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### ► **To cite this version:**

Maé Guinet, Bernard Nicolardot, Anne-Sophie Voisin. Multifunctional analysis of ecosystem services relative to the nitrogen fluxes provided by ten legume crops. European Conference on Crop Diversification, Sep 2019, Budapest, Hungary. <hal-02380011>

**HAL Id: hal-02380011**

**<https://institut-agro-dijon.hal.science/hal-02380011v1>**

Submitted on 26 Nov 2019

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## **Multifunctional analysis of ecosystem services relative to the nitrogen fluxes provided by ten legume crops**

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### **1 Introduction**

In the context of agroecological transition, ecosystem services should be maximized to ensure agriculture production (Tibi and Therond, 2017). The provision of ecosystem services relies in particular on the reintroduction of spatial and temporal biodiversity (Isbell et al., 2011). Indeed, species diversity provides a variety of functions supporting ecosystem services. Legumes deliver unique and/or complementary functions to those of other groups of species. Specifically, they should play a key role in the provision of ecosystem services relative to nitrogen (N) fluxes when reintroduced in cropping systems mainly based on cereals. Indeed, legumes enable the production of protein-rich seeds. They also have the unique ability to establish a symbiosis with specific soil Rhizobium bacteria that leads to N accumulation during their growth through the symbiotic N<sub>2</sub> fixation process (Guinet et al., 2018). Legumes also supply N to the following crops through the mineralization of their N-rich residues (Angus et al., 2015). Nevertheless, some negative impacts can result from the introduction of legumes in cropping systems. Hence, their lower ability to retrieve soil inorganic N compared to cereals (Hauggaard-Nielsen et al., 2001) and the desynchronization between N supply by legume residues and the N demand of the following crop can lead to N losses by leaching during legumes growth cycle and/or after their harvest.

As a large diversity of grain legumes exist, an important issue is to characterize and distinguish them based on their ability to deliver functions supporting ecosystem services relative to nitrogen in order to assist the choice of legume species according to the expected objectives. However, the choice of grain legumes according to the provision of those ecosystem services remains difficult due to a lack of references for a diversity of species. The objectives of our study was i) to quantify N fluxes induced by legumes and to identify several explanatory plant traits of these N fluxes in order to establish the functional profiles of ten grain legume crops and ii) to better understand the synergy and trade-off between the different N fluxes and hence the explanatory plant traits.

### **2 Materials and Methods**

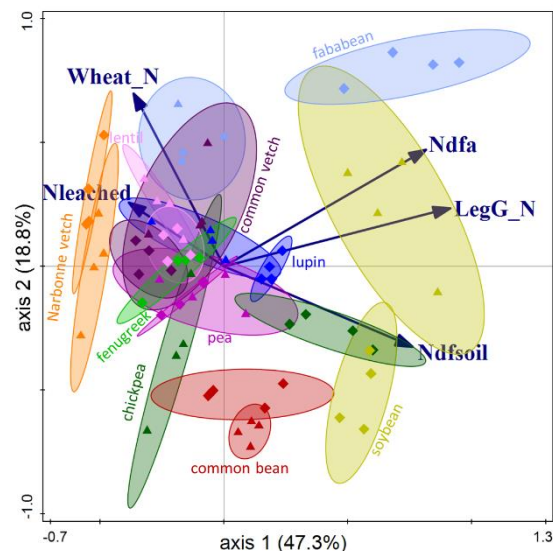
Two field experiments lasting two years were carried at the INRA experimental site of Bretenière (Dijon, France) in 2014-2015 and 2016-2017. The first year (2014 and 2016), nine legumes were cultivated in absence of N fertilization: chickpea, common bean, common vetch, faba bean, lentil, lupin, Narbonne vetch, pea and soybean. In 2016, fenugreek was also cultivated. Seeds of the ten legumes were inoculated at sowing with species-specific strains of N<sub>2</sub>-fixing bacteria to ensure symbiotic N<sub>2</sub> fixation. Two cereals were also sown as pre-crops for comparison with legumes. Legume and cereal seeds were harvested and 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 unsuitable for seed production for these two species. The second year, winter wheat was sown in October as a following crop for each of the legume and cereal pre-crops, and was not supplied with N fertilization. Wheat was then harvested in July 2015 and 2017, respectively.

During the two year legume – wheat succession, five N fluxes were measured or estimated using the STICS model: 1) amount of N in harvested legume seeds, 2) amount of N derived from the air in legume shoots through the symbiotic N<sub>2</sub> fixation process, 3) amount of N derived from the soil in legume shoots through soil inorganic N uptake by the roots, 4) amount of N leached between legume harvest and wheat harvest, and 5) amount of N in the shoots of the following wheat. Legume plant traits considered as explanatory for these N fluxes were measured on legumes during the two field experiments or during a greenhouse experiment where the same ten legume species were cultivated in rhizotrons. For the ten legumes species, a redundancy analysis was performed using the five N fluxes as response variables and the plant traits as explanatory variables in order to study the link between the N fluxes and identify the most explanatory plant traits.

### 3 Results

Our results indicate that the amount of N in legume seeds and the amount of N derived from the air were positively correlated with four plant traits: legume shoot biomass, nitrogen harvest index (amount of seed N / total amount of shoot N), mean seed weight and seed N concentration. The two previous N fluxes were uncorrelated with the amount of N in the following wheat, which was negatively correlated with the legume residue C:N ratio. A clear antagonism between the amount of N leached after legume harvest and the amount of soil inorganic N retrieved by legumes was found. The latter N flux was positively associated with the root lateral expansion rate of legumes and the belowground nodule mass fraction (nodule biomass / belowground biomass).

Those profiles enabled to distinguish legumes with high ability to retrieve soil inorganic N during their growth cycle and with low N losses by leaching after their harvest (chickpea, common bean, and soybean) against species with low ability to retrieve soil inorganic N and with higher risks of N leaching (common vetch, faba bean, fenugreek, lentil, pea and Narbonne vetch). Yet, the latter legume species tended to induce higher amounts of N in the shoots of the following wheat in comparison to chickpea, common bean, and soybean. Finally, faba bean in 2016 and soybean in 2014 had the highest amounts of N derived from the air as well as the highest amounts of N in seeds compared to the other species.



**Figure 1.** Redundancy analysis (RDA) biplot of N fluxes and legume pre-crop treatments for the 2014-2015 experiment (▲) and the 2016-2017 experiment (◆). Ellipses show the 95% confidence interval associated with each legume pre-crop treatments in both experiments.

**LegG\_N:** amount of N in harvested legume seeds; **Ndfa:** amount of N derived from the air; **Ndfsoil:** amount of N derived from the soil; **Nleached:** amount of N leached between legume harvest and wheat harvest; **Wheat\_N:** amount of N in the shoots of the following wheat.

### 4 Discussion and Conclusions

Based on the simultaneous study of five N fluxes and several plant traits considered as determinant for the provision of the N fluxes, ten grain legume species were distinguished according to their functional profile. Hence, the characterization of grain legume species according to a combination of plant traits enable to evaluate their potential abilities to deliver N functions and the resultant ecosystem services. Yet, negative impacts such as N leaching could be compensated by adequate agricultural practices such as legume-cereal mixture or the establishment of cover-crop during the fallow period. Lastly, variation within each legume species could be characterised in the future by measuring plant traits that were identify in this study, to select the right genotype in order to improve N transfers.

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