean +7.8 mg N kg^{-1} soil), i.e., showy rattlebox (10) and castor bean (21); and negative for 5 mixtures (mean -16.5 mg N kg^{-1} soil), i.e., jack bean (6), gray mucuna (7), vetch (12), pea (16) and dwarf pigeonpea (22) (Fig. 4b; Supplementary data D). All Poaceae mixtures showed additive effects for mineralized N in soil, as the observed deviations from additivity were not significant (Fig. 4b). Da varied in the range -0.2 to +0.2. For most of the species, a non-additive effect of mixing on C mineralization was not associated with a non-additive effect on N mineralization.

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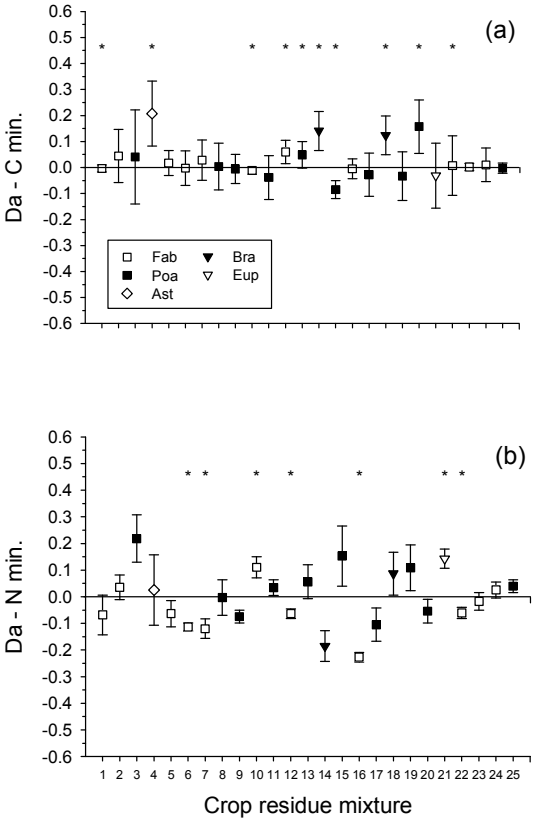

Fig. 4: Average deviation from additivity (Da) calculated for C mineralized in residue (a) and net N mineralization in soil (b) in 25 litter mixtures. Asterisks indicate significant difference of means according to Tukey's test (P<0.05). Fab: Fabaceae; Poa: Poaceae; Ast: Asteraceae; Bra: Brassicaceae; Eup: Euphorbiaceae. See Table 1 for the litter mixture species studied. Bars indicate the standard deviation of the means for mineralized C and N.

We also analyzed the mixtures all together (Fig. 5). There was a significant linear correlation between observed and expected C mineralization for the 25 mixtures (Y=1.02x; r^2=0.81; P<0.05) (Fig. 5a) showing that on average there was no interaction for C mineralization. Mixtures with a cumulative C mineralization > ~1000 mg C-CO_2 kg^{-1} soil exhibited really a ratio (observed:expected) close to 1. These mixtures were mainly of Fabaceae species. For most of the mixtures with cumulative mineralization < ~1000 mg C-CO_2 kg^{-1}, the observed C mineralization differed more from the expected mineralization and was either higher or lower depending on the species. These mixtures were primarily of Poaceae species. Regarding soil N, there was also a significant linear correlation between observed and expected net N mineralization at day 120 (Y=0.85x; r^2=0.94; P<0.05) (Fig. 5b) indicating that on average the observed net mineralization was 15% lower than the expected one. The results were confirmed when N mineralization values (observed or expected) were normalized, i.e., expressed in N mineralized per g of C mineralized for each mixture, to take into account possible differences in decomposition (Y=0.86x; r^2=0.95; P<0.05).

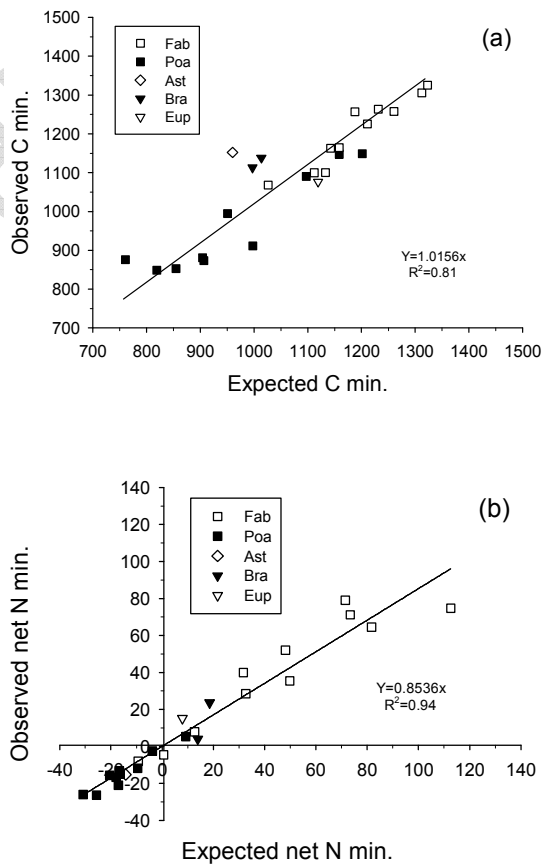

Fig. 5: Observed vs. expected C mineralization (mg C-CO_2 kg^{-1} soil) (a) and net N mineralization (mg N kg^{-1} soil) (b). Fab: Fabaceae; Poa: Poaceae; Ast: Asteraceae; Bra: Brassicaceae; Eup: Euphorbiaceae.

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The effect of similarity in functional traits on non-additive effects was analyzed by linear regression between Da and S_{ij} for the 25 mixtures. Da for C mineralization was related neither to any single-trait similarity coefficient nor to the full aggregated similarity coefficient (data not shown). For N mineralization, Da was slightly but significantly related to total N, water-soluble N, Van Soest SOL and water-soluble C (data not shown). The best relationship was obtained using only soluble N (r^2=0.25; P<0.01). Da increased (from negative values to positive values) with increasing weighted S_{ijk} for total N, indicating that synergistic effects on N mineralization occurred with decreasing heterogeneity in the N content of the mixtures (r^2=0.24; P<0.01). Using only the 7 species that had a significant non-additive effect on net N mineralization (6 Fabaceae and one Euphorbiaceae), we observed that Da was negatively related with the N content of the mixtures (Fig. 6a) and positively related to S_{ij} for total N (Fig. 6b).

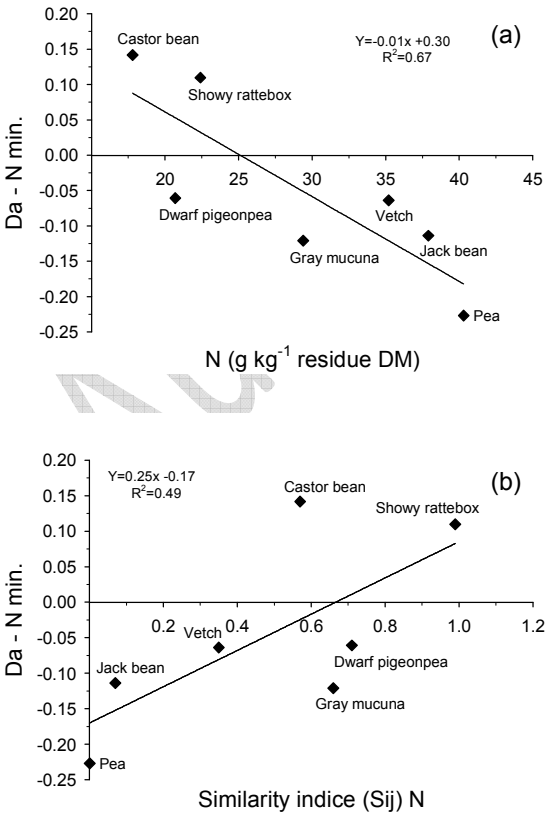

Fig. 6: Relationships between deviations from additivity (Da) calculated for net N mineralization and (a) mixture initial total N and (b) mixture heterogeneity coefficient of similarity for total N (S_{ij}). The relationship was established for 7 mixtures (leaf + stem) showing significant synergistic (positive Da) or antagonistic (negative Da) non-additive effects for N mineralization. S_{ij} for total N was a similarity coefficient calculated according to Gower and Legendre (1986).

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Table 3: Multiple regression analyses with the relationships between C mineralization and the chemical composition of residues.

Residue		k (day^{-1})[b]	r^{2c}	C_o (%)[d]	r^2
Leaf	All[a]	+0.57 N -0.49 Csw +0.80 Nsw	0.78***	+0.73 N	0.79***
	Fabaceae	-2.15 SOL -0.82 HEM -1.64 CEL +1.04 N +1.18 POL	0.91***	-1.23 HEM -0.88 CEL +0.41 N +1.10 Csw	0.95***
	Poaceae	-0.28 HEM -0.45 CEL +0.61 N	0.97***	+0.69 SOL	0.78***
Stem	All	-0.32 HEM -0.40 CEL +0.52 N	0.80***	+0.54 CEL +0.76 Csw	0.42***
	Fabaceae	+0.84 SOL	0.70***	+1.76 SOL +1.34 CEL	0.60***
	Poaceae	-0.81 LIG	0.66***	+0.68 Csw	0.46***
Mixture	All	-0.80 HEM -0.48 CEL -0.30 LIG	0.78***	+0.74 N	0.54***
	Fabaceae	-0.73 HEM	0.53***	-0.63 HEM -1.1 CEL -0.95 POL	0.87***
	Poaceae	+0.90 N	0.82***	+0.71 N	0.92***

[a] Residues from 5 botanical families (Fabaceae, Poaceae, Brassicaceae, Asteraceae and Euphorbiaceae).
[b] Mineralization constant rate.
[c] Coefficient of determination. ***P<0.001.
[d] Potentially mineralizable C pool.

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4. Discussion

The main objective of this study was to quantify the effect of mixing crop residues having different chemical qualities on their decomposition and on the net N mineralization in the soil. The chemical quality of mixtures can be characterized by the average chemical composition and by the degree of chemical heterogeneity of the mixture, as has been discussed by Harguindeguy et al. (2008). We obtained a large range of average chemical quality by using mixtures of leaves and stems from 25 different species, primarily including Fabaceae (legumes) and Poaceae (Gramineae). The mixtures resulted in different chemical compositions both between species for the same organ (leaf or stem) and between leaves and stems for a given species. For example, most Fabaceae species (legumes) were collected in the vegetative phase and were characterized by high levels of N and soluble compounds. Conversely, most grass species were harvested at maturity, and their tissues were richer in cellulose, hemicellulose and lignin, as expected (Bertrand et al., 2009). The striking result is the relatively low diversity of the chemical composition among Poaceae species and between leaves and stems for a given Poaceae species, and greater diversity for the Fabaceae species.

We used the Gower coefficient of similarity proposed by Gower and Legendre (1986) and De Bello et al. (2013) to quantify the chemical heterogeneity of mixtures. The results confirmed the marked typology observed with the PCA analysis, based on the botanical family to which the species belonged (S_{ij}=0.4 for Fabaceae and S_{ij}=0.8 for Poaceae mixtures), and the results identified species for which both organs were similar in all measured traits (e.g., S_{ij}=0.93 for rice) or different (e.g., S_{ij}=0.28 for oilseed radish). Given the characterizations, it is understandable that mixtures with low heterogeneity (mainly Poaceae mixtures in this study) suffered little or no effect of the mixing of leaves and stems, as has been observed especially for N mineralization. However, this study was limited to the examination of chemical features and did not account for differences in physical properties, which can also change the decomposition of residues left on the surface of the soil. This is the case, for example, for the capacity of water retention by plant residues (Iqbal et al., 2013) or the micro environmental conditions of residues (Makkonen et al., 2013), which were shown to influence the decomposition within residue mixtures.

The C mineralization was strongly dependent on the initial chemical composition, which confirmed the findings of numerous studies on annual crops (Trinsoutrot et al., 2000; Jensen et al., 2005) and perennial crops from natural ecosystems, for which the relationship between functional traits and decomposition have been extensively studied (e.g., Berglund et al., 2012). Within the two main families, the determinants of mineralization differed: most of the leaves underwent immediate decomposition (mainly in Fabaceae species), which was related to the relatively high SOL, total N, POL and water soluble fractions in leaves, in agreement with other studies, confirming the importance of soluble fractions (mainly in early stages) associated to available N, in plant residue decomposition (Reinertsen et al., 1984; Cobo et al., 2002; Abiven et al., 2005; Thippayarugs et al., 2008). In contrast, leaves and stems of the Poaceae family exhibited gradual decomposition, resulting in more gradual and stronger N immobilization than exhibited by Fabaceae species. These results can be attributed to the presence of higher cellulose and hemicellulose contents but also to low N content (total and soluble) combined with limited N availability in the soil. The lignin content did not appear to regulate the residue decomposition rates in soil as shown by the multiple regression analysis between C mineralized and initial chemistry (Table 3). Using 47 different crop residues, Trinsoutrot et al. (2000) also found that lignin does not explain the mineralization constant *k*. Using

249 different above-ground residues from crops, Jensen et al. (2005) found that holocellulose (CEL + HEM) was the factor that explains the greatest variability of C mineralization.

But under the conditions of decomposition at the soil surface with soil having initially a low mineral N content, as in this experiment, we assume that the low availability of N in the low-N residues had also influenced the kinetics of decomposition, because the potential microbial needs for N were not filled by N derived from the residues nor by the diffusion of soil N towards the decomposing layer (Garnier et al., 2008). Indeed, the amount of mineral N in the soil under the residue layer changed dramatically over the course of the decomposition process and greatly depended on the nature of the residues. This indicates that exchanges between the residue layer and the underlying soil were effective both in terms of (i) diffusion of N and/or C from the residues to the soil, which provoked N accumulation or N immobilization and (ii) diffusion of soil N to the residue layer to feed N decomposers. Therefore, N content in residue tissues played an important role in the processes of decomposition and N mineralization, particularly with residues at the soil surface and under conditions of low soil mineral N availability. This result was also confirmed by the strong relationship obtained between the total N content of residues and the net N mineralization in soil at day 120.

This study showed that the effects of mixing plant residues were different depending on the process considered: here C mineralization, considered as a proxy of decomposition, and net N mineralization which is more complex to interpret as the dynamics of soil mineral N content and the final N balance, result from different processes (decomposition and N release, diffusion, N immobilization in soil and in the residue layer). In fact, the literature shows often a synergistic effect of mixed residues on the degradation of C, which was often measured by mass loss (Gardner and Cardon, 2004). However, our results with C mineralization showed a majority of additive responses, and across the 25 species, there was no significant difference between observed and expected C mineralized at day 120 (Fig. 5a). It is therefore not surprising that none of the chemical similarity indices (including tissue N or C:N ratio) appeared significantly related to the response (additive or non-additive) for C mineralization, due to the overall weak effect of mixing on C mineralization. This result may seem surprising but in fact corresponds to the findings of other authors (e.g., Harguindeguy et al., 2008; Makkonen et al., 2013). Therefore we conclude that the mineralization of C from mixtures was fairly well explained by their average initial chemical traits. This conclusion is also supported by the high coefficients of determination of the equations that we obtained by stepwise regression between indices of C mineralization (C_0 and k) and chemical traits for mixtures of each botanical family. The heterogeneity of chemical traits had little or no effect on C mineralization of the mixtures studied.

Regarding N, Da calculated by species also showed that the non-additivity occurred only for 7 residue mixtures (28% of the mixtures studied) and was mostly negative (5 species). This trend applied to a few Fabaceae mixtures with initial high N content and a great heterogeneity of the mixture for N content trait, and was confirmed by the regression obtained between observed and expected net N mineralization for the 25 mixtures. This result supports the idea of a main interaction effect of residue nutrient contents, especially N, as has been shown in several studies (Hättenschwiler et al., 2005; Berglund et al., 2012). The fact that the negative deviation for N was not accompanied by a positive deviation for C suggests that the lower net accumulation of N in the soil did not correspond to an increased decomposition of the mixture. Our conclusion is that available N from the high N component of the mixture was immobilized in greater quantities by decomposer organisms growing

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on the low N component of the mixture, as a response to an increase availability of N to decomposers. Recous et al. (1995) showed with maize residues, that the N immobilization ratio, i.e. the amount of N immobilized by unit of C decomposed (or mineralized) could increase with increasing availability of N to the decomposers. An analysis of chemical characteristics of the components of the mixtures at the end of experiments but also the use of isotope tracing for N and C could help answer this question (Berglund et al., 2012) Therefore the main explanatory factors for non additivity of net N mineralized appeared to be the average high N content of the mixtures combined with a high degree of heterogeneity for this chemical trait, which enhanced the N immobilization. It concerned mostly the Fabaceae residues in this study. Several factors could alter the observed relationships. On the one hand we made this experiment with a single soil. In conditions of decomposition at the soil surface, the type of soil would mainly affect N availability (particularly the initial amount of mineral N and/or the amount of organic matter potentially mineralized) and the soil water properties. The diffusion of mineral N from the soil to the residue layer could therefore be increased in other soils, modifying the intensity of interactions for N. Also the incorporation of crop residues into the soil would increase the immobilization of N (Aita et al., 2012), decreasing also net N mineralization. But we believe that the conclusions regarding the hierarchy of responses (average chemical composition vs. chemical heterogeneity) and the differences observed between C and N interactions would still be valid in other experimental conditions.

5. Conclusions

This study showed that mixtures of plant residues consisting of leaves and stems of 25 species of annual crops induced a broad range of chemical composition and chemical heterogeneity of the mixtures. With regard to the effects of the functional traits of plant litters on important functions of agro-ecosystems, such as C or N mineralization, the results indicated that the synergistic, antagonistic or simply additive effects of mixtures were different depending on whether the residue-C mineralization or the soil mineral N were considered. In our experimental conditions, these effects were relatively low, on average. The occurrence of non-additive or additive effects depended first on the chemical quality of the mixtures (particularly the nutrient availability, like the presence of N that might control C decomposition but also alter N immobilization), while the intensity and sign of interactions (positive or negative) were primarily linked to the chemical heterogeneity of the mixtures. The results suggested that the availability of mineral N involved in the interactions between the mixture components played an important role, confirming the results of most recent works. The characterization of mixtures according to the botanical family and the type of plant organ was appropriate for interpreting the overall composition of residues considered individually or in combination, and their effects on mineralization. The results obtained in laboratory conditions suggest that the decomposition of mixtures of crop residues on the soil surface can be fairly accurately expected from the initial chemical quality of the components of the mixtures. On the other hand, the quality of these residue mixtures, and particularly their N content, will more influence the net availability of N and this phenomenon can be increased in situations where plant residues are not incorporated into soil by plowing, as explored in this work.

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Appendix A. Supplementary data: Coefficient of similarity between leaf and stem calculated for each chemical characteristics and each species using Gower's coefficient (S_{ijk}) and aggregated similarity coefficient (S_{ij}) calculated as the mean of S_{ijk} for the whole set of characteristics.

Species	Similarity coefficient (S_{ijk})									
	SOL[a]	HEM	CEL	LIG	C	N	Csw	Nsw	POL	S_{ij}
1 – Bean	0.09	0.28	0.16	0.17	0.29	0.52	0.24	0.38	0.54	**0.30**
2 – Soybean	0.00	0.29	0.06	0.00	0.80	0.67	0.11	0.37	0.58	**0.32**
3 – Maize	0.95	0.94	0.96	0.74	0.27	0.95	0.76	0.99	0.97	**0.84**
4 – Sunflower	0.47	0.89	0.16	0.92	0.66	0.54	0.37	0.62	0.00	**0.51**
5 – Sunn hemp	0.04	0.27	0.00	0.42	0.20	0.13	0.29	0.44	0.75	**0.28**
6 – Jack bean	0.31	0.55	0.28	0.48	0.44	0.37	0.00	0.83	0.81	**0.45**
7 – Gray mucuna	0.44	0.90	0.33	0.38	0.34	0.20	0.25	0.94	0.91	**0.52**
8 – Millet	0.72	0.91	0.72	0.60	0.00	0.70	0.95	0.85	0.85	**0.70**
9 – Sorghum	0.80	0.89	0.79	0.82	0.95	0.62	0.90	0.90	0.62	**0.81**
10 – Showy rattlebox	0.25	0.62	0.16	0.42	0.93	0.39	0.74	0.94	0.65	**0.56**
11 – Black oat	0.79	0.85	0.77	0.90	0.85	0.80	0.41	0.95	0.92	**0.80**
12 – Vetch	0.59	0.65	0.64	0.59	0.76	0.45	0.58	0.84	0.76	**0.65**
13 – Wheat	0.98	0.79	0.96	0.87	0.56	0.89	0.58	0.82	0.72	**0.80**
14 – Oilseed radish	0.17	0.42	0.22	0.18	0.20	0.41	0.16	0.00	0.73	**0.28**
15 – Rye	0.80	0.98	0.78	0.67	0.73	0.86	0.95	0.78	0.77	**0.81**
16 – Pea	0.61	0.97	0.53	0.44	0.88	0.24	0.19	0.66	0.79	**0.59**
17 – Triticale	0.95	0.39	0.65	0.84	0.85	0.81	0.80	0.78	0.97	**0.78**
18 – Oilseed rape	0.06	0.00	0.28	0.14	0.54	0.60	0.39	0.45	0.72	**0.35**
19 – Barley	0.99	0.81	0.99	0.78	0.59	0.86	0.62	0.78	0.93	**0.82**
20 – Ryegrass	0.86	0.79	0.94	0.84	0.44	0.85	0.70	0.82	0.95	**0.80**
21 – Castor bean	0.13	0.41	0.03	0.54	0.54	0.38	0.68	0.85	0.20	**0.42**
22 – Dwarf pigeonpea	0.24	0.65	0.02	0.73	0.32	0.08	0.55	0.73	0.64	**0.44**
23 – Blue lupine	0.45	0.49	0.29	0.78	0.93	0.00	0.33	0.23	0.68	**0.46**
24 – Native lupine	0.37	0.39	0.48	0.43	0.98	0.17	0.35	0.60	0.64	**0.49**
25 – Rice	0.95	0.95	0.98	0.90	0.90	0.94	0.83	0.97	0.92	**0.93**

[a] SOL: Soluble fraction (Van Soest); HEM: Hemicellulose; CEL: Cellulose; LIG: Lignin; C: Total organic carbon; N: Total nitrogen; Csw: Water-soluble carbon; Nsw: Water-soluble nitrogen; POL: Soluble polyphenols.

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Appendix B. Supplementary data: Weighted coefficient of similarity between leaf and stem calculated for each chemical characteristics and each species using Gower's coefficient weighted (S_{pij}) and aggregated similarity coefficient (S_{ij}) calculated for the whole set of characteristics. The weighted coefficients were calculated taking into account the proportion of leaf and stem masses in the mixtures.

Species	Similarity coefficient (S_{pij})									
	SOL[a]	HEM	CEL	LIG	C	N	Csw	Nsw	POL	S_{ij}
1 – Bean	0.51	0.46	0.52	0.29	0.74	0.78	0.82	0.73	0.56	**0.60**
2 – Soybean	0.52	0.37	0.39	0.00	0.61	0.91	0.79	0.77	0.67	**0.56**
3 – Maize	0.87	0.00	0.47	0.52	0.21	0.95	0.92	0.89	0.88	**0.63**
4 – Sunflower	0.79	0.82	0.39	0.75	0.65	0.79	0.84	0.83	0.09	**0.66**
5 – Sunn hemp	0.97	0.19	0.00	0.12	0.00	1.00	0.76	0.98	0.96	**0.55**
6 – Jack bean	0.00	0.96	0.99	0.97	0.36	0.07	0.01	0.46	0.53	**0.48**
7 – Gray mucuna	0.78	0.81	0.59	0.43	0.80	0.66	0.86	0.97	1.00	**0.77**
8 – Millet	0.98	0.29	0.54	0.53	0.39	0.97	0.75	0.99	1.00	**0.71**
9 – Sorghum	0.79	0.87	0.99	0.92	0.85	0.70	0.91	0.89	0.47	**0.82**
10 – Showy rattlebox	0.94	0.55	0.31	0.32	0.39	0.99	0.96	0.84	0.94	**0.69**
11 – Black oat	0.88	0.84	0.86	0.91	0.93	0.89	0.78	0.99	0.92	**0.89**
12 – Vetch	0.41	0.93	1.00	0.92	0.60	0.35	0.46	0.57	0.55	**0.64**
13 – Wheat	0.86	0.90	0.84	0.81	0.73	0.96	0.94	0.94	0.79	**0.86**
14 – Oilseed radish	0.70	0.49	0.48	0.26	0.60	0.76	0.86	0.59	0.82	**0.62**
15 – Rye	0.93	0.24	0.49	0.41	0.32	0.99	0.74	0.98	0.97	**0.67**
16 – Pea	0.26	0.84	0.96	0.90	0.43	0.00	0.00	0.00	0.56	**0.44**
17 – Triticale	0.98	0.86	0.73	0.82	0.80	0.90	0.99	0.90	0.99	**0.89**
18 – Oilseed rape	0.85	0.04	0.36	0.10	0.32	0.90	0.98	0.85	0.93	**0.59**
19 – Barley	0.99	0.88	0.99	0.85	0.97	0.91	0.84	0.86	0.91	**0.91**
20 – Ryegrass	0.96	0.41	0.70	0.73	0.55	0.96	0.99	0.95	0.85	**0.79**
21 – Castor bean	0.33	0.63	0.60	0.74	0.97	0.57	0.85	0.89	0.00	**0.62**
22 – Dwarf pigeonpea	0.73	0.45	0.30	0.42	0.52	0.71	0.94	1.00	0.77	**0.65**
23 – Blue lupine	0.43	0.75	0.77	0.70	0.79	0.20	0.56	0.37	0.53	**0.57**
24 – Native lupine	0.52	0.61	0.78	0.64	0.97	0.44	0.72	0.73	0.56	**0.66**
25 – Rice	0.96	0.97	0.99	0.93	0.99	0.96	0.93	0.98	0.90	**0.96**

[a] SOL: Soluble fraction (Van Soest); HEM: Hemicellulose; CEL: Cellulose; LIG: Lignin; C: Total organic carbon; N: Total nitrogen; Csw: Water-soluble carbon; Nsw: Water-soluble nitrogen; POL: Soluble polyphenols.

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Appendix C. Supplementary data: Cumulative residue-C mineralized and kinetic coefficients calculated from a simple exponential decay function for leaves, stems and mixture (leaf + stem) of plant residues incubated at 25ºC during 120 days.

Species	Leaves			Stems			Leaf + Stem		
	C min. measured[a] %	C_0[b] %	k[c] day^{-1}	C min. measured %	C_0 %	k day^{-1}	C min. measured %	C_0 %	k day^{-1}
1 – Bean	61.5 Ba*	57.5	0.083	45.7 Cc	42.0	0.047	54.6 Bb	50.6	0.064
2 – Soybean	60.7 Ba	57.4	0.053	40.2 Db	41.4	0.024	49.9 Cab	47.8	0.033
3 – Maize	43.9 Ea	46.9	0.022	35.9 Fa	39.9	0.016	39.4 Da	41.1	0.022
4 – Sunflower	57.0 Ca	56.1	0.047	42.1 Db	56.1	0.012	57.7 Aa	60.7	0.022
5 – Sunn hemp	59.3 Ca	56.4	0.074	52.7 Ba	54.9	0.027	55.3 Ba	52.9	0.055
6 – Jack bean	62.4 Ba	59.4	0.064	56.0 Aa	54.2	0.043	60.5 Aa	58.2	0.059
7 – Gray mucuna	61.5 Ba	56.5	0.066	57.1 Aa	53.1	0.051	60.5 Aa	56.0	0.059
8 – Millet	65.9 Aa	63.6	0.046	51.0 Bb	53.7	0.023	55.2 Bb	54.1	0.030
9 – Sorghum	52.4 Da	50.3	0.058	50.4 Ba	47.6	0.037	51.2 Ba	49.0	0.051
10 – Showy rattlebox	56.4 Ca	54.1	0.088	53.8 Ba	49.6	0.061	53.9 Ba	49.2	0.072
11 – Black oat	61.9 Ba	61.6	0.045	46.6 Cc	44.8	0.036	54.2 Bb	53.2	0.042
12 – Vetch	62.1 Ba	57.8	0.099	50.9 Bb	47.6	0.109	58.4 Aa	54.6	0.104
13 – Wheat	53.3 Da	51.3	0.038	42.7 Db	40.7	0.028	47.9 Cab	47.5	0.028
14 – Oilseed radish	60.2 Ca	57.6	0.128	45.6 Cb	43.3	0.043	57.7 Aa	54.7	0.076
15 – Rye	51.6 Da	50.5	0.031	42.2 Db	49.4	0.016	42.0 Db	43.5	0.023
16 – Pea	64.8 Aa	61.8	0.086	61.5 Aa	56.9	0.071	63.4 Aa	58.5	0.083
17 – Triticale	49.1 Da	49.3	0.035	36.3 Eb	39.1	0.022	40.9 Db	41.3	0.028
18 – Oilseed rape	61.6 Ba	59.1	0.108	48.1 Bb	47.2	0.049	57.8 Aab	55.0	0.068
19 – Barley	46.6 Ea	48.5	0.028	39.8 Db	39.8	0.025	41.6 Da	41.8	0.032
20 – Ryegrass	51.0 Da	52.3	0.030	26.0 Fc	25.8	0.028	39.8 Db	41.8	0.021
21 – Castor bean	62.5 Ba	60.4	0.048	43.2 Db	39.7	0.034	53.3 Bab	50.3	0.048
22 – Dwarf pigeonpea	59.8 Ca	54.7	0.069	48.9 Bb	45.3	0.035	51.9 Bab	49.3	0.040
23 – Blue lupine	67.1 Aa	64.3	0.109	58.7 Ab	53.8	0.065	63.0 Aab	59.4	0.089
24 – Native lupine	61.8 Ba	57.4	0.078	52.6 Bb	48.9	0.051	57.7 Aab	54.4	0.066
25 – Rice	55.8 Ca	54.7	0.035	38.2 Ec	38.4	0.032	46.9 Cb	46.6	0.035

[a] The % of added C mineralized from residues plant after 120 days.
[b] Potentially mineralizable C pool.
[c] Mineralization constant rate.
* Mean values with different large letters in the column are different by Scott-Knott test (P<0.05) and small letters in the lines by Tukey test (P<0.05).

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Appendix D. Supplementary data: Cumulative CO_2-C evolved and soil mineral N of mixtures (leaf + stem residues) of 25 crop species, observed at day 14 and day 120 of incubation, and expected values calculated using results of single leaf and stem treatments, assuming additivity between mixture components.

Species	Carbon min. (% added C)				Nitrogen min. (mg N kg^{-1} soil)			
	Measured	Expected[a]	Measured	Expected	Measured	Expected	Measured	Expected
	14 days		120 days		14 days		120 days	
1 – Bean	625	634 ns	1100	1133 *	22.9	25.8 ns	60.9	65.5 ns
2 – Soybean	412	428 ns	1068	1027 ns	15.1	15.2 ns	45.1	43.6 ns
3 – Maize	237	220 ns	849	819 ns	11.3	12.8 ns	26.9	22.1 ns
4 – Sunflower	274	227 *	1152	960 *	15.5	16.1 ns	37.7	37.3 ns
5 – Sunn hemp	458	513 ns	1163	1142 ns	31.6	33.2 ns	65.1	69.7 ns
6 – Jack bean	706	691 ns	1257	1260 ns	82.4	93.7 *	133.6	150.8 *
7 – Gray mucuna	690	658 ns	1264	1231 ns	52.1	66.8 *	104.5	118.9 *
8 – Millet	432	404 ns	1148	1158 ns	18.9	24.0 ns	42.8	43.0 ns
9 – Sorghum	578	556 ns	1090	1097 ns	24.0	26.7 ns	48.3	52.2 ns
10 – Showy rattlebox	681	688 ns	1100	1112 *	34.7	27.9 *	82.9	74.7 *
11 – Black oat	516	524 ns	1149	1202 ns	12.8	11.7 ns	39.6	38.3 ns
12 – Vetch	893	843 *	1257	1188 *	71.1	77.3 *	121.2	129.5 *
13 – Wheat	345	355 ns	995	951 *	5.5	5.6 ns	28.3	26.8 ns
14 – Oilseed radish	709	591 *	1113	997 *	14.7	18.9 ns	46.1	56.7 ns
15 – Rye	278	308 *	911	998 *	13.6	13.0 ns	38.9	34.0 ns
16 – Pea	811	829 ns	1305	1312 ns	83.9	98.3 *	129.2	167.1 *
17 – Triticale	309	306 ns	881	904 ns	16.1	15.2 ns	33.4	37.4 ns
18 – Oilseed rape	640	586 ns	1138	1014 *	28.8	32.1 ns	78.1	72.2 ns
19 – Barley	309	277 *	873	907 ns	10.7	9.2 ns	37.6	34.1 ns
20 – Ryegrass	274	255 *	876	761 *	7.3	9.3 ns	39.0	41.3 ns
21 – Castor bean	543	537 ns	1076	1119 ns	16.1	18.2 ns	58.2	50.9 *
22 – Dwarf pigeonpea	551	582 ns	1165	1159 *	34.2	37.8 *	71.6	76.2 *
23 – Blue lupine	886	883 ns	1325	1323 ns	53.5	74.5 *	121.9	124.2 ns
24 – Native lupine	709	688 *	1226	1211 ns	59.9	57.2 ns	102.9	100.3 ns
25 – Rice	343	334 ns	853	855 ns	8.9	10.7 ns	34.1	32.8 ns

[a] Expected values are calculated according to the proportion of leaves and stems in the mixtures (see Table 1). Asterisks in the lines indicate significant difference of means by Tukey test ($P<0.05$). Not significant (ns).

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