



## Cover crop crucifer-legume mixtures provide effective nitrate catch crop and nitrogen green manure ecosystem services

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### ABSTRACT

During the fallow period, crucifers grown as catch crops are known to effectively reduce nitrate leaching, while legumes act mainly as green manure by releasing large amounts of mineral nitrogen (N) for the subsequent cash crop once incorporated into the soil. Crucifer-legume cover crop mixtures could be an effective solution for obtaining these two ecosystem services because they combine advantages of both species. However, crucifers might be a poor companion crop due to their high competition for abiotic resources and a potential allelopathic effect on legumes when grown with them. The aim of our study was to assess performances of a wide range of bispecific crucifer-legume mixtures to provide both catch crop and green manure services. A two-year experiment was conducted at two sites (near Toulouse and Orléans, France) where cultivars from eight crucifer species (rape, white mustard, Indian mustard, Ethiopian mustard, turnip, turnip rape, radish and rocket) and nine legume species (Egyptian clover, crimson clover, common vetch, purple vetch, hairy vetch, pea, soya bean, faba bean, and white lupin) were tested in sole-crop and bispecific mixtures (substitutive design of 50%-50% sole crops). We measured cover crop biomass and N acquisition to assess the soil nitrate catch crop service and N green manure service for the subsequent cash crop. In all experiments, compared to bare soil, crucifer-legume mixtures and crucifer sole cover crops provided the same level of nitrate catch crop service by reducing soil mineral N by an average of 59%, while legume sole cover crops reduced it by at least 35%, which is significant. In addition, within 6 months after termination, crucifer-legume mixtures mineralised more N (mean of 22 kg N ha<sup>-1</sup>) and thus had a larger N green manure effect for the subsequent cash crop than crucifer sole cover crops (mean of 8 kg N ha<sup>-1</sup>). This was due to greater N acquisition and a lower C:N ratio of crucifer-legume mixtures; even though crucifers always had advantage in acquiring N, legumes acquired enough N to provide an effective green manure service. These results were consistent in all of our experiments, which represent a wide range of crucifer-legume cover crops, demonstrating their generality. They also demonstrate the compatibility and complementarity of these species when grown together. In conclusion, combining crucifers and legumes as cover crops is an effective solution for obtaining multi-ecosystem services related to N recycling by providing both nitrate catch crop and N green manure services.

### 1. Introduction

Intensive agricultural practices and bare soils during fallow periods can cause nitrate pollution of ground water. Cover crops grown during the autumn-winter period between two cash crops in annual rotation are an effective solution to decrease nitrate leaching (nitrate pollution mitigation service) due to their ability to capture soil mineral nitrogen (SMN) (Meisinger et al., 1991; Constantin et al., 2011). Cover crops can also provide a green manure service by releasing acquired nitrogen (N) to the subsequent cash crop after incorporation into the soil

(Kramberger et al., 2009; Justes et al., 2012). The green manure service has been found to be inversely proportional to the C:N ratio of cover crops (Quemada and Cabrera, 1995; Justes et al., 2009). Compared to bare soil, non-legume species grown as catch crops effectively decrease nitrate leaching, while leguminous species or legumes are the most efficient to ensure N green manure (Thorup-Kristensen et al., 2003). Non-legume species can capture up to 70% of SMN during the fallow period (Justes et al., 2012). Among them, Brassicaceae species or crucifers such as turnip rape (*Brassica rapa*), white mustard (*Sinapis alba*) and fodder radish (*Raphanus sativus*) are often considered the most

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effective catch crops due to their rapid increase in root depth and density (Thorup-Kristensen, 2001; Thorup-Kristensen et al., 2003), which allows them to capture a large amount of N soon after sowing, decreasing nitrate leaching and water pollution (Thomsen and Hansen, 2014). Crucifers provide less green manure service than legumes due to their moderate C:N ratio (range = 15–25), which induces slower mineralisation of the cover crop residues after their incorporation into the soil (Justes et al., 2009). Legumes usually provide the most effective green manure service due to their low C:N ratio (range = 10–15), which induces faster mineralisation, and of a larger proportion, of their acquired N present in their residues returning to the soil (Tonitto et al., 2006). Some of the N that legumes acquire comes from atmospheric N<sub>2</sub> fixation, meaning that exogenous N is added to the agroecosystem (Peoples et al., 1995). Certain legumes can also provide a catch crop service, although not as much as crucifers, and can decrease SMN during the fallow period by up to 60% compared to that under bare soil, depending on the legume species and site (Tonitto et al., 2006; Tribouillois et al., 2016; Wortman, 2016).

Cover-crop mixtures composed of legume and non-legume species could provide both catch crop and green manure services simultaneously by combining advantages of both sole crop species (Ranells and Waggener, 1997; Kramberger et al., 2009; Tosti et al., 2014). The green manure service of cover crop mixtures generally lies between those of non-legume sole crops and legume sole crops because mixtures have lower C:N ratios and acquire more N than non-legume sole crops (Kuo and Sainju, 1998; Tosti et al., 2014; Tosti et al., 2012). Mixtures also provide a catch crop service similar to that of non-legume sole crops (Möller et al., 2008; Tosti et al., 2014; Tribouillois et al., 2016). It has been shown that three months after sowing, a mixture with turnip rape and white mustard provided more catch crop service than one with the non-legumes foxtail millet (*Setaria italica*), bristle oat (*Avena strigosa*), Italian ryegrass (*Lolium multiflorum*) or phacelia (*Phacelia tanacetifolia*), and maintained a good green manure service (Tribouillois, 2014), indicating that crucifers are good candidates for providing efficient nitrate catch crop effect in mixtures.

Mixtures also increase N-related ecosystem services because of their high resource use efficiency, due to niche complementarity in using abiotic resources such as light, water and nutrients (Jensen, 1996). Overall, compared to crucifer sole crops higher N acquisition in crucifer-legume mixtures has been demonstrated for grain production (Andersen et al., 2005) and cover crops (Tribouillois et al., 2016; Wendling et al., 2017). However, legumes grown in mixtures may suffer from the competition engendered by crucifers (Szumigalski and Van Acker, 2008; Wortman et al., 2012; Tribouillois et al., 2016). Crucifers could be a poor companion crop for two main reasons: 1) they strongly compete for water, nutrients and light due to their rapid root and shoot growth and 2) they can have an allelopathic effect on legumes due to their production of glucosinolates, which are exuded by roots and transformed in the soil into biocides such as isothiocyanates (Chew, 1988; Matthiessen and Kirkegaard, 2006). Competition for abiotic resources varies greatly among crucifer species. For example, species and cultivars in the crucifer family have differences in shoot and root architecture and plant ontogeny that can induce different interactions with the intercropped legume. Crucifers' production of glucosinolates can vary greatly in the types of molecules and their concentrations (Kirkegaard and Sarwar, 1998) which generate potential and actual allelopathic effects on the legume. As a consequence, the balance between compatibility and incompatibility between crucifer and legume species in mixtures must be studied to determine the most effective bispecific mixtures.

From a practical viewpoint of cover cropping, few experiments have been published that consider root biomass and N content of crucifer-legume mixtures. Roots can contain a high proportion of the N of certain crucifer species, such as fodder radish and turnip, because their taproots have different C:N ratios than their shoots (e.g. Thorup-Kristensen et al., 2003). This indicates that roots must be considered to

accurately predict the N green manure service generated by the N released by mineralisation of cover crop residues incorporated into the soil, which determines N availability for the subsequent cash crop.

The aim of our study was to analyse crucifer-legume mixtures – or Brassicacea-Leguminous mixtures- composed of a wide range of species and cultivars. Our study assessed two key aspects of cover crops: 1) levels of green manure and catch crop ecosystem services provided as sole crops and in mixtures and 2) the type of interaction between the two species (complementarity or competition) involved in N acquisition. Three hypotheses were tested:

- 1) Mixtures have higher N acquisition, a lower C:N ratio, and more green manure service than crucifer sole crops.
- 2) Mixtures provide more catch crop service than legume sole crops and almost the same level of service than crucifer sole crops, and then provide multi-ecosystem services.
- 3) The most suitable compromise between catch crop and green manure services depends on interspecific interactions that maximise the complementarity of both species.

Recent studies have highlighted that climate, soil type and N availability at sowing have the greatest influence on mixtures performances (Tribouillois et al., 2016). Accordingly, we assessed performances of crucifer-legume mixtures by conducting field experiments from late summer to late autumn for two years at two sites with different soil and climate conditions, which allowed us to assess the generality of species-mixture functioning.

## 2. Materials and methods

### 2.1. Experimental design and cover crop management

Four field experiments were conducted in two years (2014 and 2015) at two sites: 1) the Lamothe experimental farm of INP-EI Purpan, located in Seysses, 20 km south of Toulouse, south-western France (43.506° N, 1.237° E), and 2) the La Vannelière research station of Jouffray Drillaud, located 50 km south-east of Orléans, central France (47.776° N, 2.098° E). Experiments conducted in 2014 and 2015 are referred to as L2014 and L2015, respectively, at Lamothe (L) and as V2014 and V2015, respectively, at La Vannelière (V). According to the Köppen climate classification, Lamothe (Toulouse) has a humid subtropical climate and La Vannelière (Orléans) has an oceanic climate (Table 1). At all four site-years, the experiment was a completely randomised design that was replicated with three replicates in blocks. Surface area of the elementary plot, containing 10 rows for each treatment, was 18.0 m<sup>2</sup> for L2014 and V2014, 22.5 m<sup>2</sup> for L2015 and 20.0 m<sup>2</sup> for V2015. To avoid plant-plant competition effects between adjacent treatments, only the six rows in the middle of the plot were harvested and used for soil measurements.

Cover crop species were selected for their ability to grow rapidly during the autumn in a short-term fallow period. Crucifer and legume species and cultivars were selected for their diversity in shoot/root architecture and precocity. All mixtures (Table 2) contained one crucifer and one legume (bispecific mixtures) and were designed to minimise competition according to expert knowledge and recently published information (Tribouillois et al., 2016). The following species were used: 1) the crucifers or Brassicacea species of rape (*Brassica napus*), white mustard (*Sinapis alba*), Indian mustard (*Brassica juncea*), Ethiopian mustard (*Brassica carinata*), turnip (*Brassica rapa* subsp. *rapa*), turnip rape (*Brassica rapa* subsp. *oleifera*), radish (*Raphanus sativus*) and rocket (*Eruca sativa*) and 2) the legumes or Leguminous species of Egyptian clover (*Trifolium alexandrinum*), crimson clover (*Trifolium incarnatum*), common vetch (*Vicia sativa*), purple vetch (*Vicia benghalensis*), hairy vetch (*Vicia villosa*), pea (*Pisum sativum*), soya bean (*Glycine max*), faba bean (*Vicia faba*), and white lupin (*Lupinus angustifolius*).

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