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RESEARCH ARTICLE

Provision of contrasted nitrogen-related ecosystem services among grain legumes

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Abstract

Legumes deliver unique functions that are complementary to those of other groups of species (cereals and oil-rich crops), providing many ecosystem services related to nitrogen. However, the choice of grain legumes according to their ability to provide these ecosystem services remains difficult due to a lack of references for a variety of species. During two legume – wheat successions established between 2014 and 2017, five nitrogen pools were measured, and considered as proxies of nitrogen functions supporting ecosystem services and dis-services. Nitrogen pools were analyzed together with several of their explanatory shoot and root characteristics (i.e. plant traits and properties). For the first time, a wide range of grain legumes could be characterized by their contrasted functional profiles relative to nitrogen. For each species, synergies and trade-offs between the different nitrogen functions were highlighted and related to the explanatory plant characteristics. Shoot and root characteristics explained 76.1% of the variability of nitrogen functions among legumes species. Chickpea, common bean, and soybean had high capacity to take up soil nitrogen during their growth cycles, reducing the risk of nitrogen losses after their harvest. These species were characterized by a high root lateral expansion rate and their capacity to invest a large proportion of belowground biomass in nodules. Conversely, common vetch, faba bean, lentil, pea and Narbonne vetch, were less able to take up soil nitrogen, with higher risks of nitrogen losses, but these species induced high amounts of nitrogen in the following wheat crop and were characterized by high crop residue nitrogen concentration. Larger amounts of nitrogen fixed and exported in seeds were measured for species characterized by high shoot dry matter, high nitrogen harvest index, high seed nitrogen concentration, and large seeds. Hence, this study should facilitate the selection of legume species according to the expected objectives.

Keywords Functional profile · Plant traits · Multifunctionality index · Symbiotic nitrogen fixation · Seed nitrogen · Iorganic nitrogen uptake · Nitrogen leaching · Pre-crop nitrogen benefits

1 Introduction

To overcome environmental issues (water soil and air pollution, biodiversity loss, etc.) and resource scarcity in the context of climate change, cropping systems must enable the production of agricultural goods through a better resource use efficiency while minimizing negative impacts on the environments (Tilman et al. 2002).

In the context of agroecological transition, ecosystem services should be maximized to ensure agricultural production while reducing farm inputs (MEA 2005; Power 2010; Tibi and Therond 2017). The provision of ecosystem services relies in particular on the reintroduction of spatial and temporal biodiversity (Isbell et al. 2011) in farming landscapes and in cropping fields. Indeed, species diversity in agricultural ecosystem ensures a variety of ecological functions, resulting from ecological processes, and providing ecosystem services. Synergy between ecological functions induced by different species allows: i) more efficient resource use, ii) the simultaneous provision of a wider range of ecosystem services, and iii) better adaptation to environmental disruptions.

Although legumes deliver unique and complementary ecological functions to those of other groups of species (Peoples et al. 2019) grain and forage legumes only represent 3% of the French agricultural land (Voisin et al. 2014). Consequently, legumes should play a leading role in the provision of ecosystem services relative to nitrogen (N) when reintroduced in

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cropping systems that are mainly based on cereals and oleaginous species. Legumes deliver provisioning services through the production of protein-rich seeds and forages. Legumes also support N supply to crops, thanks to their unique ability to establish a symbiosis with specific soil rhizobia bacteria that are able to fix atmospheric N_2, thus providing N to the plant (Guinet et al. 2018). Moreover, legumes supply N to the following crops of the succession, through the mineralization of their N-rich crop residues (Peoples et al. 2019). However, the introduction of legumes in cropping systems can result in some dis-services, due to soil N losses in the environment by leaching and gas emissions during their growth cycle and after their harvest (Peoples et al. 2019; Plaza-Bonilla et al. 2015). Indeed, more N is left in the soil after legume harvest, due to their lower ability to take up soil N compared to cereals (Hauggaard-Nielsen et al. 2001). Moreover, the desynchronization between N supply to the soil by legume residue mineralization and the N demand of the following crop, can lead to soil N losses during the fallow period. Such soil N losses are highly dependent on i) rainfall amount and distribution during these periods, ii) soil water holding capacity and iii) soil microbial activity (Peoples et al. 2019).

While ecosystem services delivered by legumes have been widely studied, especially in comparison to cereals (Peoples et al. 2019), little is known about the differences that may exist among a wide range of legume species. Given the large diversity of grain legumes, it is important to characterize and distinguish them based on their ability to deliver ecological functions that provide ecosystem services relative to N. Hence, it would facilitate the selection of grain legumes according to the objectives desired (Damour et al. 2014) and carry out appropriate management of N resources in cropping systems that include legumes. Synergies and trade-offs between the different ecological N functions delivered by legumes must be identified to determine the ability of different species to simultaneously provide multiple ecosystem services while limiting dis-services (Bennett et al. 2009; Lescourret et al. 2015).

Nitrogen functions occurring during and after legume growth can be approximated by N pools resulting from N processes integrated over a given period of time. However, the systematic measurement of N pools under a wide range of environmental conditions and diversity of species is costly and time consuming. To overcome these constraints, species can be characterized by traits that determine the effect of plants on ecological functions (Garnier and Navas 2012). Plant traits are commonly defined as "any morphological, physiological or phenological feature measurable at the individual level from the cell to the whole-organism level, without reference to the environment or any other level of organization" (Violle et al. 2007). Based on this approach, species can be characterized and classified as a function of a combination of similar trait attributes (i.e. the specific value of each trait in a given environment and at a given time), which can be considered as predictors of the ecological functions and the resulting ecosystem services or dis-services.

Based on the framework of trait – ecological function – ecosystem services, our objective was to characterize and distinguish ten grain legume species according to their provision of ecosystem services or dis-services relative to N (Fig. 1). A three-step approach was carried out to: i) quantify a set of five N functions (approximated by N pools) delivered by legumes and providing a series of ecosystem services or dis-services; they were measured over a two-year legume – cereal succession, ii) identify and measure explanatory plant characteristics of the different N functions, and iii) establish the functional profile of these ten legume species in order to determine their ability to deliver several simultaneous ecosystem services relative to N.

2 Materials and methods

2.1 Conceptual framework

This study was based on a framework of trait – ecological function – ecosystem services, using well established concepts applied to study agro-ecosystems (Lescourret et al. 2015; Tibi and Therond 2017). Agro-ecosystems can be characterized by their structural and functional components. The structural components include the physical, geochemical (here rainfall, temperature and soil characteristics) and the biological (here legume species identity) compartments. The functional components of agro-ecosystems are the biophysical and biological processes occurring in the ecosystem (here N-cycling processes, i.e. transformation of one form of N into another) occurring in the soil – plant – air continuum during and after legume growth. Legume characteristics that are assumed to influence the intensity of N processes were measured either at the plant level (referred as plant traits; Violle et al. 2007) or at the population level (referred as plant properties; Damour et al. 2014).

Ecological functions are the result of close interactions between natural processes (here N processes) and structural components (here legumes) that support ecosystem services. In this study, ecological N functions, delivered by legumes, were approximated by N pools resulting from N processes integrated over specific periods of time in a two-year legume - cereal succession (e.g. the amount of N fixed over the legume growth cycle, measured in legume shoots at plant physiological maturity). The resulting ecosystem services were classified in four categories as suggested by the MEA (2005): provisioning, regulating, cultural and supporting services. The first three categories directly affect human well-being, while the fourth category maintains the other three. We also considered dis-services, defined as the negative impacts for humans, resulting from ecosystem functioning.

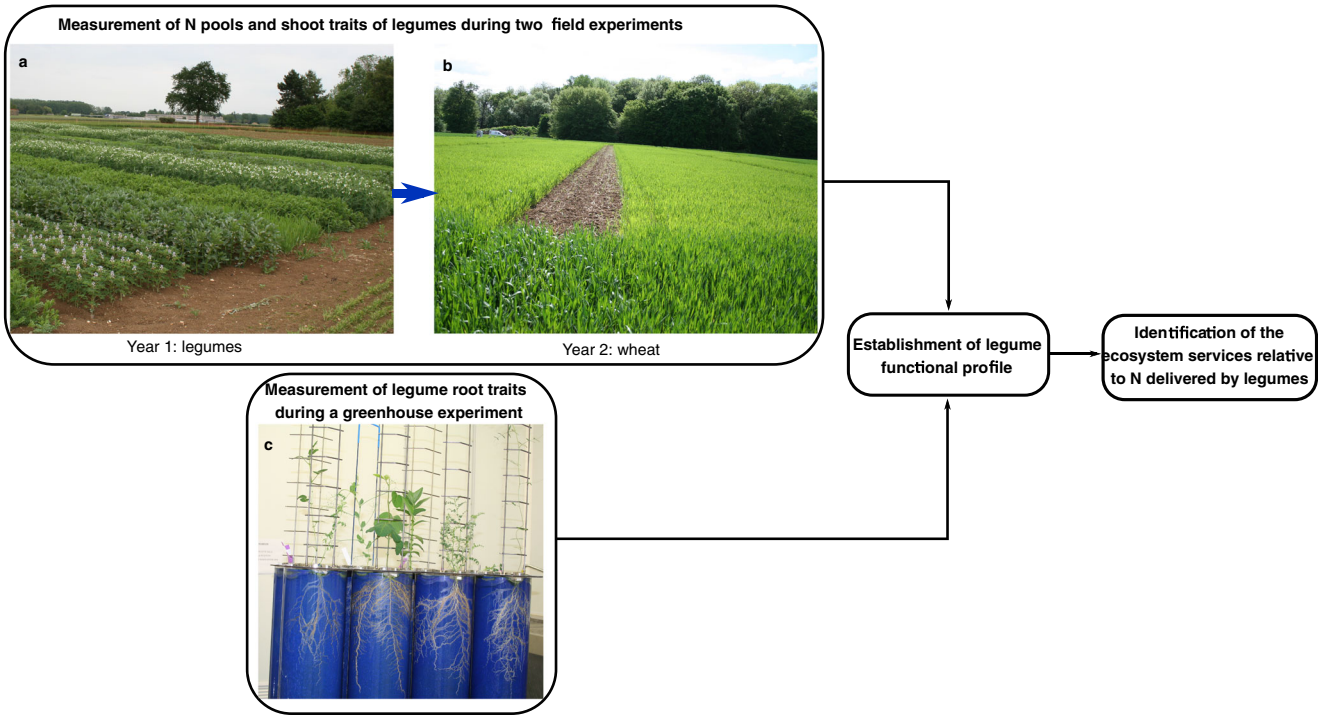


Fig. 1 Conceptual framework diagram. **a** Plots of grain legumes at flowering showing the diversity of shoot characteristics. From left to right: lupin, lentil, common vetch, lupin, fababean, common vetch, pea (© Marlène LEFEBVRE, May 2014). **b** Plots of wheat following legume pre-crops (© Marlène LEFEBVRE, May 2015). **c** Roots of legumes cultivated in rhizotrons (© Maé GUINET, February 2016)

2.2 Experimental set up

2.2.1 Field experiments

Two field experiments, each lasting two years, were carried at the INRAE experimental site of Bretenière (Dijon, France, 47.241 N, 5.115 E, 206 m a.s.l.) in 2014-2015 (Experiment I) and 2016-2017 (Experiment II) in two neighboring fields with very similar soil characteristics. The soil is a Cambisol with a clay surface layer (depth 0.65±0.15 m) developed on an alluvial coarse layer. Soil characteristics are: clay = 469 g kg^{-1}, silt = 471 g kg^{-1}, sand = 60 g kg^{-1}, organic C = 19.3 g kg^{-1}, organic N = 1.75 g kg^{-1}, pH = 7.5, $CaCO_3$ < 1 g kg^{-1}. The site is subject to a semi-continental climate characterized by cold winters (average daily temperature of 3°C and average monthly rainfall of 44 mm) and hot summers (average daily temperature of 19°C and average monthly rainfall of 73 mm). The first years of each experiment (2014 and 2016) were characterized by contrasted rainfall distribution during the year. Between March and June, rainfall was unusually low in 2014 (102 mm) and unusually high in 2016 (399 mm), compared to the mean rainfall over the last 20 years during the same period (255 mm). In contrast, between July and November, rainfall was unusually high in 2014 (541 mm) compared to 2016 (303 mm) and to the mean rainfall over the last 20 years during the same period (362 mm).

During the first year (2014 and 2016), nine grain legumes were sown either in March (*Vicia sativa* L., 1753: common vetch, *Vicia faba* L., 1753: faba bean, *Lens culinaris* (L.) Coss & Germ., 1845 subsp. *lens*: lentil, *Lupinus albus* L., 1753: lupin and *Pisium sativum* L., 1753: pea) or in May (*Cicer arietinum* L., 1753: chickpea, *Phaseolus vulgaris* L., 1753: common bean, *Vicia narbonensis* L., 1753: Narbonne vetch, and *Glycine max* (L.) Merr., 1917: soybean), based on the physiological requirements of each species. In 2016, fenugreek (*Trigonalla foenum-gracum* L., 1753) was also sown in March. One cultivar per species was chosen as it was the most cultivated cultivar in France and/or the most adapted to the climatic conditions in Burgundy (Table 1). At sowing, inorganic N contents in the upper 60 cm of the soil were 69 kg N ha^{-1} in March 2014, 84 kg N ha^{-1} in May 2014, 94 kg N ha^{-1} in March 2016 and 115 kg N ha^{-1} in May 2016. Seeds of the ten legumes were inoculated upon sowing, with species-specific strains of N_2-fixing bacteria, with at least 10^5 and 10^6 viable rhizobia per seed, for small and large seeded legumes, respectively. The liquid inoculant was mixed with adhesive carrier (contained in the FORCE 48 packaging, BASF) to ensure the inoculant stuck to the seeds. The rhizobia strains were known or previously tested in the greenhouse to ensure symbiotic N_2 fixation (Table 1). These strains were obtained from the "Agro-environmental microorganisms of interest" collection maintained at UMR Agroecologie, INRAE, Dijon. Further information on the

Table 1 Cultivar, sowing density, row spacing and Rhizobium strain, for the ten legume crops and two cereals

Pre-crop species	Cultivar	Sowing density (seeds m^{-2})	Row spacing (cm)	Rhizobium strain
Common vetch	Candy	100	20	*Rhizobium leguminosarum* bv. *Viciae MIAE01212 (MSDJ469, P221)*
Faba bean	Espresso	45	25	*Rhizobium leguminosarum* bv. *Viciae MIAE01211 (MSDJ822, FH34)*
Fenugreek	Fenu-fix	180	16	*Ensifer* sp. (*Trigonella*) *MIAE06333 (MSDJ3531)*
Lentil	Anicia	250	20	*Rhizobium leguminosarum* bv. *Viciae MIAE01212 (MSDJ469, P221)*
Lupin	Feodora	70	25	*Bradyrhizobium* sp. (*Lupinus*) *MIAE00428 (MSDJ718, LL13)*
Pea	Kayanne	80	25	*Rhizobium leguminosarum* bv. *Viciae MIAE01212 (MSDJ469, P221)*
Barley	Irina	350	15	none
Chickpea	Twist / Vulcano	70 / 55	20/20	*Mesorhizobium* sp. (*Cicer*) *MIAE04996 (MSDJ2193, 3HOa8)*
Common bean	Flavert	30	25	*Rhizobium etli MIAE05216 (MSDJ2414, GD164)*
Narbonne vetch	Clara	35	20	*Rhizobium leguminosarum* bv. *Viciae MIAE01212 (MSDJ469, P221)*
Soybean	Sultana (000)	70	25	*Bradyrhizobium diazoefficiens MIAE00426 (MSDJ1996, G49)*
Sorghum	Québec	30	35	none

references of the rhizobia strains can be found in Guinet et al. (2018).

To estimate symbiotic N_2 fixation, the ^{15}N isotope dilution method was used with unfertilized N cereals as reference non-fixing crops: barley (*Hordeum vulgare* L., 1753; for legumes sown in March) and sorghum (*Sorghum bicolor* (L.) Moench, 1794; for legumes sown in May). To this end, 5 kg N ha^{-1} of NH_4NO_3 labeled with 1% ^{15}N was dissolved in water and spread on the soil at a rate of 300 L ha^{-1} at legumes and cereals sowing. Considering the very small amount of N applied, this treatment is hereafter referred to as the unfertilized treatment. An additional N treatment was applied to both cereals (barley and sorghum) to compare the N functions delivered by N-fertilized cereals with those delivered by legumes. Nitrogen fertilization was applied as solid NH_4NO_3 at rates of 60 and 75 kg N ha^{-1} in 2014 (3rd and 28th April, respectively) for barley. In 2016, due to the extremely wet conditions in April and May, only 70 kg N ha^{-1} were applied on barley on the 19th of April to reduce the risk of N leaching. N fertilizer was applied twice during sorghum growth at rates of 50 kg N ha^{-1} each in 2014 (9th May and 13th June) and 2016 (7th June and 7th July). Legumes and cereals were cultivated on 1.5 m wide and 12 m long plots with four replicates for each species and N fertilization rates for cereals (i.e. 4 replicates for each N fertilized and unfertilized cereals) in a fully randomized design. Each plot was surrounded by a 25 cm alley to limit inter-plot hedge effect. Sowing density and row spacing of each crop can be found in Table 1.

After harvesting the seeds of legumes and N-fertilized cereals, residues were chopped and incorporated into the soil. No seeds were harvested for Narbonne vetch in 2014 and 2016 and for chickpea in 2016, due to climatic conditions that were unsuitable for seed production for these two species. In these cases, whole plants were considered as shoot residues and are referred to as 'cover-crops'(cc) hereafter. Winter wheat (*Triticum aestivum* L., 1753; cv. Rubisko; 350 seeds m^{-2}; 16 cm row spacing) was sown in October as a following crop for each of the legume and N-fertilized cereal pre-crops, and was not supplied with N fertilizer. Wheat was then harvested in July 2015 and 2017, respectively.

2.2.2 Greenhouse experiment

A greenhouse experiment was carried out in 2016 at the Plant Phenotyping Platform for Plant and Microorganism Interaction (4PMI) at INRAE Dijon (France) to measure root traits of the same ten grain legumes, using the same rhizobia strains as in the field experiments. Legumes (4 replicates per species) were cultivated in cylindrical RhizoTubes©, measuring 49.5 cm high and 18 cm diameter, in which roots were confined in a two-dimension zone between an inner permeable membrane and an outer transparent tube to enable root phenotyping (Jeudy et al. 2016). The core of the RhizoTubes© was filled with an inert substrate (i.e. 60% attapulgite and 40% clay pebbles). The liquid inoculant was applied at root emergence (3 days after sowing) with at least 10^5 and 10^6 viable rhizobia per seed, for small and large seeded legumes, respectively. Plants were watered three times a day with 50 mL of nutrient solution the first six days and five times a day with 150 mL for the rest of the experiment. The nutrient solution (0.80 mM K_2HPO_4, 1.00 mM $MgSO_4$, 2.5 mM $CaCl_2$, 0.7 mM K_2SO_4, 0.20 mM NaCl, 50 μM iron Fe III-(EDTA), 32 μM H_3BO_3, 10 μM $MnSO_4$, 0.77 μM $ZnSO_4$, 0.15 μM $H_{24}N_6O_{24}Mo_7$ and 0.32 μM CuSO4) contained only traces of N, to ensure symbiotic N_2 fixation was the only N source. During the 16 h photoperiod, plants were continuously illuminated with a lower threshold of

300 μmol m^{-2} s^{-1} provided by supplemental illumination using 400 W lamps (HPS Plantastar, OSRAM, Munich, Germany) when incident solar radiation dropped below 300 W m^{-2}. Mean day and night temperatures were 22±1 °C and 19±1 °C, respectively.

2.2.3 Sampling

In the first year of each field experiment (2014 and 2016), shoot samples of legume and cereal (in both N-fertilized and unfertilized treatments) were collected at physiological maturity, in the central rows of the plot, on subplots of 1 m^2 to avoid hedge effect. After this stage, no more dry matter accumulation occurs in the seed and seed water content is below 55% and 35% for legumes and cereals, respectively. After separating seed and straw, samples were oven-dried for 48 h at 80°C to determine dry matter. Samples were milled and N concentration and ^{15}N enrichment were measured using a mass spectrometer combined with an elemental analyzer (ANCA-GLS, Sercon Ltd., Crewe, UK). For both years, the cellulose and soluble compound concentrations of shoot residues (i.e. straw) were determined using a standard procedure (AFNOR 2018) derived from the method proposed by Van Soest (1963).

In the years following legumes and N-fertilized cereals (2015 and 2017), wheat shoots were sampled at physiological maturity on subplots of 1 m^2 to determine shoot dry matter and N concentration, using the same analysis method as previously described.

During the greenhouse experiment, root images were taken five times a week to monitor root expansion. Maximum root lateral expansion was measured using Matlab (2015), and lateral expansion rate (Ler) against time was calculated by linear regression analysis. Legume plants were harvested 27 days after sowing (late vegetative stage). Shoots, roots and nodules were separated and oven-dried to determine the dry matter of the different organs.

2.3 N pools

A set of five N pools for the ten legumes and the two N-fertilized cereals (i.e. proxy of N functions) was determined during the two field experiments (Table 2). Cereals were used as a baseline to contrast the range of values measured for legumes with those measured for cereals cultivated under the same pedoclimatic conditions. All N pools were calculated in kg N ha^{-1}:

- The total amount of N in legume and cereal seeds (Nseed), characterizes the provisioning service, through the process of N accumulation in seeds. In 2014 and 2016, Nseed was calculated by multiplying seed dry matter by seed N concentration, both measured at legume and cereal physiological maturity;
- The total amount of N in legume shoots derived from the air (Ndfa) over the legume growth cycle underpins the supporting service of N supply to legumes, through the symbiotic N_2 fixation process. In 2014 and 2016, Ndfa was calculated at legume physiological maturity by multiplying the total amount of N in shoots by the percentage of N derived from the air (%Ndfa). For each legume, %Ndfa was calculated by isotopic dilution, using the following equation (1) (Rennie and Rennie 1983):

$$\%Ndfa = 100 \times \frac{\delta^{15}\text{N non fixing reference crop} - \delta^{15}\text{N legume}}{\delta^{15}\text{N non fixing reference crop} - B} \quad (1)$$

where $\delta^{15}N$ legume is shoot ^{15}N enrichment of the legume crop, $\delta^{15}N$ non-fixing reference crop is that of barley or sorghum in the unfertilized N treatment; and B is the isotopic fractionation factor associated with symbiotic N_2 fixation processes. Reported observed values range from -0.5 to -1.5, varying with species, plant age and growing stage, (Unkovich and Pate 2000). Due to ^{15}N supply in our experiment, $\delta^{15}N$ enrichment of the soil was increased in average to 41.3, as measured by δ^{15} N of non-fixing reference crop at maturity. Therefore, compared to soil $\delta^{15}N$ enrichment, *B* value can be neglected in equation (1). It was thus set to zero, as suggested by Rennie and Rennie (1983) when using the isotope dilution method. Stabilization of soil $\delta^{15}N$ over time was assessed by collecting shoot samples of the reference cereals (barley and sorghum) five times over their growth cycle.

For the two N fertilized cereals, Ndfa was set to zero as cereals have no access to atmospheric N_2.

- The total amount of N in wheat shoots (Nwheat) cultivated after legumes and cereals characterizes the supporting service of N supply to the following crop by legume and cereal pre-crops. The total amount of N in the following wheat does not directly provide the amount of N derived from pre-crop residue mineralization process. However, as wheat was not N fertilized, the differences in the amounts of N in wheat shoots between pre-crop treatments (ten legumes and two cereals) partially revealed differences in the residue N mineralization among pre-crops. The total amount of N accumulated in wheat over its growth cycle was calculated by multiplying the amount of shoot dry matter by the shoot N concentration measured at wheat physiological maturity in 2015 and 2017;
- The total amount of N in legume and cereal shoots derived from the soil (Ndfsoil), characterizes the ability of legumes and cereals to take up soil N during their cycle. Ndfsoil was considered as an indicator of the amount of inorganic N that was not lost by leaching or gas emissions

Table 2 Ecosystem services–ecological function–plant trait framework applied for the comparison of ten grain legumes

Ecosystem services and dis-services	N pools (indicator of the ecological functions)	Functions	Process	Plant traits or plant properties
Provisioning service				
Production of rich-N seeds	Amount of N in legume seed (Nseed)	Produce rich-N seeds	Accumulation of N in seeds	Nitrogen Harvest Index (NHI) Seed Nitrogen Concentration (SNC) mean Seed Weight (mSW) number of Seeds (S_numb)
Supporting services of N supply to crops				
N supply to legumes	Amount of N in legume shoots derived from the air (Ndfa)	Supply N to the system	Symbiotic N_2 fixation	shoot Dry Matter (DMs) belowground Nodule Mass Fraction (NMFb)
N supply to the following crop	Amount of N in the shoots of the following wheat (Nwheat)	Increase the amount of N available for the following wheat	N mineralization of legume residues	Residue Nitrogen concentration (Res_Nc) Residue Cellulose concentration (Res_CEL) Residue Soluble Compounds concentration (Res_SC)
Regulating service of water quality towards nitrates				
Reduction of water contamination by nitrates during legume cycle	Amount of N in legume shoots derived from the soil (Ndfsoil)	Retain inorganic N in the soil-plant continuum	Soil inorganic N uptake	root Lateral Expansion Rate (Ler) Root Mass Fraction (RMF)
Regulating service of climate change mitigation				
Reduction of nitrous oxide emissions during legume cycle	Amount of N in legume shoots derived from the soil (Ndfsoil)	Retain inorganic N in the soil-plant continuum	Soil inorganic N uptake	root Lateral Expansion Rate (Ler) Root Mass Fraction (RMF)
Dis-service of water pollution by nitrates				
Water pollution by N leaching after legume crops	Amount of N leached after legume harvest (NLeached)	Generate N losses outside the agricultural ecosystem	Soil inorganic N leaching	Residue Nitrogen concentration (Res_Nc) Residue Cellulose concentration (Res_CEL) Residue Soluble Compound concentration (Res_SC) root Lateral Expansion Rate (Ler) Root Mass Fraction (RMF)

during the legume and cereal cycle, thus contributing to the regulating services of water quality towards nitrates and climate change mitigation. It was assumed that higher was the amount of N left in the soil after legume harvest (i.e. low ability of crop to uptake soil N), higher was the risk of N losses under rainy conditions. Ndfsoil was calculated at legume physiological maturity in 2014 and 2016, by subtracting the amount of N in legume shoots derived from the air (Ndfa) from the total amount of N in legume shoots. For N fertilized cereals, the amount of N derived from the soil (Ndfsoil) was distinguished from the amount of N derived from the fertilizer (Ndffertilizer). For both years (2014 and 2016), at cereal physiological maturity the amount of N in unfertilized cereals was subtracted from the total amount of N of the same cereal fertilized with N, in order to estimate the amount of N derived from the fertilizer. The amount of N derived from the soil was assumed to be similar between the N-fertilized cereal and the unfertilized cereal. For legumes, Ndffertilizer was set to zero as no N fertilization was applied.

– The total amount of N leached between the legume or the cereal pre-crop harvest and the wheat harvest (NLeached) is an indicator of the potential dis-service of water pollution by nitrates. The amount of N leached was simulated with the agronomic model STICS (Brisson et al. 2003) for the ten legume and the two cereal pre-crops for both experiments I (2014-2015) and II (2016-2017). A complete description of the model initialization and the simulations is available in Guinet (2019).

2.4 Explanatory plant traits and plant properties

Several plant traits and plant properties considered as explanatory for the different N functions considered here were measured for the ten grain legumes during the two field experiments and the greenhouse experiment (Table 2).

2.4.1 Explanatory plant properties of N accumulation in legume seeds

The total amount of N in legume seeds varies according to seed mass and seed N content. Two plant properties were considered as explanatory for seed mass: i) the number of seeds per square meter (S_num), and ii) mean seed weight (mSW). Two additional plant properties were considered as explanatory for the plant investment of N in seeds: i) seed nitrogen concentration (SNC) and ii) nitrogen harvest index (NHI), calculated as the ratio between the total amount of N in seeds and the total amount of N in shoots (straw + seed).

2.4.2 Explanatory plant traits and properties of N supply to crops

The N requirement for growth is considered as one of the main drivers of the amount of atmospheric N_2 fixed by legumes (Anglade et al. 2015). Thus, shoot dry matter (DMs) was measured at legume physiological maturity to characterize legume crop N requirements for growth. In addition, the belowground nodule mass fraction (NMFb), calculated as the ratio between nodule biomass and belowground biomass (nodule + root), was measured on plants cultivated in RhizoTubes©. This latter trait characterizes the tradeoff in biomass distribution between the two organs that enable the acquisition of N by legumes: nodules (for symbiotic N_2 fixation) and roots (for soil N uptake). Root traits values can be found in Guinet et al. (2018).

Residue N mineralization is considered an important process for explaining differences in the total amount of N in shoots of wheat cultivated after the ten grain legumes. Many studies have highlighted the effect of residue biochemical characteristics on residue N mineralization (Kumar and Goh 2003) Three biochemical characteristics were selected to explain the differences in legume residue N mineralization and consequently the amount of N in the following wheat: i) residue nitrogen concentration (Res_Nc), ii) residue cellulose concentration (Res_CEL) and iii) residue soluble compound concentration (Res_SC).

2.4.3 Explanatory plant traits of N losses mitigation

Soil N uptake by legumes modulates the amount of N left in the soil and potentially lost by leaching or gas emissions. The amount of soil N taken up by legumes varies according to the ability of the root system to explore the soil. Root lateral expansion rate (Ler) has been highlighted as a determinant for explaining the differences in soil N uptake within a wide range of legume species (Guinet et al. 2018). Root mass fraction (RMF), calculated as the ratio between root biomass and the total plant biomass, characterizes the investment of plant biomass in roots (i.e. the organs enabling soil N uptake). Both latter traits were measured on plants cultivated in RhizoTubes© because of the difficulty to measure these traits in field experiments.

2.4.4 Explanatory plant traits and properties of N leaching

The amount of N leached after legume harvest depends on the amount of inorganic N in the soil after harvest, which is potentially modulated by the ability of legumes to take up soil inorganic N, and by legume residue N mineralization. Thus, plant traits and plant properties affecting soil N uptake by legumes and residue N mineralization were considered as

explanatory plant traits of the amount of N leached after legume harvest (section 2.4.2 and 2.4.3).

2.5 Calculation of multifunctionality

The multiple threshold approach suggested by Byrnes et al. (2014) was applied to assess the multifunctionality of the ten grain legumes using the five N pools described above (Nseed, Ndfa, Nwheat, Ndfsoil, Nleached). The objective of this approach is to provide an index for each legume species, to assess their ability to deliver multiple ecosystem services relative to N.

First, values of Nleached were transformed using equation 2 to capture the desired direction of the effect (i.e. high transformed values indicate low N losses by leaching).

$$\text{Nleached_r}\ i,x = -\text{Nleached}\ i,x + \max(\text{Nleached}\ x) \qquad (2)$$

where Nleached i,x is the amount of N leached between the harvest of legume i and the harvest of the following wheat in experiment x (Experiment I or II); max(Nleached x) is the maximal amount of N leached considering all legume pre-crops in experiment x (i.e. 123 kg N ha^{-1} after the common vetch harvest in experiment I and 49 kg N ha^{-1} after the common vetch harvest in experiment II).

The multifunctionality index was calculated at different thresholds, ranging from 5 to 100% of the maximum values observed for each N pool. The maximum value was the mean of the nine highest observations for each N pool across all legume treatments and experiments. The multifunctionality index corresponds to the number of N pools that simultaneously exceed a given threshold. For example, a treatment would receive a multifunctionality score of 5 for the 50% threshold if Nseed was higher than 156 kg N ha^{-1}, Ndfa was higher than 156 kg N ha^{-1}, Nwheat was higher than 47 kg N ha^{-1}, Ndfsoil was higher than 43 kg N ha^{-1}, and Nleached_r was higher than 34 kg N ha^{-1} (Fig. 2). For each legume species, the multifunctionality index was calculated without distinguishing the two field experiments. Since chickpea had no seed production in 2014 whereas it did in 2016, the multifunctional index was calculated separately for both experiments.

2.6 Contribution of legumes to soil N

The potential benefit of grain legumes for soil N (i.e., the net N balance) is calculated as the difference between the amount of N derived from the air (Ndfa) and the amount of N in legume seed (Nseed) (Evans et al. 2001). A positive net N balance indicates an input of N to the soil due to the symbiotic N_2

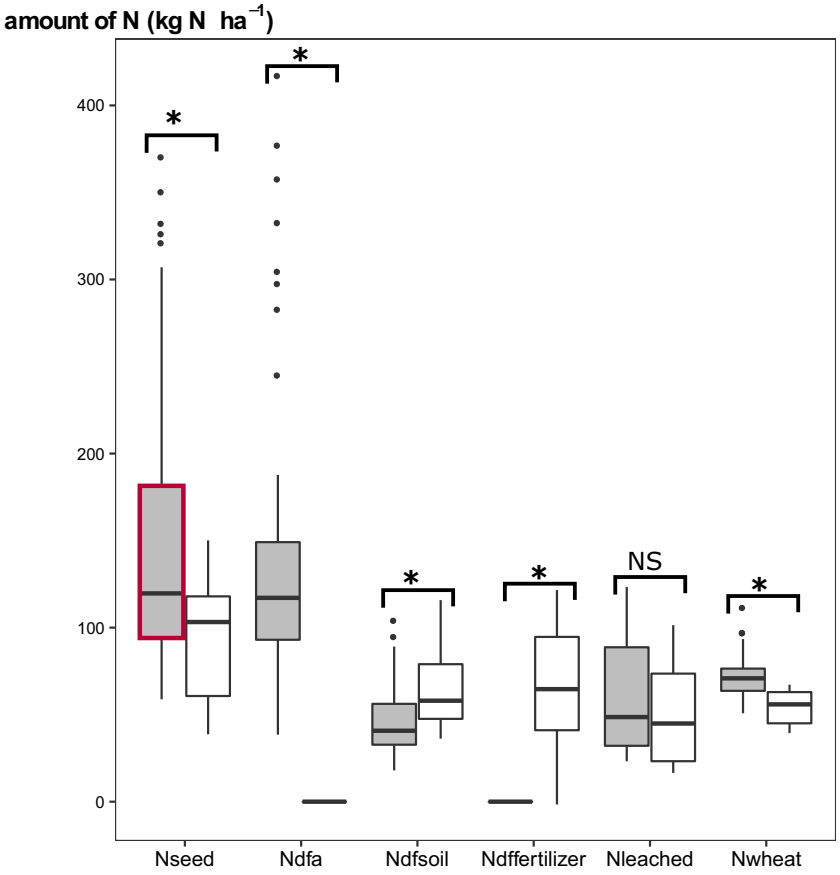


Fig. 2 N pool ranges for legumes (gray) and N-fertilized cereals (white), measured during two field experiments lasting 2 years. Amount of N in seed (Nseed); amount of N in shoots derived from the air (Ndfa); amount of N in shoots derived from the soil (Ndfsoil); amount of N in shoots derived from the fertilizer (Ndffertilizer); amount of inorganic N leached between the pre-crop harvest and the harvest of the following wheat (NLeached); amount of N in the shoots of the following wheat (Nwheat). The red edges of the boxplot indicate that only legumes with seed production were considered. For Nseed, Ndfsoil, Nleached, and Nwheat, $*p < 0.001$ indicates a significant difference between legumes and cereals, and for Ndfa and Ndffertilizer a significant difference with zero

fixation process. The net N balance was calculated in 2014 and 2016 for each of the ten grain legumes.

2.7 Data analysis

All calculations and data analyses were performed with R version 3.5.1. (R Development Core Team 2016).

Each N pool was regressed against all crop species (both legumes and N-fertilized cereals) to ensure the precise estimation of the performance of each crop and meet assumptions of linear regression. Custom contrasts were adjusted with the {emmeans} package version 1.3.4 to test two hypotheses: (i) all crop species show similar performance and (ii) the average performance of all legume species is similar to the average performance of N-fertilized cereal species. Cereals were removed from the dataset for the analysis of Ndfa, since N_2 fixation is not relevant for cereals (i.e., equal to zero, no variability). Likewise, legumes were removed from the dataset for the analysis of Ndffertilizer because no N fertilization was applied on legumes. In these two cases, the average performances of legume and cereal crops were simply compared with zero.

Two-way analysis of variance (ANOVA) was performed to test for year, legume species, and year × legume species interaction effects on each of the five N pools. Normality and homoscedasticity assumptions were visually assessed through quantile-quantile (QQ) and residual vs. fitted plots, respectively. If the effects were significant, contrasts among pre-crops were adjusted using the package {emmeans}version 1.3.4.

The multifunctionality index was calculated using the "getFuncsMaxed" function from the {multifunc} package developed by Byrnes et al. (2014).

A redundancy analysis was performed to determine the synergy and trade-off between N functions (approximated by N pools) and identify plant traits and properties that best explained the variability of N functions among the ten grain legume species. N functions were considered as the response variables while plant traits and plant properties were considered as the explanatory variables. The net N balance was added in the RDA as a supplementary variable. The effect of plant traits and plant properties on N functions was tested using a Monte Carlos permutation test (999 permutations). The RDA was performed using Canoco v. 5.10 (Šmilaueur and Lepš 2014) on the variables standardized beforehand.

3 Results and Discussion

3.1 N pool variability

In comparison with N-fertilized cereals, on average legumes exported 55% more N in seeds (Nseed; contrast on legumes–cereals, estimate = 51.5, SE = 12.9, df = 69, t-ratio = 3.99, $p < 0.001$) and led to 30% more N in the following wheat (Nwheat; contrast on legumes–cereals, estimate = 16.3, SE = 2.29, df = 79, t-ratio = 7.12, $p < 0.001$; Fig. 2). Conversely, soil N uptake by legumes was 27% less than by cereals (contrast on legumes–cereals, estimate = − 17.7, SE = 3.08, df = 79, t-ratio = − 5.74, $p < 0.001$). Legumes can also be distinguished from cereals according to their N nutrition. In addition to soil N uptake, N requirements are either fulfilled by symbiotic N_2 fixation for legumes or by N fertilizer uptake for cereals. No significant differences were observed regarding N leaching occurring after legume or cereal pre-crops. These results confirm the ecosystem services relative to N provided by legumes in comparison to cereals (production of N-rich seed and N supply to the following crops). However, they also highlight the potential higher risk of N losses after legumes due to their lower ability to take up soil N (even if in this study, no significant differences in N leaching between legumes and cereals were observed).

Although general differences between legumes and cereals could be determined, considerable variability among legume species and years was also highlighted for all five N pools (Fig. 2; Table 3). The highest variability was observed for the amount of N exported in legume seeds (Nseed) and for the amount of N in legume shoots derived from the air (Ndfa). For both N pools, the highest values were measured for soybean in 2014 and 2016 and for faba bean in 2016 while the lowest values were measured both years for Narbonne vetch, common bean, common vetch, and lentil. The amount of soil N uptake was significantly higher for soybean and common bean for both years, and for chickpea in 2016 compared with all other legume species (on average 73 vs 38 kg N ha^{-1}). The amount of N leached after legume harvest was only significantly different between legumes in 2014 with common vetch, lentil, faba bean, lupin, and pea having the highest values. In 2014, the amounts of N lost by leaching were much higher compared to 2016 because of unusually high rainfall between July and November which might have exacerbated the differences between legumes. Unlike 2015, significant differences between species were highlighted for the amount of N in the following wheat in 2017. Faba bean and Narbonne vetch induced the highest amounts of N in wheat in contrast to soybean and chickpea.

Thereafter, our study focused on the ten legume species in order to (i) determine synergies and tradeoffs among the five N functions (approximated by N pools) and (ii) establish and compare the functional profiles of the ten legume species.

3.2 Explanatory plant traits and plant properties of the N functions

Plant traits and plant properties explained 76.1% of the N pool variability. On the first two axes of the RDA, 66.1% of the N pools variability was explained by plant traits and plant

Table 3 N pools as affected by legume species and years. Values are mean ($n = 4$). Lowercase letters refer to contrasts between pre-crops. Mean value sharing the same letter ('a' and/or 'b') are not different according to Tukey's test (ns: non-significant, *$p < 0.05$, **$p < 0.01$, ***$p < 0.001$)

	Nseed		Ndfa		Ndfsoil		Nleached		Nwheat	
	(kg N ha^{-1})									
	2014	2016	2014	2016	2014	2016	2014	2016	2015	2017
Chickpea		167 c	117 bc	140 bc	40 b	84 a	59 b	23	64	64 c
Common bean	126 c	99 d	90 cd	81 de	69 a	64 ab	60 b	31	60	67 bc
Common vetch	91 c	62 d	100 cd	87 de	43 b	27 d	123 a	49	67	79 abc
Fababean	116 c	316 a	129 bc	364 a	45 b	58 bc	103 a	28	78	84 ab
Fenugreek		93 d		119 cd		29 d		30		70 bc
Lentil	93 c	93 d	99 cd	123 cd	39 b	29 d	122 a	32	77	75 abc
Lupin	168 b	177 bc	147 b	174 b	38 b	39 cd	91 a	35	74	65 bc
Narbonne vetch			60 d	72 e	30 b	39 cd	77 ab	44	76	93 a
Pea	130 bc	88 d	112 bc	113 cde	44 b	31 d	89 a	45	70	66 bc
Soybean	327 a	216 b	290 a	172 b	67 a	82 a	48 b	34	67	61 c
Species	***		***		***		***		***	
Year	ns		***		ns		ns		***	
Species * year	***		***		**		*		***	

properties (47.3% and 18.8% on the first and second axes, respectively; Fig. 3a). Much of the N pool variability was explained by shoot dry matter (DMs: 38.4%, $p = 0.001$) while belowground nodule mass fraction (NMFb: 8.4%, $p = 0.001$), seed nitrogen concentration (SNC: 8.3%, $p = 0.001$), residue soluble compound concentration (Res_SC: 6.9%, $p = 0.001$), and residue nitrogen concentration (Res_Nc: 6.3%, $p = 0.001$) each explained between 6 and 11% of the variability. The remaining plant traits and properties each explained less than 5% of the variability of N pools, with only a significant effect of root lateral expansion rate (Ler: 3.1%, $p = 0.001$), nitrogen harvest index (NHI: 2.2%, $p = 0.002$), and root mass fraction (RMF: 1.0%, $p = 0.049$).

3.3 Synergy between seed N and N_2 fixation

The RDA biplot highlighted a clear positive correlation between the amount of N in legumes derived from the air (Ndfa) and the amount of N in legume seeds (Nseed; Fig. 3a). Both N pools were positively correlated with four plant traits: legume shoot dry matter (DMs), nitrogen harvest index (NHI), mean seed weight (mSW), and seed N concentration (SNC). The amount of N accumulated in legume seeds (Nseed) first depends on plant growth, which drives the plant's N requirements and its accumulation of N. Based on the variation among species and experiments, the positive relationship between the amount of N fixed by legumes and legume shoot dry matter led to a mean value of 25 kg of N fixed for every ton of shoot dry matter. This value is equivalent to the maximum value reported by Peoples et al. (2019). Second, the amount of N in seeds depends on the amount of N remobilized from vegetative organs to seeds during seed filling at the end of the plant growth cycle (Schiltz et al. 2005). The latter process seems to be modulated by seed size and seed N concentration.

3.4 Underlying N functions of legumes affecting the performances of the following wheat

When considered independently, the amount of N derived from the air (Ndfa) and the amount of N in legume seeds (Nseed) were not correlated with the amount of N in the following wheat (Nwheat; Fig. 3a). However, a positive relationship was highlighted between the amount of N in the following wheat and the net N balance (i.e., calculated as the difference between Ndfa and Nseed). Thus, the contribution of legumes to soil N through the symbiotic N_2 fixation process increases the amount of N in the following wheat.

Situations enhancing the symbiotic N_2 fixation process tend to induce a positive net N balance (Evans et al. 2001). The increase of symbiotic N_2 fixation occurs when little soil N is available during the legume growth cycle, as most legume N requirements are then fulfilled through the symbiotic N_2 fixation process. Likewise, legume species with low ability to take up soil N mostly rely on the symbiotic N_2 fixation process to fulfill their N requirements (Guinet et al. 2018). Therefore, the higher the legume uptake of soil N during the growth cycle (Ndfsoil), the lower the net N balance and thus the amount of N in the following wheat (Nwheat; Fig. 3a). As expected, the amount of soil N taken up by legumes (Ndfsoil)

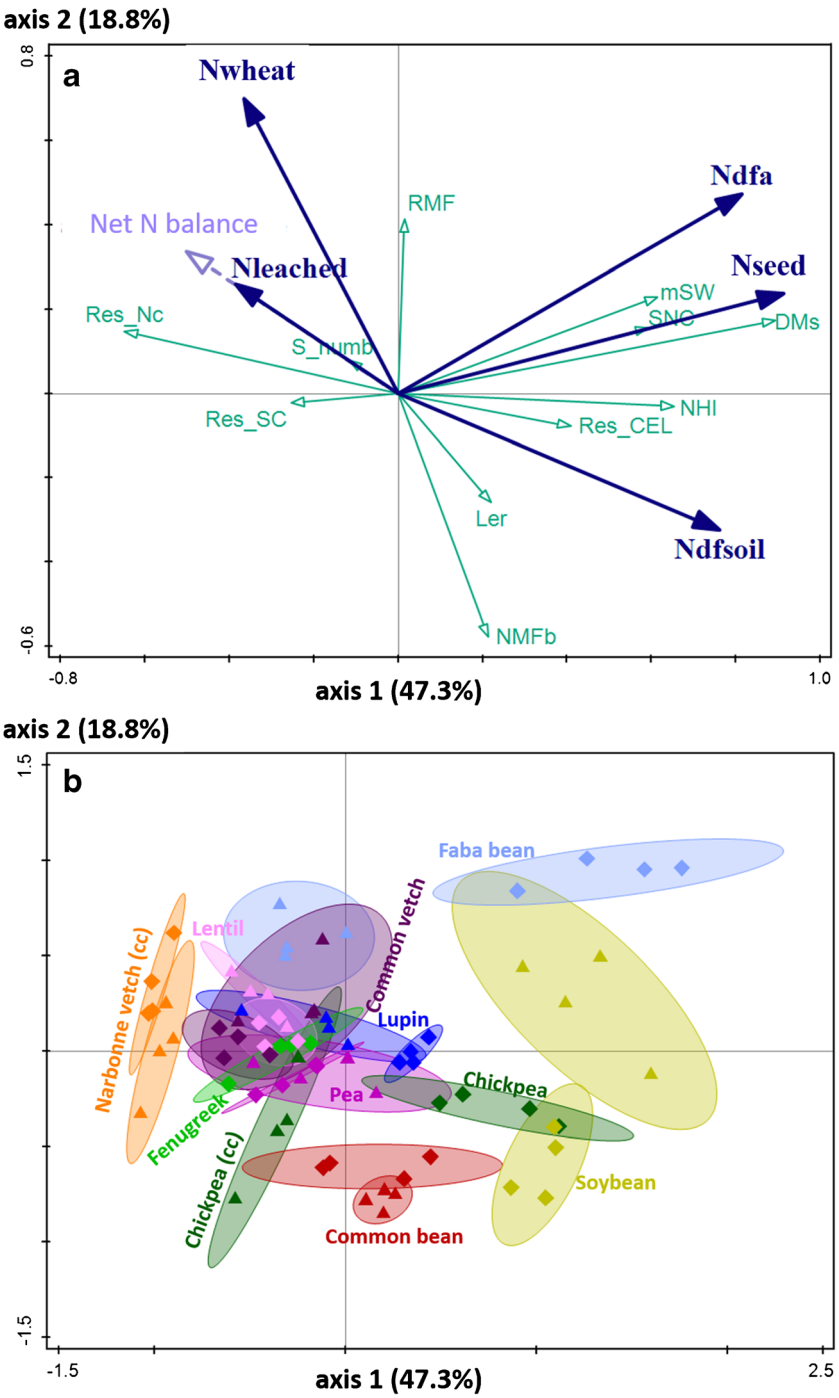


Fig. 3 **a** Redundancy analysis (RDA) biplot on the first two axes, illustrating the relationship between N pools (response variables: thick dark blue arrows) and plant traits and properties (explanatory variables: fine light blue arrows). The apparent N balance was added as an explanatory variable (purple dotted arrow). Nitrogen harvest index (NHI); seed nitrogen concentration (SNC); mean seed weight (mSW); number of seeds (S_numb); shoot dry matter (DMs); belowground nodule mass fraction (NMFb); residue nitrogen concentration (Res_Nc); residue cellulose concentration (Res_CEL); residue soluble compound concentration (Res_SC); root lateral expansion rate (Ler); root mass fraction (RMF). Amount of N in legume seed (Nseed); amount of N in legume shoots derived from the air (Ndfa); amount of N in the shoots of the following wheat (Nwheat); amount of N in legume shoots derived from the soil (Ndfsoil); amount of N leached after legume harvest (NLeached). **b** Scatter diagram with the first two axes of the RDA. The ellipses represent a 95% confidence interval for each legume species and both experiments: experiment I: (▲) and experiment II (♦). Species for which there was no seed production are indicated by cc: cover-crops

was positively correlated with the root lateral expansion rate (Ler). Ndfsoil was also positively correlated with the belowground nodule mass fraction (NMFb), considered as a proxy for C allocation trade-off between roots and nodules. A high biomass allocation to nodules reflects a high C cost associated with nodule establishment, maintenance, and metabolism, but with a depressive effect on root and shoot development (Bourion et al. 2007). This tradeoff suggests that legumes for which nodules induce a high C cost (high NMFb) primarily take up soil N through the development of their root system in order to minimize the C cost associated with N acquisition. Conversely, no relation was established between the amount of soil N taken up by legumes (Ndfsoil) and root mass fraction (RMF). As highlighted by Dunbabin et al. (2003) plant traits characterizing root architecture may be more appropriate for explaining the differences in the ability of legumes to take up soil N, than simply considering root biomass.

High amounts of N derived from the air (Ndfa) are not sufficient to ensure a positive net N balance. For a given amount of N derived from the air (Ndfa), the net N balance results from the tradeoff between N exported in legume seeds and N supply to the soil by legume straws left in the field. Legumes having a high seed N concentration (SNC) also have straws characterized by low N concentrations (Res_Nc) and high fractions of structural polysaccharides such as cellulose (Res_CEL; Fig. 3a). Consequently, straws having a lower N mineralization potential (Palm et al. 2001), thus, release less inorganic N for the following wheat to take up. Residue N mineralization results from the interaction between climatic conditions, soil physical, chemical and biological characteristics, and residue biophysical characteristics. Thus, the provision of N to the following crops through residue mineralization is highly complex compared with synthetic fertilizer provisions, leading to a potential desynchronization between N supply and N requirement of the following crop. However, residue N incorporated in soil organic matter contributes to long-term soil fertility (Palmer et al. 2017).

3.5 Tradeoff between soil N uptake and N leaching

We found a clear negative correlation between the amount of N in legume shoots derived from the soil (Ndfsoil) and the amount of N leached after the legume harvest (Nleached; Fig. 3a). In accordance with Plaza-Bonilla et al. (2015), this relationship indicates that legume species with a good ability to take up soil N during their growth cycle reduce the risk of N leaching after their harvest by decreasing the amount of inorganic N left in the soil. Our experimental site is characterized by frequent rain episodes during the autumn-winter period. During this same period, because the soil is either left bare or cropped with the following wheat at its early stages, with only very low N requirements, the inorganic N left in the soil after the legume harvest is greatly exposed to N leaching. Nitrogen leaching to groundwater aquifers and rivers causes water pollution and eutrophication (Palmer et al. 2017). In waterlogged soils, denitrification of soil inorganic N left after legume crops might also generate nitrous oxide emissions. Nitrous oxide is a major greenhouse gas contributor to climate change (Peoples et al. 2019). Thus, while increasing soil N after legumes can positively influence the supporting service of N supply to the following wheat, it may also generate disservices associated with N losses.

However, considerable losses of N by leaching do not necessarily lead to the smallest amounts of N in the following crop, as shown by the positive relationship between the amount of N leached after the legume harvest (Nleached) and the amount of N in the following wheat (Nwheat; Fig. 3a). Even when a proportion of soil N is leached after legume harvest, the N requirements of the following wheat can also be covered by soil and residue organic N mineralization. The latter two processes mainly occur during the spring period, as temperatures rise. During the spring, the significant N requirements of the following wheat ensure the uptake of N released by these two processes and contribute to reducing N leaching in comparison with the autumn-winter period.

3.6 Functional profiles of the ten grain legumes

For the first time, ten grain legume species were evaluated in the same pedoclimatic conditions. They were characterized by contrasted functional profiles relative to N functions (Fig. 3b, Table 3). On the one hand, chickpea in 2016 (i.e., with seed production), common bean, and soybean had a high ability to take up soil N during their growth cycles (Ndfsoil). As a consequence, they limited the potential dis-service of water pollution by nitrates thanks to the low N losses by leaching after their harvest (Nleached). Yet, these three species contributed little to the supporting service of N supply to the following crop, as they induced low amounts of N in the following wheat (Nwheat). The three latter species were characterized by a high root lateral expansion rate (Ler) and belowground nodule mass fraction (NMFb). The high ability of chickpea and common bean to take up soil N could partially be explained by poor symbiotic N_2 fixation efficiency. In the last decades, common bean genotypes have been bred and selected in high fertility soil that likely resulted in low N_2 fixation efficiency (Van Kessel and Hartley 2000). Moreover, even if chickpea was inoculated with rhizobia upon sowing, the selection of highly efficient and competitive rhizobia strains in the northeast part of France is still at stake for this species. Conversely, in our study, soybean was bred to nodulate with highly efficient rhizobia strains and seems to be able to efficiently acquire N from symbiotic N_2 fixation and soil N uptake.

On the other hand, Narbonne vetch, lentil, common vetch, and faba bean had low ability to take up soil N (Ndfsoil) and induced higher amounts of N leaching after their harvest (Nleached), hence contributing to the dis-service of water pollution by nitrates. However, the latter legume species tended to better contribute to the supporting service of N supply to the following crop in comparison with chickpea, common bean, and soybean. Indeed, their high residue N concentrations (Res_Nc) induced higher amounts of N in the shoots of the following wheat.

Compared with the other species, faba bean in 2016 and soybean in 2014 had the highest amounts of N derived from the air (Ndfa) as well as the highest amounts of N in seeds (Nseed), underpinning the supporting service of N supply to legumes and the provisioning service. These two species were characterized by high shoot dry matter (DMs) and nitrogen harvest index (NHI).

Finally, pea and lupin had an intermediate position (i.e., a mean value for all five N pools in comparison with the other

legume species), meaning that these two species enabled a compromise between the different ecosystem services and dis-services relative to N.

3.7 Legume multifunctionality at different levels of intensity

For thresholds (i.e., percentage of the maximum observed level of each N pool) ranging between 5 and 25%, chickpea considered as cover crop (cc), common vetch and Narbonne vetch (cc) had a multifunctionality index of 4, meaning that at least one of the five N pools was below the 25% threshold (Fig. 4). For Narbonne vetch and chickpea cc, this was explained by the absence of seed production (i.e., the amount of N in seeds was nil for these two species) while for common vetch, the high N losses due to leaching after harvest led to a value of zero for Nleached_r. For the same range of thresholds, all the other legume species had the maximum multifunctionality index of 5.

For all species, the mean number of N pools exceeding the threshold decreased as the threshold increased, supporting the existence of trade-offs between N functions, and limiting the ability of grain legumes to simultaneously provide multiple ecosystem services at high levels (Blesh 2018). Hence, the choice of grain legumes must be determined according to the most desired ecosystem services. In our study, the same threshold was assigned to the five N pools. To go further in the assessment of ecosystem services provided by legumes,

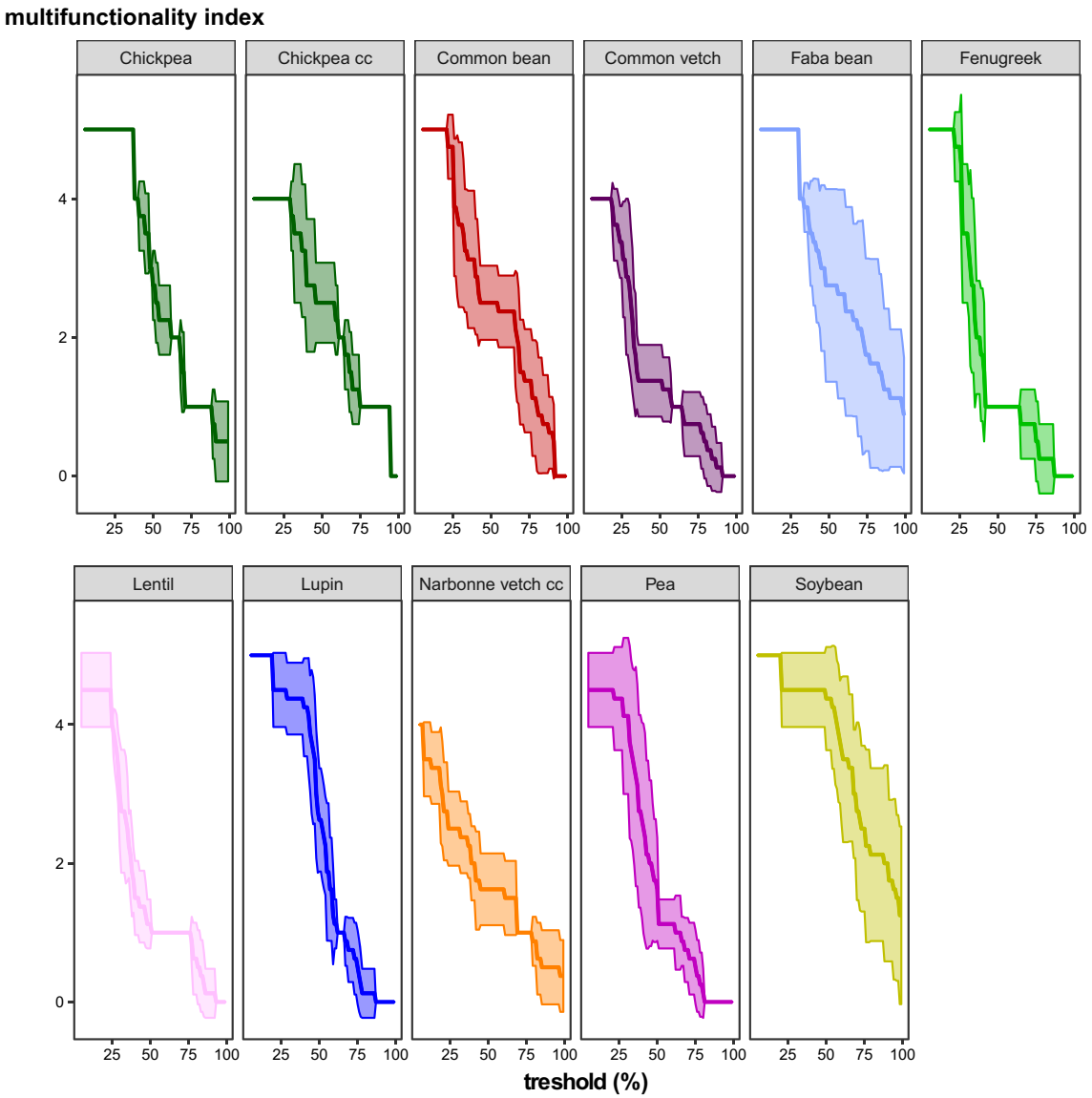


Fig. 4 Multifunctionality index for thresholds ranging from 5 to 100% for ten legume crops. The envelopes correspond to the standard deviation. Species for which there was no seed production are indicated by cc: cover-crops (i.e., Narbonne vetch in 2014 and 2016 and chickpea in 2014). For this reason, chickpea with seed production was distinguished from chickpea with no seed production to calculate the multifunctionality index

collaborative work with farmers could be developed to define relevant thresholds for each N function, in order to select legume species according to the specific goals desired by farmers. The multifunctional index approach is an innovative way to characterize species for their differential ability to deliver multiple functions at different levels of intensity with a unique indicator.

4 Conclusion

In this study, the simultaneous measurements of five N pools and several explanatory plant traits enabled for the first time to distinguish ten grain legume species according to their functional profiles. Hence, the characterization of grain legume species according to a combination of plant traits assesses their potential abilities to deliver N functions and support the resulting ecosystem services. Legumes with a high ability to take up soil N during their growth cycles and inducing low N losses by leaching after their harvest (chickpea, common bean, and soybean) were distinguished from species with a low ability to take up soil N, thus posing higher risks of N leaching, but inducing high amounts of N in the following wheat (common vetch, faba bean, lentil, pea, and Narbonne vetch). In given situations, the establishment of these profiles should facilitate the selection of appropriate legume species according to the intended objectives and appropriately adjust N management in cropping systems that include grain legumes.

The profiles in this study were established in the Burgundy region of France. Characterizing the same legume species in different environments might require re-evaluating trait attributes or the integration of new traits that would be better adapted to the environmental constraints of the new situation. Ecosystem services relative to N are influenced by climatic conditions, which can affect N functions (i) directly, such as N leaching, or (ii) indirectly, by impacting plant traits and plant properties that determine the provision of N functions. However, the functional profiles were relatively well maintained between both experiments that were nonetheless characterized by contrasting climatic conditions.

Lastly, ecosystem services and dis-services are modulated by agricultural practices. For example, the establishment of cover crops during the fallow period, or the choice of a following crop with an earlier sowing date and with higher early stage N requirements than wheat (e.g., rapeseed oil), could substantially reduce N leaching losses after the legume harvest.

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Compliance with ethical standards

Conflict of interest The authors declare that they have no conflict of interest.

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