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Influence of root and leaf traits on the uptake of nutrients in cover crops

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Abstract

Aims Cover crops play an important role in soil fertility as they can accumulate large amounts of nutrients. This study aimed at understanding the nutrient uptake capacity of a wide range of cover crops and at assessing the relevance of acquisition strategies.

Methods A field experiment was conducted to characterize 20 species in terms of leaf and root traits. Plant traits were related to nutrient concentration and shoot biomass production with a redundancy analysis. Acquisition strategies were identified using a cluster analysis.

Results Root systems varied greatly among cover crop species. Five nutrient acquisition strategies were delineated. Significant amounts of nutrients (about 120 kg ha^{-1} of nitrogen, 30 kg ha^{-1} of phosphorus and 190 kg ha^{-1} of potassium) were accumulated by the species in a short period. Nutrient acquisition strategies related to high accumulations of nutrients consisted in either high shoot biomass and root mass and dense tissues, or high nutrient concentrations and root length densities. Species with high root length densities showed lower C/N ratios.

Conclusions The same amounts of nutrients were accumulated by groups with different acquisition strategies. However, their nutrient concentrations offer different perspectives in terms of nutrient release for the subsequent crop and nutrient cycling improvement.

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Introduction

In the perspective of a more sustainable agriculture, integrated nutrient management combined with renewable nutrient sources is required. Integrated nutrient management aims at improving nutrient use by crops while decreasing losses (Frossard et al. 2009). It includes good timing of external supply, efficient nutrient recycling and the use of crops with high nutrient acquisition and use efficiency (Frossard et al. 2009).

Thoughtful integration of cover crops in the rotation can provide many services including the enhancement of nutrient cycle efficiency. Indeed, cover crops are able to accumulate large amounts of nutrients and can

therefore prevent their loss. For example, 40 % to 70 % of N can be recycled in a system using cover crops compared to bare fallow systems (Tonitto et al. 2006). Absorption by the cover crops is particularly important for highly mobile, and thus leachable, nutrients, such as nitrate, but is also highly relevant for less mobile nutrients that can be lost through runoff or soil erosion.

In addition to nutrient recycling, several cover crop species are able to increase the amounts of available nutrients in the soil, especially in poor nutrient conditions. For example, it has been shown that the exudation of P-mobilizing compounds by the roots of P-efficient species, such as some Brassicaceae (Hunter et al. 2014) or some Fabaceae (e.g. Kamh et al. 1999; Nuruzzaman et al. 2005) makes recalcitrant P available for the subsequent crop. The amount of N available for the subsequent crop can also be increased by the integration of Fabaceae in the crop rotation. It has been reported that in only 3 months, Fabaceae can fix up to 143 kg ha^{-1} of N via biological N fixation (Büchi et al. 2015).

The major part of the nutrients accumulated by the cover crops is subsequently released for the main crop through the decomposition of the biomass and the mineralization of the residues. Mineralization rate is highly dependent on residue quality, which evolves with time, and can be appraised through the C/N ratio. Justes et al. (2009) observed that a C/N ratio below 26 should be favorable for mineralization. Above this threshold, immobilization of the nutrients occurs. For P, similar threshold exists and P is generally mineralized when the P concentration is higher than 3 mg g^{-1} (Damon et al. 2014).

However, uptake capacity differs widely among species. A characterization of plant traits involved in nutrient accumulation is required to guide the choice of cover crops for improving nutrient recycling efficiency. Leaf and root traits have been frequently related to plant ability to acquire, use and conserve resources, and have been used to qualify plant acquisition strategy (Reich et al. 2003). Several studies have evidenced a trade-off between plant characteristics allowing resource acquisition or conservation (e.g. Wright et al. 2004; Diaz et al. 2004; Fort et al. 2013). At the leaf level species with high nutrient uptake capacity are characterized by high specific leaf area, high leaf N concentration and low leaf dry matter content, while conservative species exhibit opposite characteristics (Grime et al. 1997; Diaz et al. 2004). Despite the high importance of roots for nutrient acquisition, root traits have not received as much attention as

above-ground characteristics. Nevertheless, it has been reported that high specific root length is associated with high root N concentration, and promotes high nutrient acquisition capacity (Tjoelker et al. 2005; Roumet et al. 2006). On the contrary, high root mass, dense tissues and large diameters favor resource conservation (Eissenstat et al. 2000; Craine et al. 2001).

In order to maximize cover crop benefits on nutrient cycle, it is crucial to characterize and to understand mechanisms involved in nutrient accumulation. The objectives of the present study are therefore to 1) characterize twenty cover crop species in terms of root and leaf traits 2) investigate the relationship between plant traits and nutrient uptake capacity, 3) delineate nutrient acquisition strategies on the basis of plant traits and nutrient concentration and 4) to discuss these results in terms of nutrient recycling efficiency by cover crops.

Materials and methods

Site description and experimental design

The study was carried out in 2013 at Agroscope Changins (46° 23' 59.7"N - 06° 14' 24.9"E, 426 m asl) in Switzerland on a Cambisol (FAO classification system) with 256 g kg^{-1} of clay and 274 g kg^{-1} of sand in the top 20-cm soil layer. The average total annual precipitation is 999 mm and the mean temperature 10.2 °C (30-year averages, 1981–2010). To further characterize soil fertility, soil analyses were performed. Soil organic matter (SOM), total N (N_{tot}), mineral N (N_{min}), cation-exchange capacity (CEC), total P, K, Mg, zinc, copper and manganese (P_{tot}, K_{tot}, Mg_{tot}, Zn_{tot}, Cu_{tot}, Mn_{tot}), organic P (P_{org}) and available nutrient forms (P_{Olsen}, K_{AA}, Mg_{AA}, Zn_{DTPA}, Cu_{DTPA}, Mn_{DTPA}) were measured for the layers 0–5 cm, 5–20 cm and 20–50 cm at mid-August (Table 1).

The field experiment was conducted with twenty species that are commonly used or can potentially be used as cover crops under Swiss conditions (Table 2). All the studied species are frost sensitive and are killed by the frost during standard Swiss winter. The preceding crop, alfalfa, was sprayed with glyphosate 5 days before cover crop sowing. The soil was plowed and harrowed. Cover crops were sown in 10 m^2 plots at the beginning of August using an experimental seeder with 13.5 cm row spacing at 2 cm depth. Seeding rates were adapted from recommended rates in such a way that species with

Table 1 Physical and chemical soil characteristics of the experimental field at three different soil depths

	Clay (g kg⁻¹)	Sand (g kg⁻¹)	pH[a] H₂O	SOM[a] (g kg⁻¹)	N_tot[a] (g kg⁻¹)	N_min[b] (kg ha⁻¹)	CEC[a] (meq kg⁻¹)	P_tot[a] (mg kg⁻¹)	P_org[c] (mg kg⁻¹)	P_Olsen[d] (mg kg⁻¹)	K_tot[a] (g kg⁻¹)	K_AA[e] (mg kg⁻¹)	Mg_tot[a] (g kg⁻¹)	Mg_AA[e] (mg kg⁻¹)	Zn_tot[a] (mg kg⁻¹)	Zn_DTPA[f] (mg kg⁻¹)	Cu_tot[a] (mg kg⁻¹)	Cu_DTPA[e] (mg kg⁻¹)	Mn_tot[a] (mg kg⁻¹)	Mn_DTPA[f]
0–5 cm	250	277	6.9	25.4	1.70	9.8	121	692	273	15.7	18.30	194	9.36	103	73.6	1.01	28.5	1.96	86.5	20.5
5–20 cm	258	273	7.1	24.4	1.61	26.0	123	686	265	18.2	17.96	170	9.62	105	74.5	0.86	27.8	1.88	84.1	15.9
20–50 cm	319	216	7.9	13.6	0.97	30.2	111	516	162	6.8	19.83	112	11.23	150	80.3	0.71	30.3	1.08	97.9	14.2

[a] pH-water, SOM, total N, CEC and total P, K, Mg, Zn, Cu and Mn measured according to the Swiss standard methods (FAL et al. 2004)

[b] Nmin extracted with water and calcium chloride

[c] Organic-P measured after soil incineration at 550 °C during 1 h and extraction of the ashes with 0.5 M H2SO4 (Saunders and Willians 1955)

[d] Olsen P measured according to Olsen et al. (1954)

[e] KAA and MgAA extracted with ammonium acetate according to the Swiss standard methods (FAL et al. 2004)

[f] ZnDTPA, CuDTPA and MnDTPA extracted with diethylenetriaminepentaacetic acid (DTPA) according to the Swiss standard methods (FAL et al. 2004)

similar characteristics had the same plant density, and to ensure a good performance. The experimental design followed a randomized block design with three replicates. During the period from sowing (beginning of August) to biomass sampling (end of October), the mean temperature was 16.1 °C and the cumulated rainfall was 339 mm. The experimental field was irrigated twice, seven and nine days after sowing, with 15 mm water to ensure a good plant emergence. No fertilization was applied.

Shoot biomass, nutrient concentration and nutrient uptake

Shoot biomass production was assessed at 85 days after sowing (532 growing degree-days (GDD) $T_{base} = 10$ °C). This corresponds to the arrival of cold temperatures stopping cover crop growth. Species can present differences in maturity at this time. Above-ground parts were harvested at the ground surface level from 0.25 m² squares per plot. The biomass was dried for 72 h at 55 °C, weighed and analyzed to determine N, P, K, calcium (Ca), Mg and carbon (C) concentration. N and C were assessed after combustion (Dumas 1831) and P, K, Ca and Mg were measured by ICP-AES after incineration and solubilization in hydrofluoric acid. Nutrient uptake in the above-ground biomass was calculated as the product of nutrient concentration and shoot biomass. Another plant sampling was made earlier in the growing period, at 48 days after sowing (386.8 GDD $T_{base} = 10$ °C) in order to take into account species maturity differences.

Leaf collection and trait measurement

Leaf traits were measured at 42 days after sowing (359 GDD $T_{base} = 10$ °C), before any flowering, on the youngest mature leaves following the standard protocol of Pérez-Harguindeguy et al. (2013). Fifteen leaves per plot were collected and rehydrated in demineralized water, in the dark at 4 °C during 15 h. After rehydration procedure, leaves were weighed to determine water-saturated leaf mass, and leaf area was assessed with a leaf area meter (LI 3000 C, LICOR). Leaves were then oven-dried for 48 h at 55 °C and weighed to determine dry mass. Leaf dry matter content (LDMC, mg g⁻¹) was estimated by the ratio of dry mass to fresh mass, and specific leaf area (SLA, mm mg⁻¹) was calculated as the ratio between area and dry mass. The mean value over

Table 2 Botanical family, cultivar, targeted plant density and maturity stage at the final harvest date of the studied cover crops

Latin name	Common name	Cultivar	Targeted plant density (pl m^{-2})	Code	Growth stage at final harvest date
Brassicaceae					
Brassica juncea	Indian mustard	Vitasso	500	b1	Vegetative
Brassica rapa campestris	Turnip rape	Nokonova	500	b2	Flowering
Raphanus sativus longipinnatus	Daikon radish	Structurator	80	b3	Vegetative
Raphanus sativus oleiformis	Forage radish	Pegletta	200	b4	Fruit development
Sinapis alba	White mustard	Albatros	300	b5	Fruit development
Fabaceae					
Lens nigricans	Lentil	Lenti-fix	200	f1	Vegetative
Pisum sativum	Field pea	Arkta	150	f2	Vegetative
Trifolium alexandrinum	Berseem clover	Tabor	500	f3	Vegetative
Vicia faba	Faba bean	Fuego	80	f4	Flowering
Vicia sativa	Common vetch	Candy	200	f5	Vegetative
Poaceae					
Avena strigosa	Oat	Pratex	400	p1	Flowering
Setaria italica	Foxtail millet	Extenso	400	p2	Vegetative
Sorghum sudanense	Sorghum	Hay-king	200	p3	Vegetative
Asteraceae					
Guizotia abyssinica	Niger	Azofix	300	a1	Vegetative
Helianthus annuus	Sunflower	Iregi	80	a2	Flowering
Other families					
Cannabis sativa	Hemp	Fedora	200	o1	Fruit development
Fagopyrum esculentum	Buckwheat	Lilea	200	o2	Senescence
Linum usitatissimum	Flax	Princess	500	o3	Flowering
Phacelia tanacetifolia	Phacelia	Balo	500	o4	Flowering
Salvia hispanica	Chia	Unknown	500	o5	Vegetative

the 15 leaves was computed for each species in each replicate.

Root collection and trait measurement

Two soil cores (diameter 5.7 cm, 50 cm depth) for each plot were taken before soil frost, at 118 days after sowing (544 GDD T_{base} = 10 °C). Each core was taken within the rows, centered on one plant. The soil cores were divided into three depths: 0–5 cm, 5–20 cm and 20–50 cm and stored at −18 °C until washing. After defrosting in warm water, soil samples were washed onto a 0.9 mm sieve with an elutriation system (Gillison's Variety Fabrication, USA). Roots were then separated by hand from organic debris and dead roots of previous crop. Image analysis using WinRHIZO 4.1 (Regent Instruments, Quebec) was performed to determine root length, area and mean diameter. Each root sample was then dried for 72 h at 55 °C and weighed. Based on the traits assessed by image analysis and dry mass, other root traits were derived. Root length density (RLD, cm cm^{-3}) was calculated as the total root length within one core divided by the soil volume. Specific root length (SRL, m g^{-1}) was computed by the ratio of root length to dry mass. Root tissue density (RTD, g cm^{-3}) was obtained with root dry mass divided by root volume. A mean value was calculated for each species in each replicate using the data of the two soil cores.

Root nutrient concentration (N, P, K, Ca, Mg and C) was measured on aggregated samples made of the six core samples per species, in order to have enough material for the analysis. The amount of root material was not sufficient for field pea and only N concentration was

assessed for this species. The analyses were done according to the same methods as for shoot biomass. As the soil cores were only made within cover crop rows, a conservative estimation of root biomass per hectare was computed considering that there were no roots between cores. The root mass per hectare was extrapolated from the root mass of the cores using the number of plant per row according to the sowing density for each species. Nutrient uptake was determined using this estimation of root biomass per hectare.

Data analysis

Analyses of variance (ANOVA) were performed on all the traits to test the difference between species. The experimental design corresponded to a randomized block design. The block were analyzed as a random factor. Mg concentration C/N ratio, leaf area, SLA, LDMC, root mass and RTD were log-transformed before analysis in order to meet the application conditions of ANOVA. When species effect was significant ($p < 0.05$), values of least significant difference (LSD) were calculated to compare the species means, using the R package agricolea (De Mendiburu 2014). The non-parametric Kruskal-Wallis test was used for shoot Ca concentration as it did not fulfill the required conditions.

In addition, the relative contribution of nutrient concentration and shoot biomass to the variation in nutrient uptake among the species was analyzed using the method described in Moll et al. (1982). This method is based on a partitioning of the variation of a variable in its components. It assesses the contribution of the components to the sum of squares of the product. The analysis is performed on the logarithms in order to linearize the product. The mean values for each species across the three replicates were used for this analysis.

To relate plant traits to shoot biomass and nutrient concentrations, a redundancy analysis (RDA), using the vegan R package (Oksanen et al. 2013), was computed with plant traits as explanatory variables, and shoot biomass and nutrient concentrations as response variables. Daikon radish was excluded from the analysis due to its very particular root system, namely a big taproot with a diameter of about 5 cm. Part of this root is aboveground and was harvested in the shoot biomass, making the comparison with other species difficult in terms of uptake capacity (the RDA including daikon radish is shown in the online resource 1). The scores of the two first RDA axis were used in a cluster analysis in order to delineate groups of species sharing the same root and leaf characteristics and with similar pattern of nutrient acquisition. The analysis was done on the centroid score of each species in order to avoid the potential classification of the replicates of a species in different groups. The complete linkage clustering of the gclus R package (Hurley 2012) was used for this analysis. The performance of the groups in terms of shoot biomass, nutrient concentration and uptake was tested with analyses of variance. When the group effect was significant a LSD at $p < 0.05$ was calculated.

All statistical analyses were performed with R 3.1.1 (R Core Team 2014).

Results

Growth conditions

Soil tillage before cover crop sowing and irrigation allowed a regular plant emergence and the major part of the species reached more than 80 % of their targeted sowing density. Despite lower emergence rates for some species, a high soil cover percentage was achieved by all the species. Growth conditions were also favorable after plant emergence. SOM content, total N and CEC were satisfactory on the whole soil profile despite lower values in the 20–50 cm layer (Table 1). The analysis revealed that available N (N_{min}) was globally high. We observed that the available forms of P and K decreased considerably with soil depth. While the availability of these nutrients was satisfactory according to the Swiss fertilization guidelines (Sinaj et al. 2009) in 0–20 cm, it was limited in 20–50 cm. On the contrary, Mg amounts, in both forms, were higher in deeper layers, but sufficient in all three layers. The results showed also that micronutrients (Zn, Cu and Mn) were not limiting (Table 1). Thus, these non-limiting soil fertility conditions combined to soil tillage before cover crop sowing and irrigation allowed good growth conditions.

Shoot biomass production, nutrient concentration and nutrient uptake

Growth dynamic differences were observed among the species (Table 2). Buckwheat reached flowering at 28 days after sowing (272 GDD $T_{base} = 10$ °C) and was already senescent at the final harvest date, at 85 days (359 GDD $T_{base} = 10$ °C). White mustard, hemp and

oilseed radish reached also flowering very quickly (42, 48 and 52 days after sowing, respectively) and started to set seeds at the final harvest date. At this date, flowering was observed for other cover crops such as oat or faba bean (Table 2).

Shoot biomass, nutrient concentration and nutrient uptake varied widely among families and species (Table 3). The highest biomass production was attained at the final harvest date for most of the species (Online resource 2). On average, the cover crops produced 5.8 t ha^{-1} and biomass ranged from 2.8 t ha^{-1} for buckwheat and lentil to more than 10 t ha^{-1} for sunflower. High biomass production was also reported for faba bean (7.8 t ha^{-1}) and niger (7.6 t ha^{-1}). Buckwheat was the only species showing a decrease (−2.1 t ha^{-1} on average) in biomass production between the first and the final harvest date.

Field pea, common vetch and lentil showed clearly the highest N concentration with about 35 g kg^{-1} (Table 3). Faba bean showed significantly lower N concentration than the other Fabaceae. For species from other families, N concentration ranged from 22.5 g kg^{-1} for hemp to 13.5 g kg^{-1} for white mustard. Several species, such as turnip rape or field pea, presented P concentration higher than 5 g kg^{-1}. K concentration ranged from 10.9 g kg^{-1} (buckwheat) to 47.3 g kg^{-1} (niger). Turnip rape (37.8 g kg^{-1}) showed significantly higher Ca concentration than the other species and the highest Mg concentration was reached by hemp (3.84 g kg^{-1}). High P, K and Ca concentrations were also observed for daikon radish and phacelia.

Translated into total nutrient uptake, more than 150 kg ha^{-1} of N were accumulated by faba bean, berseem clover, common vetch and sunflower (Table 3). Daikon radish showed the highest uptake of P (32.5 kg ha^{-1}) and Ca (211 kg ha^{-1}) and niger accumulated considerably more K than the other species (359 kg ha^{-1}). With more than 25 kg ha^{-1}, the Asteraceae showed significantly higher Mg accumulation than the other species.

Significant differences in C/N ratio were also observed between species (Table 3). Pea and common vetch showed the lowest C/N ratios (11.0 and 12.4 respectively) at 85 days after sowing. Low ratios were also observed for lentil (13.6) and berseem clover (15.7), and other non Fabaceae species such as daikon radish, turnip rape, phacelia and hemp. In contrast, several species (white mustard, sunflower, buckwheat, oilseed radish, Indian mustard and sorghum) showed

high C/N ratio, exceeding 26. At the first harvest date, all the species showed C/N ratios below 26. Fabaceae species showed the lowest ratios (<12). Low C/N (<14) were also observed for foxtail millet, niger, flax, phacelia and chia.

The partitioning of nutrient uptake as the product of shoot biomass and nutrient concentration gave contrasting results. For N and P, variation in biomass contributed more to uptake variation than concentration (73 % and 70 % respectively). On the contrary, concentration variation had the greatest influence on variation in Ca (65 %) and Mg (54 %) accumulation. For K, a rather equal contribution of biomass and concentration variation was observed (49 % and 51 % respectively).

Leaf traits

At the leaf level, significant differences between species were observed (Table 4). Very low leaf area (8 mm^2) was measured for flax and lentil, while turnip rape and daikon radish showed the largest area (185 and 179 mm^2 respectively). Turnip rape was also characterized by the lowest SLA (18.7 mm mg^{-1}) and the lowest LDMC (79 mg g^{-1}). Similar value of LDMC was observed for daikon radish (82 mg g^{-1}). At the other extreme, field pea showed significantly higher SLA (44.5 mm mg^{-1}) than the other species, while hemp had the highest LDMC (243 mg g^{-1}).

Root traits, nutrient concentration and nutrient uptake

Contrasting root systems were observed among the cover crop species (Table 4 and Fig. 1). Oat showed the highest total root length with 94.5 m of roots in the soil core (0–50 cm). High root length was also observed for Indian mustard, turnip rape, oilseed radish and phacelia. These species presented particularly high root density in the topsoil. Intermediate or low values of root mass were measured for these high length root systems (Table 4). On the contrary, faba bean and sunflower showed low total root length but high root mass (2.31 and 2.61 g respectively), especially in the 0–5 cm layer (about 70 % of total root mass was in the first five centimeters). Daikon radish presented comparable root system with high root mass up to 20 cm, but in contrast to the previous species, daikon radish had high root length in 20–50 cm. Field pea showed the lowest total root length (30.6 m) and root mass (0.24 g).

Table 3 Mean values ± standard deviation and analyses of variance of shoot biomass, nutrient concentration and uptake and C/N ratio for the twenty cover crop species

	Species	Shoot biomass (t ha⁻¹)	N concentration (g kg⁻¹)	P concentration	K concentration	Ca concentration	Mg concentration	N uptake (kg ha⁻¹)	P uptake	K uptake	Ca uptake	Mg uptake	C/N
b1	Indian mustard	6.6 ± 0.3	17.0 ± 1.7	2.77 ± 0.24	34.0 ± 2.1	17.6 ± 2.3	1.08 ± 0.21	112 ± 14	18.2 ± 2.5	223 ± 21	116 ± 20	7.2 ± 1.8	26.7 ± 3.3
b2	Turnip rape	4.4 ± 0.6	20.4 ± 1.1	5.37 ± 0.23	37.7 ± 2.7	37.8 ± 0.4	1.77 ± 0.06	90 ± 8	23.7 ± 3.4	167 ± 34	167 ± 23	8.8 ± 0.7	20.5 ± 1.3
b3	Daikon radish	6.3 ± 2.0	22.2 ± 1.5	5.30 ± 0.85	42.8 ± 4.5	33.7 ± 2.3	1.68 ± 0.32	139 ± 35	32.5 ± 5.0	265 ± 57	211 ± 52	10.3 ± 1.8	18.6 ± 1.3
b4	Oilseed radish	6.4 ± 0.5	15.9 ± 1.6	3.94 ± 0.23	39.1 ± 2.8	19.3 ± 1.5	1.52 ± 0.08	102 ± 17	25.2 ± 3.2	250 ± 33	123 ± 17	9.7 ± 0.9	28.0 ± 3.4
b5	White mustard	6.7 ± 0.7	13.5 ± 1.8	2.21 ± 0.36	20.6 ± 1.6	13.0 ± 1.6	0.90 ± 0.16	90 ± 7	14.6 ± 0.7	138 ± 25	86 ± 5	5.9 ± 0.5	35.3 ± 4.8
f1	Lentil	2.8 ± 1.2	34.6 ± 0.8	5.11 ± 0.33	30.8 ± 4.0	12.1 ± 0.8	2.15 ± 0.08	98 ± 42	14.7 ± 6.5	90 ± 46	34 ± 14	6.0 ± 2.4	13.6 ± 0.1
f2	Field pea	3.3 ± 0.5	36.2 ± 3.5	5.20 ± 0.51	31.6 ± 0.4	18.8 ± 8.0	3.28 ± 0.78	120 ± 28	17.0 ± 1.9	104 ± 16	61 ± 27	10.8 ± 2.8	11.0 ± 0.2
f3	Berseem clover	6.6 ± 1.3	28.5 ± 0.7	3.29 ± 0.14	25.3 ± 0.7	22.1 ± 2.7	2.10 ± 0.38	188 ± 36	21.7 ± 3.9	167 ± 31	145 ± 24	13.8 ± 3.1	15.7 ± 0.8
f4	Faba bean	7.8 ± 0.8	22.1 ± 1.0	3.49 ± 0.18	24.9 ± 2.7	8.8 ± 1.2	1.60 ± 0.16	172 ± 15	27.2 ± 3.6	194 ± 28	69 ± 16	12.5 ± 2.5	21.4 ± 1.0
f5	Common vetch	4.6 ± 1.2	35.9 ± 0.4	4.01 ± 0.38	32.5 ± 4.3	13.2 ± 1.6	2.31 ± 0.21	164 ± 46	18.1 ± 3.9	146 ± 25	59 ± 11	10.5 ± 2.3	12.4 ± 0.4
p1	Oat	6.5 ± 0.1	18.9 ± 2.2	3.41 ± 0.05	33.2 ± 1.3	7.4 ± 1.4	1.54 ± 0.14	123 ± 17	22.1 ± 0.6	215 ± 13	48 ± 10	10.0 ± 1.1	24.1 ± 3.1
p2	Foxtail millet	4.5 ± 0.6	20.4 ± 1.2	2.50 ± 0.18	36.5 ± 1.2	8.1 ± 0.8	2.63 ± 0.20	90 ± 8	11.2 ± 2.2	163 ± 27	36 ± 7	11.8 ± 2.3	22.4 ± 1.6
p3	Sorghum	5.8 ± 2.1	18.4 ± 5.5	3.55 ± 0.19	26.8 ± 4.9	6.6 ± 0.7	2.41 ± 0.35	100 ± 18	20.4 ± 6.5	150 ± 39	37 ± 10	13.5 ± 3.1	26.4 ± 6.9
a1	Niger	7.6 ± 1.1	17.2 ± 3.9	3.80 ± 0.40	47.3 ± 5.0	24.1 ± 0.9	3.36 ± 0.30	129 ± 11	28.8 ± 3.7	359 ± 41	183 ± 20	25.4 ± 1.5	25.0 ± 5.3
a2	Sunflower	10.3 ± 0.9	15.4 ± 2.6	2.92 ± 0.19	25.3 ± 3.6	13.5 ± 0.6	2.98 ± 0.18	157 ± 14	30.0 ± 1.0	258 ± 15	139 ± 7	30.6 ± 0.9	29.8 ± 6.0
o1	Hemp	4.8 ± 0.2	22.5 ± 2.2	4.89 ± 0.08	12.0 ± 0.3	30.0 ± 2.7	3.84 ± 0.06	108 ± 15	23.5 ± 1.3	57 ± 4	144 ± 19	18.4 ± 1.1	20.7 ± 2.1
o2	Buckwheat	2.8 ± 0.4	16.4 ± 2.6	2.70 ± 0.51	10.9 ± 2.5	16.7 ± 3.5	2.37 ± 0.09	46 ± 15	7.7 ± 2.7	30 ± 3	45 ± 5	6.6 ± 0.8	28.9 ± 4.0
o3	Flax	5.4 ± 1.1	21.2 ± 1.2	3.48 ± 0.18	25.1 ± 0.7	10.7 ± 1.4	1.61 ± 0.21	114 ± 16	18.8 ± 3.8	136 ± 24	59 ± 18	8.8 ± 2.8	22.4 ± 1.7
o4	Phacelia	6.3 ± 1.3	21.3 ± 2.2	4.83 ± 0.52	42.1 ± 3.8	29.3 ± 2.1	1.27 ± 0.11	132 ± 26	30.4 ± 7.2	261 ± 33	185 ± 45	7.9 ± 1.2	20.6 ± 2.3
o5	Chia	6.0 ± 1.7	19.3 ± 3.7	4.64 ± 0.36	36.1 ± 1.5	18.6 ± 1.1	3.33 ± 0.32	116 ± 37	27.5 ± 6.0	216 ± 53	113 ± 35	19.9 ± 5.2	23.7 ± 4.2
	Mean	5.8	21.9	3.87	30.7	18.1	2.19	120	21.7	179	103	12.4	22.4
	p value	<0.001	<0.001	<0.001	<0.001	<0.001[b]	<0.001[a]	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001[a]

[a] Analysis performed on log-transformed data

[b] Kruskal-Wallis test

Table 4 Mean values ± standard deviation and analyses of variance of leaf and root (0–50 cm) traits for the twenty cover crop species

		Leaf area (mm²)	SLA (mm² mg⁻¹)	LDMC (mg g⁻¹)	Root length (m)	Root area (cm²)	Root diameter (mm)	Root mass (g)	RLD (cm cm⁻³)	SRL (m g⁻¹)	RTD (g cm⁻³)
b1	Indian mustard	73 ± 27	27.8 ± 1.9	114 ± 1	78.6 ± 2.2	467 ± 8	0.19 ± 0.01	0.89 ± 0.23	6.16 ± 0.17	99 ± 27	0.40 ± 0.09
b2	Turnip rape	185 ± 16	18.7 ± 2.3	79 ± 7	76.6 ± 4.4	483 ± 49	0.20 ± 0.01	1.19 ± 0.27	6.00 ± 0.34	72 ± 12	0.48 ± 0.06
b3	Daikon radish	179 ± 26	22.7 ± 0.4	82 ± 4	47.4 ± 12.3	432 ± 80	0.30 ± 0.03	4.77 ± 1.21	3.71 ± 0.96	11 ± 0	1.52 ± 0.36
b4	Oilseed radish	146 ± 19	21.9 ± 0.9	97 ± 2	73.5 ± 8.7	454 ± 36	0.20 ± 0.01	1.11 ± 0.18	5.76 ± 0.68	74 ± 22	0.51 ± 0.11
b5	White mustard	45 ± 9	33.7 ± 1.2	148 ± 4	45.0 ± 8.8	269 ± 46	0.19 ± 0.01	1.15 ± 0.47	3.52 ± 0.69	47 ± 18	0.89 ± 0.41
f1	Lentil	8 ± 1	36.8 ± 4.1	153 ± 19	44.1 ± 3.6	379 ± 58	0.27 ± 0.04	0.49 ± 0.12	3.46 ± 0.28	93 ± 23	0.19 ± 0.01
f2	Field pea	49 ± 3	44.5 ± 2.6	111 ± 5	30.6 ± 3.3	214 ± 31	0.22 ± 0.02	0.24 ± 0.04	2.40 ± 0.26	138 ± 15	0.19 ± 0.02
f3	Berseem clover	15 ± 1	30.9 ± 0.8	141 ± 3	46.4 ± 4.4	403 ± 42	0.28 ± 0.02	0.81 ± 0.24	3.64 ± 0.35	63 ± 21	0.29 ± 0.05
f4	Faba bean	78 ± 8	32.3 ± 1.4	113 ± 5	38.0 ± 2.3	435 ± 58	0.37 ± 0.05	2.31 ± 0.35	2.98 ± 0.18	18 ± 3	0.62 ± 0.28
f5	Common vetch	25 ± 3	41.1 ± 1.1	129 ± 1	42.0 ± 5.6	335 ± 57	0.25 ± 0.02	0.45 ± 0.12	3.29 ± 0.44	97 ± 21	0.21 ± 0.01
p1	Oat	27 ± 3	28.2 ± 1.1	194 ± 5	94.5 ± 3.3	694 ± 69	0.23 ± 0.02	0.88 ± 0.14	7.41 ± 0.26	114 ± 12	0.22 ± 0.01
p2	Foxtail millet	38 ± 2	28.0 ± 1.0	206 ± 5	59.1 ± 14.4	388 ± 106	0.21 ± 0.01	0.60 ± 0.14	4.63 ± 1.13	99 ± 10	0.31 ± 0.04
p3	Sorghum	95 ± 1	29.9 ± 0.4	201 ± 6	60.2 ± 14.4	532 ± 115	0.28 ± 0.01	1.42 ± 0.15	4.72 ± 1.13	44 ± 15	0.39 ± 0.09
a1	Niger	68 ± 7	38.3 ± 3.5	105 ± 3	61.6 ± 3.3	513 ± 29	0.27 ± 0.00	1.43 ± 0.41	4.83 ± 0.26	45 ± 9	0.42 ± 0.09
a2	Sunflower	118 ± 15	28.9 ± 1.6	120 ± 5	42.5 ± 3.6	391 ± 72	0.29 ± 0.03	2.61 ± 0.81	3.33 ± 0.28	20 ± 6	0.87 ± 0.04
o1	Hemp	39 ± 2	23.2 ± 0.8	243 ± 15	42.3 ± 6.9	342 ± 50	0.26 ± 0.01	1.41 ± 0.56	3.32 ± 0.54	35 ± 15	0.63 ± 0.21
o2	Buckwheat	19 ± 2	38.0 ± 0.5	148 ± 3	45.3 ± 9.8	310 ± 63	0.22 ± 0.01	0.50 ± 0.13	3.55 ± 0.77	92 ± 20	0.30 ± 0.04
o3	Flax	8 ± 1	39.9 ± 0.8	146 ± 6	55.5 ± 15.3	437 ± 138	0.25 ± 0.02	0.56 ± 0.18	4.35 ± 1.20	102 ± 5	0.21 ± 0.05
o4	Phacelia	37 ± 4	30.4 ± 2.9	108 ± 8	67.3 ± 5.0	418 ± 40	0.20 ± 0.01	0.71 ± 0.20	5.27 ± 0.39	105 ± 25	0.35 ± 0.05
o5	Chia	51 ± 7	33.8 ± 1.0	153 ± 7	70.9 ± 6.7	565 ± 59	0.25 ± 0.02	1.85 ± 0.69	5.56 ± 0.53	46 ± 22	0.50 ± 0.13
Mean		65	31.4	140	56.1	423	0.25	1.27	4.39	71	0.47
p value		<0.001[a]	<0.001[a]	<0.001[a]	<0.001	<0.001	<0.001	<0.001[a]	<0.001	<0.001	<0.001[a]

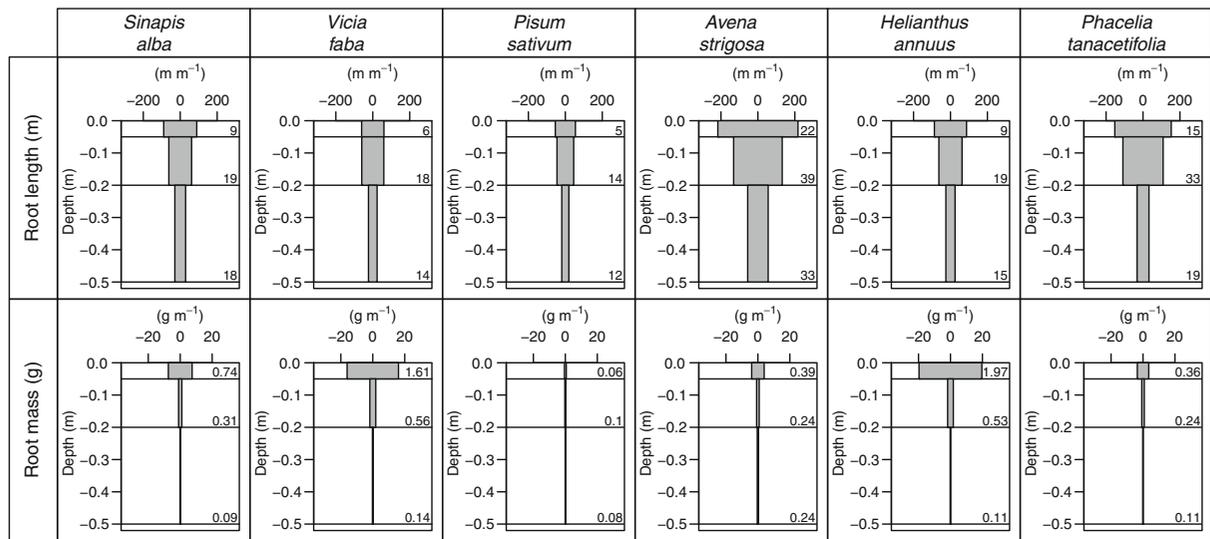[a] Analysis performed on log-transformed data

Fig. 1 Total root length (m) and root mass (g) in the 0–5, 5–20 and 20–50 cm layers of six representative species. The surface of each rectangle corresponds to the value of the respective root trait and can be calculated by the product of rectangle width (x axis) and the soil thickness (m). This value is given on the right side of the rectangle

Significant differences were also observed among species for nutrient concentrations in the roots (Table 5). Fabaceae showed the highest N concentration, with an average of 26.8 g kg^{-1}. For the other species, N concentration varied from 7.4 g kg^{-1} for sunflower, to 20.2 g kg^{-1} for foxtail millet. Particularly high P and K concentrations (5.35 and 39.17 g kg^{-1} of P and K, respectively) were measured for daikon radish. Values were much lower for the other species, ranging from 1.01 g kg^{-1} (sunflower) to 2.97 g kg^{-1} (berseem clover) for P, and from 2.97 g kg^{-1} (hemp) to 12.71 g kg^{-1} (turnip rape) for K. The lowest C/N ratios were observed for the Fabaceae (16 on average). In contrast, Asteraceae species, white mustard, turnip rape and chia showed very high C/N ratio, above 40.

Based on a conservative estimation of root biomass per hectare, significant differences in nutrient accumulation were observed among species (Table 5). Daikon radish accumulated the highest amounts of nutrients in the roots per hectare (65.0 kg ha^{-1} of N, 20.43 kg ha^{-1} of P and 149.5 kg ha^{-1} of K). Faba bean accumulated at least 38.0, 4.36 and 18.9 kg ha^{-1} of N, P and K respectively. The amounts of nutrients stored in the roots were much lower for the other species. N accumulation ranged from 11.6 kg ha^{-1} (field pea) to 40.8 kg ha^{-1} (berseem clover). P accumulation varied from 1.05 kg ha^{-1} (buckwheat) to 5.71 kg ha^{-1} (turnip rape), and K, from 3.0 kg ha^{-1} (buckwheat) to 15.0 kg ha^{-1} (sunflower).

Influence of root and leaf traits on nutrient uptake

In the redundancy analysis, root and leaf traits explained 48 % of the variability in nutrient concentration and biomass production. Each of the two first axis explained 16 %. At the root level, a strong positive correlation was found between root mass and RTD (Fig. 2a). These roots traits were highly negatively correlated to SRL. A strong positive correlation was observed between RLD and root area, which were both negatively linked to average diameter. These traits were not or loosely related to the other root traits. At the leaf level, leaf area was negatively related to the other leaf traits, SLA and LDMC. SLA was only slightly positively correlated to SRL.

N and Mg showed high positive correlation with SRL and SLA. At the opposite, shoot biomass was positively related to RTD, root mass, root and leaf area and RLD. C concentration was positively related to average diameter and LDMC. In contrast, K and Ca were linked to leaf and root area and RLD. P was positively correlated to SRL and independent from RLD and root area.

Five groups could be individuated by the cluster analysis based on the RDA scores (Fig. 2b). The first group ('*biomass*' group), composed of sunflower (a2), faba bean (f4) and white mustard (b5), showed root systems with high mass, dense tissues, large diameter and high root area, and high shoot biomass production. The second group ('*length*' group) was characterized by

Table 5 Mean values of the conservative estimations of root biomass, nutrient concentration, C/N ratio and root nutrient uptake for the twenty cover crop species

		Root biomass (t ha^{-1})	N concentration (g kg^{-1})	P concentration	K concentration	C/N	N uptake (kg ha^{-1})	P uptake	K uptake
b1	Indian mustard	1.47 ± 0.38	12.2	2.25	7.33	37.2	17.9 ± 4.7	3.32 ± 0.86	10.8 ± 2.8
b2	Turnip rape	1.98 ± 0.45	10.6	2.89	12.71	43.0	21.0 ± 4.8	5.71 ± 1.31	25.1 ± 5.7
b3	Daikon radish	3.82 ± 0.97	17.0	5.35	39.17	26.1	65.0 ± 17.0	20.43 ± 5.20	149.5 ± 38.0
b4	Oilseed radish	1.83 ± 0.30	12.1	2.36	10.37	36.9	22.1 ± 3.6	4.32 ± 0.70	19.0 ± 3.1
b5	White mustard	1.90 ± 0.78	8.0	1.10	3.76	58.6	15.2 ± 6.3	2.09 ± 0.86	7.2 ± 2.9
f1	Lentil	0.82 ± 0.20	25.3	2.10	4.83	15.5	20.6 ± 5.0	1.72 ± 0.41	3.9 ± 0.9
f2	Field pea	0.39 ± 0.06	29.6	–	–	13.8	11.6 ± 1.9	–	–
f3	Berseem clover	1.35 ± 0.40	30.3	2.97	6.85	14.6	40.8 ± 12.1	4.00 ± 1.18	9.2 ± 2.7
f4	Faba bean	1.85 ± 0.28	20.6	2.38	10.22	22.5	38.0 ± 6.0	4.36 ± 0.67	18.9 ± 2.9
f5	Common vetch	0.75 ± 0.20	28.1	1.97	4.79	13.4	21.1 ± 5.5	1.48 ± 0.39	3.6 ± 0.9
p1	Oat	1.46 ± 0.23	15.3	1.62	4.86	25.6	22.3 ± 3.5	2.36 ± 0.38	7.1 ± 1.1
p2	Foxtail millet	1.00 ± 0.24	20.2	1.41	3.81	20.2	20.2 ± 4.8	1.41 ± 0.33	3.8 ± 0.9
p3	Sorghum	2.35 ± 0.25	13.4	1.41	4.22	29.4	31.5 ± 3.4	3.31 ± 0.35	9.9 ± 1.1
a1	Niger	2.37 ± 0.68	9.0	1.34	6.23	50.4	21.3 ± 6.1	3.18 ± 0.91	14.8 ± 4.2
a2	Sunflower	2.09 ± 0.65	7.4	1.01	7.17	66.8	16.0 ± 4.8	2.11 ± 0.66	15.0 ± 4.7
o1	Hemp	2.33 ± 0.92	15.7	1.18	2.97	27.7	36.6 ± 14.5	2.75 ± 1.09	6.9 ± 2.7
o2	Buckwheat	0.83 ± 0.21	16.4	1.26	3.64	23.6	13.7 ± 3.4	1.05 ± 0.26	3.0 ± 0.8
o3	Flax	0.92 ± 0.29	15.7	1.70	4.03	27.4	14.4 ± 4.6	1.57 ± 0.50	3.7 ± 1.2
o4	Phacelia	1.18 ± 0.33	14.0	2.69	3.69	31.1	16.6 ± 4.7	3.18 ± 0.90	4.4 ± 1.2
o5	Chia	3.07 ± 1.14	10.3	1.06	3.80	43.1	31.7 ± 11.8	3.25 ± 1.21	11.7 ± 4.3

high RLD and high root and leaf area, and assembled turnip rape (b2), niger (a1) and phacelia (o4). This group presented high P, K and Ca concentration and medium shoot biomass. The third group ('*intermediate*' group) was composed of Indian mustard (b1), oilseed radish (b4), oat (p1), chia (o5) and berseem clover (f3). These species, especially Indian mustard, oilseed radish and oat, presented characteristics of both of the two previous groups: high root and shoot mass and dense tissues combined to high RLD and high root and leaf area. The fourth group ('*diameter*' group) gathered foxtail millet (p2), sorghum (p3), buckwheat (o2) and flax (o3). These species were characterized by large root diameter, high LDMC and low P, K and Ca concentration. The fifth group ('*SLA*' group) comprised lentil (f1) common vetch (f5) and hemp (o1). It presented high SRL, high SLA and high N, P and Mg concentration but low RTD, root mass, RLD and root area. According to the cluster analysis, field pea (f2) did not belong to one of these five groups and formed a single group. It was thus added to the closest group, i.e. the *SLA* group. These groups were confirmed by the univariate analyses

of variance of shoot biomass production (Fig. 3a) and nutrient concentration (Fig. 3b–e). With more than 8 t ha^{-1} on average, the *biomass* group produced significantly more biomass than the *length*, the *diameter* and the *SLA* groups. The *SLA* group showed clearly higher N concentration than the other groups. The highest P concentration was observed in the *SLA* and the *length* group. The *length* group presented also significantly higher K concentration than the *SLA*, *diameter* and *biomass* groups, and significantly higher Ca concentration than all the other groups. No significant difference between the groups was noticed for Mg concentration (not shown).

Accumulation differences were observed among the different groups for P, K and Ca (Fig. 3g–i). Species from the *length* group accumulated significantly higher amounts of these nutrients than the *diameter* and the *SLA* groups. The *biomass* and the *intermediate* groups accumulated as much P and K as the *length* group but less Ca. No significant differences were observed among the five groups for N (Fig. 3f) and Mg (not shown) uptake.
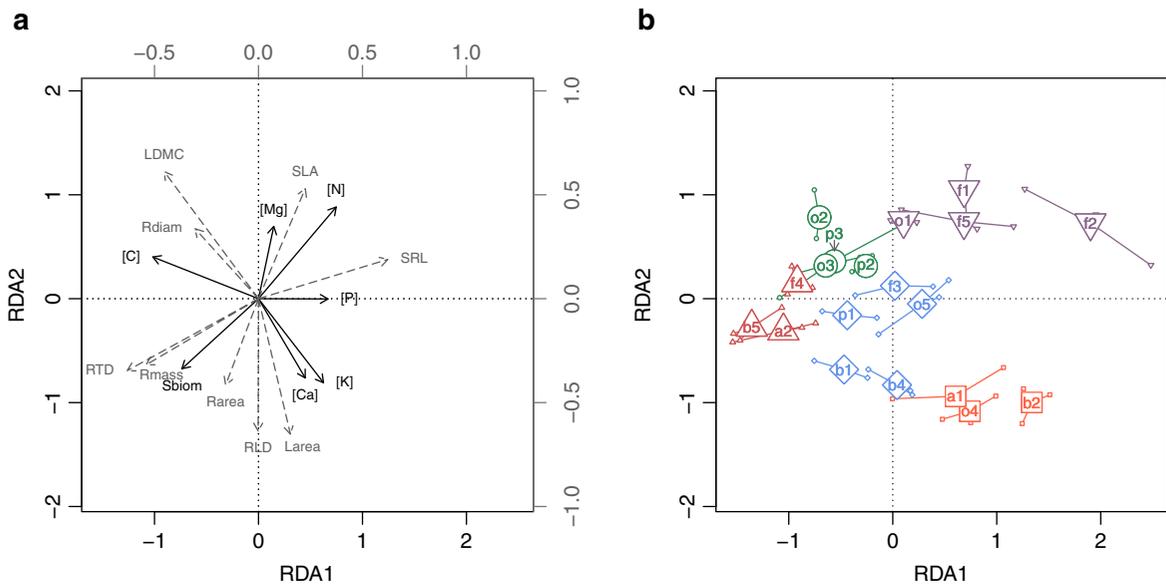
Fig. 2 **a** Redundancy analysis (RDA) between shoot and root traits (explanatory variables, *dotted grey arrows*), and shoot biomass and nutrient concentrations (response variables, *black arrows*). The bottom and left-hand scales are for the response variables, the top and right-hand scales are for the explanatory variables. **b** Species coordinates on the RDA axes. Each point corresponds to a replicate and is related to the centroid of the correspondent species indicated by the species code. Species with similar symbol belong to the same group: Δ = '*biomass*', □='*length*', ◊ = '*intermediate*', o = '*diameter*' and ∇ = '*SLA*'. For species code correspondence, see Table 2. Sbiom = shoot biomass, Larea = leaf area, SLA = specific leaf area, LDMC = leaf dry matter content, Rarea = root area, Rdiam = root diameter, Rmass = root mass, RLD = root length density, SRL = specific root length, RTD = root tissue density

As Fabaceae can access to atmospheric N through biological fixation, the redundancy analysis was redone without the Fabaceae species in order to evidence which are the most relevant traits for soil N acquisition (Online resource 3). A greater proportion of nutrient concentration and shoot biomass variability was explained by the plant traits (53 %). Only the relationships between plant traits, and N, P and Mg concentrations were modified. The positive relationship between SLA and N and Mg concentrations, evidenced for all the species, was no longer valid when the Fabaceae were removed from the analysis. Relationship between RLD and root area, and N, P and Mg concentration was also changed. These root traits were not correlated with N and Mg, and positively correlated with P.

Discussion

Influence of plant traits on nutrient uptake

Numerous studies have been conducted on the shoots of the cover crops (e.g. Brennan and Boyd 2012; Ramirez-Garcia et al. 2014) but only few investigated their root

system despite its importance (e.g. Thorup-Kristensen 2001; Bodner et al. 2013, 2014). In our study, a description of twenty cover crop species was made in terms of leaf and root traits. These plant traits were related to shoot biomass and nutrient concentration. Five nutrient acquisition strategies were delineated on the basis of plant characteristics and patterns of nutrient accumulation. These groups were composed of species from different families, showing that the main driver of this clustering may be shoot and root traits rather than taxonomy.

The *biomass* group (sunflower, faba bean and white mustard) was characterized by high shoot biomass and presented high root mass and high root tissue density, highlighting the importance of a well-developed root system for high shoot production. The parallel development of the above-ground parts and the root system could be explained by the functional co-operation between roots and shoots (Wang et al. 2006). The low specific leaf area, low specific root length, low N concentration and high root tissue density observed in this group are generally found in plants with a resource-conservative strategy, which show also slow tissue turnover and slow short term growth (Wright et al. 2004;
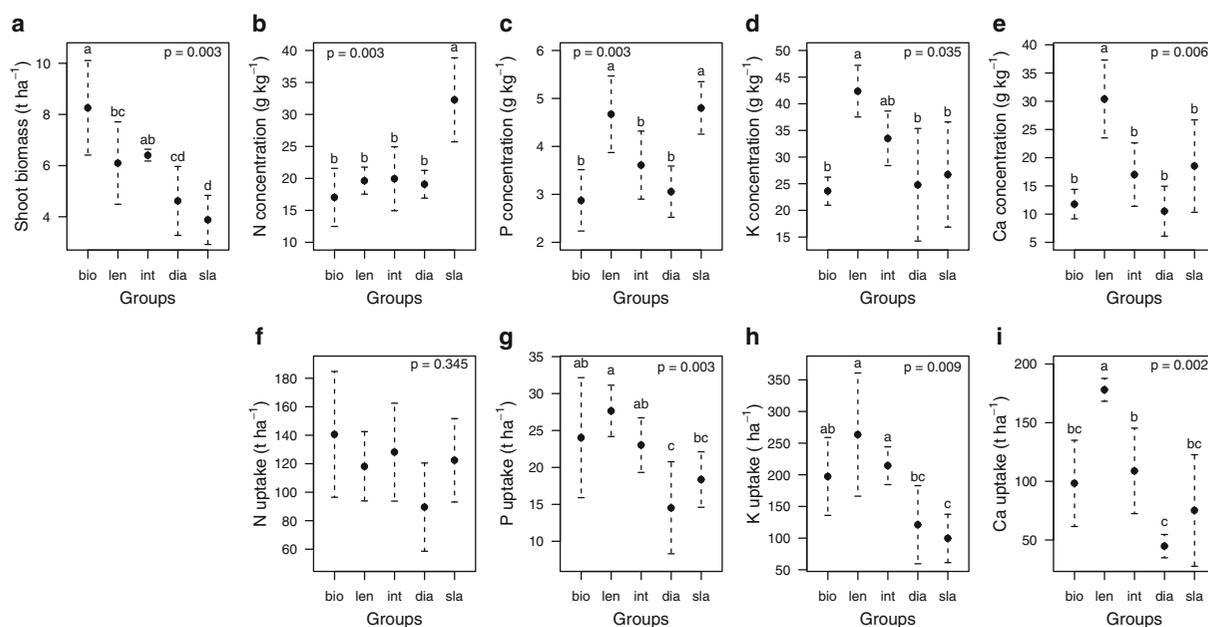
Fig. 3 Mean values and standard deviations of **a.** shoot biomass (t ha^{-1}), **b–e** nutrient concentration (g kg^{-1}) and **f–i** nutrient uptake (t ha^{-1}) of the different groups (bio = 'biomass', len = 'length', int = 'intermediate', dia = 'diameter' and sla = 'SLA'). The results of the analyses of variance are given at the top of the plot and different letters indicate that the groups are significantly different (LSD 5 %)

Tjoelker et al. 2005). For two species (white mustard and sunflower), these characteristics were associated with high C/N ratio and low P concentration that could lead to a slow decomposition of the residues. Nevertheless, despite these rather conservative characteristics, species from this group accumulated large amounts of N, P and K and had the highest shoot biomass production after a short growing period. This is most likely due to the good growing conditions of this experiment, in terms of water and nutrient availability and temperature, which favored quick growth. Also, the set of species studied here includes only species that were selected for fast growing and high nutrient uptake capacity, as expected from cover crops. Thus, even if the *biomass* group showed more conservative characteristics than the other groups, all these species are more acquisitive than wild species (Tribouillois et al. 2015).

The *length* group, which was composed of phacelia, niger and turnip rape showed high root length density and root area. Comparable root length density was observed by Bodner et al. (2013) for phacelia. In our study, these characteristics were related to high P, K and Ca concentrations. Root length density influences the acquisition of nutrients by increasing root area and is especially important for the nutrients available by diffusion such as P and K because of their restricted mobility

(Lynch 2007). In the *length* group, intermediate values of specific leaf area, specific root length, root tissue density and N concentration were observed. Based on the findings of Grassein et al. (2015), species from this group would thus be expected to be more acquisitive or less conservative than the species from the *biomass* group. In comparison to the *biomass* group, the *length* group showed lower C/N ratio and higher P concentration that are more favorable to mineralization. However, we observed that the *length* group accumulated only significantly more Ca, and as much N, P and K as the *biomass* group. As expected the *intermediate* group accumulated as much N, P and K as the *biomass* and the *length* group and showed intermediate C/N ratios and P concentration.

The *diameter* group gathered flax, buckwheat, sorghum and foxtail millet and was characterized by a high root diameter. The main differences with the *length* group were the low root length density and root area, related to low P, K and Ca concentrations. These low concentrations coupled with intermediate shoot biomass produced low nutrient accumulations compared to the other groups. The high root diameter found in this group is generally related to long root life span (Eissenstat et al. 2000), a better resistance to water stress and higher rates of water transport within the root (Cornelissen

et al. 2003). It has been shown by Fort et al. (2013) that high root diameter is associated with high root investment in deep soil layer for grassland species. They have postulated that this allows resource capture from deeper layers. However, in our study, root diameter was not related to root length or root mass in the 20–50 cm layer. Plants have likely concentrated their roots in the first twenty centimeters due to the high nutrient availability in this layer.

The last group (*SLA* group), comprising three Fabaceae species (common vetch, lentil and field pea) and hemp, was characterized by traits typically related to acquisitive strategy: low root mass, low root tissue density, high specific leaf area and high specific root length. Nevertheless, these species did not accumulate more N than the *biomass* or the *length* group, and showed relatively low accumulation of K and Ca. In contrast to the *length* group, they were characterized by low root length and low root area but showed high N and P concentrations. Thanks to biological fixation, Fabaceae species can access atmospheric N. Moreover, it has been shown that in P-depleted soils, they are able to mobilize poorly available P fractions through high phosphatase activity (Nuruzzaman et al. 2006). Thus, Fabaceae species should probably be less dependent on root length to acquire these nutrients, and even in satisfactory nutrient conditions, they should build roots with lower root length than the other species. In the RDA, we observed that plant traits explained more variation of nutrient concentration and shoot biomass when Fabaceae were removed from the analysis. This confirms that Fabaceae were less dependent on these root characteristics for nutrient accumulation in the present experimental conditions.

In this study, five nutrient acquisition strategies were observed. Among these strategies, three groups (*biomass*, *length* and *intermediate*) showed comparable high nutrient accumulation despite different plant traits and patterns of nutrient accumulation. On a short term perspective, the *length* group seems to be more interesting as the accumulated nutrients should be more quickly available through favorable C/N ratio and P concentration.

Root trait relevance for nutrient acquisition depends strongly on nutrient availability of the environment in which plants are grown. In this study, the relationships were defined in satisfactory fertility conditions. In limiting nutrient conditions, other root traits should be relevant for high nutrient uptake. For example, it was evidenced that deeper root systems facilitate nitrate

acquisition due to their high mobility (White et al. 2013). On the contrary, P availability is generally higher in the topsoil due to the input of fertilizer, the higher soil organic matter content and the relatively low mobility of P. Thus, traits associated with topsoil foraging, namely shallower growth of basal roots, adventitious rooting and greater dispersion of lateral roots, are crucial (Lynch 2007). Root hairs will also increase P acquisition capacity by expanding root area (Gahoonia and Nielsen 2004; Zhu et al. 2010). Some species such as buckwheat (Teboh and Franzen 2011) or white lupin (Kamh et al. 1999) are also able to mobilize non-available nutrients, in particular P, through solubilization by root exudates for example. Soil nutrient availability can moreover modify plant root architecture, as plants are able to adapt their root system according to nutrient conditions (López-Bucio et al. 2003). Additional experiments are thus required to determine the relevant traits for nutrient acquisition in nutrient shortage conditions and to determine the species adapted to these conditions.

Nutrient uptake by cover crops

Two ways to achieve high nutrient accumulation are commonly described: producing high shoot biomass, or exhibiting high nutrient concentrations. The calculation of the relative contribution of shoot biomass and nutrient concentration to nutrient uptake variation showed that for N and P, the contribution of biomass variation was the highest. These results revealed that species with the highest biomass production should be favored for the highest accumulations of these nutrients.

The favorable fertility conditions combined to early irrigation and soil tillage, allowed a high biomass production of the cover crops and the accumulation of substantial amounts of nutrients in only 3 months (532 GDD T_{base} = 10 °C). The highest N accumulations were observed in three Fabaceae (berseem clover, faba bean and vetch) with more than 160 kg ha^{-1}. These values are similar to the values reported by Büchi et al. (2015) for a comparable growing period, in the same site. Other species such as phacelia, daikon radish, sunflower and niger managed to accumulate almost as much N as the best performing Fabaceae. All these species accumulated also the highest P amounts, more than 30 kg ha^{-1}, thereby exceeding what is classically found in literature. Eichler-Löbermann et al. (2008) reported that cover crops can take up to 5.5 kg ha^{-1} of P in the aboveground parts, while Liu et al. (2015) showed up to

15 kg ha^{-1} in the whole plant under very good field conditions. These differences were most likely due to variation in biomass production. Indeed, biomass production of phacelia was about 6 t ha^{-1} in our study and barely more than 1 t ha^{-1} with similar (Eichler-Löbermann et al. 2008) or lower (Liu et al. 2015) P concentration. White and Weil (2011) also showed that P accumulation of daikon radish was dependent on biomass production, ranging in their study from 5.9 to 25 kg ha^{-1} of P. Cover crop biomasses observed in this experiment were in the range of biomass measured in other experiments in the same area on several different years. Thus, nutrient uptake measured here are likely representative of favorable cultivation conditions in this region. We observed that cover crops can additionally accumulate high amounts of other nutrients: more than 250 kg ha^{-1} of K, about 200 kg ha^{-1} of Ca and up to 31 kg ha^{-1} of Mg. Few studies have quantified the uptake of K, Ca and Mg in cover crops despite their essential functions for plants, such as stomatal regulation for K, chlorophyll synthesis for Mg and root extension for Ca (Hawkesford et al. 2012). Biomass production and nutrient uptake increased up to the final harvest for all the species, except for buckwheat. This species presents a very short life cycle and was already senescent at the final harvest date. It is thus more adapted to shorter cover cropping situation, e.g. when cover crop sowing is delayed or before an early subsequent crop.

Significant quantities of nutrients are also stored in the roots. The highest accumulations (about 65, 20 and 149 kg ha^{-1} of N, P and K respectively) were observed in daikon radish with 3.8 t ha^{-1} root biomass. These results are higher than what is found in literature most likely due to higher plant development thanks to the favorable growing conditions. White and Weil (2011) observed that root biomass of daikon radish was around 1 t ha^{-1} and root P uptake ranged from 3.6 to 7 kg ha^{-1}. On average, the other species accumulated about 23, 3 and 10 kg ha^{-1} of N, P and K in their roots. In contrast, based on our shoot N uptake and the shoot/root N ratio published by Unkovich and Pate (2000), estimations of root N uptake are higher than that obtained with our conservative estimates. This suggests that our estimates are likely in the range of values generally observed in other studies. It is essential to take into account root uptake for the balance of nutrient cycle in crop rotation.

In contrast to mineral fertilizers, nutrients accumulated by cover crops are released progressively and cover crop mineralization can be coordinated with subsequent crop needs. For this purpose, C/N ratio can be used as an indicator of the dynamics and the rate of mineralization (Justes et al. 2009). With C/N ratio values above 26, it has been shown that a net immobilization of N by soil microorganisms occurs within the first weeks (Justes et al. 2009). Mineralization rate is also reduced and strong pre-emptive competition can occur (Thorup-Kristensen and Dresbøll 2010). At the end of the growing period, while several species, including Fabaceae, exhibited C/N ratios favorable to mineralization, others presented already very high ratios. Nitrogen accumulated in these species will be first immobilized. We observed that generally these species showed also P concentration below the threshold favoring mineralization, which was set at 3 mg g^{-1} of P by Damon et al. (2014). The majority of the species showing high C/N and low P concentration presented more advanced developmental stages. In order, for the main crop, to fully benefit from cover crops' accumulation, the timing of cover crop killing and incorporation is thus crucial (Thorup-Kristensen and Dresbøll 2010; Alonso-Ayuso et al. 2014). When cover crops are cultivated before a winter crop such as winter wheat, cover crop termination date is mostly defined by the crop seeding. When cover crops are cultivated before a spring crop, several options of cover crop killing timing are possible. The killing time influences both the amount of nutrients accumulated and the quality of the plant material. In fact, the C/N ratio increases considerably between the different plant developmental stages. Optimal killing time should thus be defined according to highest nutrient uptake with favorable C/N ratio and P concentration. The objective is to match with the subsequent crop needs and avoid losses and leaching, or regarding N, immobilization. In dry conditions, it is also crucial to decide cover crop killing time in function of soil water content to limit the risk of water competition with the subsequent crop (Alonso-Ayuso et al. 2014). A good management of cover crops should have positive effects on the subsequent crop in the short term, but also in the long term, by improving soil fertility through the input of large amounts of organic matter.

Conclusions

The exhaustive characterization of 20 cover crop species highlighted differences in root and leaf traits and different patterns of nutrient accumulation. Three strategies enabled accumulation of substantial amounts of all the

nutrients in a short period but among these strategies, differences in nutrient release are expected due to different C/N ratio and P concentration. Thus, in high fertility conditions and in a short term perspective, species with high nutrient concentrations and high root length density, would be recommended. In lower fertility conditions, species with other strategies might be more beneficial notably the Fabaceae species, which can access atmospheric N.

The characterization of a large set of species evidenced the possibility to integrate cover crops with high accumulation capacity to improve nutrient use efficiency in agricultural systems and to prevent losses to the environment. The new references on cover crop accumulation help to better integrate cover crops in fertilization plans.

The different root systems observed among species evidenced also the varying contributions that can be expected by cover crops for improving soil quality, for example in terms of below ground carbon inputs or soil structure.

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