



## Cultivar complementarity for symbiotic nitrogen fixation and water use efficiency in pea-oat intercrops and its effect on forage yield and quality

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### ABSTRACT

Three oat (*Avena* spp.) and three field pea [*Pisum sativum* subsp. *arvense* (L.) Asch.] cultivars were grown in pure stands and interspecific, binary mixtures under Mediterranean conditions for two growing seasons (2011–12 and 2012–13). The aim of the work was to identify traits contributing to the complementary use of soil nitrogen (N) and water in intercrops. Such traits could be used as putative selection criteria for cultivars well-adapted to intercropping systems. The <sup>15</sup>N natural abundance method was used to assess the percentage of N derived from N<sub>2</sub>-fixation (%N<sub>dfa</sub>) in pure stands and intercrops and the percentage of N transferred from pea to oat (%N<sub>trans</sub>) in intercrops. Carbon isotope discrimination ( $\Delta_{\text{pea}}$ ,  $\Delta_{\text{oat}}$ ) was used as an assessment of water use efficiency. Isotope determinations were conducted when 30% of pea flowers were open. Dry matter yield (DM) of pure stands and intercrops was estimated at forage silage maturity of peas, and forage quality traits (protein, fat, ash, crude fiber, and nitrogen free extracts concentrations) were measured. Intercropping either increased or decreased %N<sub>dfa</sub> depending on the cultivars included in the mixtures. High %N<sub>dfa</sub> by pea in mixtures was associated with a high contribution of pea in the mixture and high crude protein concentration in DM. High amounts of fixed N and N accumulated in pea were necessary to maximize protein concentration and protein yield in intercrops. These amounts were calculated to be 72.0 and 94.4 kg N ha<sup>-1</sup> for protein concentration and 124.2 and 160.8 kg N ha<sup>-1</sup> for protein yield. Oats in intercrops seemed to affect the N niche of peas.  $\Delta_{\text{oat}}$  values were positively correlated with %N<sub>dfa</sub>, the amount of fixed N in pea and the amount of N accumulated in pea. A plausible explanation for this is that high  $\Delta_{\text{oat}}$  values were indicative of a better access to soil water and nitrate (NO<sub>3</sub>-N) sources through a deeper and denser rooting system, thus increasing the competition on the companion pea which led to higher percentage of N<sub>2</sub>-fixation and consequently to higher amounts of N fixed by and accumulated in pea. High- $\Delta$  oats were less dependent on N transferred from pea as was indicated by a negative correlation between these two traits. As a consequence, high  $\Delta_{\text{oat}}$  values were associated with high protein concentration and protein yield. In contrast,  $\Delta_{\text{pea}}$  values were negatively correlated with protein concentration and protein yield. Peas, having a less competitive rooting system compared to oats, were adaptive to intercropping when they had a more conservative use of soil water (lower  $\Delta_{\text{pea}}$ ), and thus avoided competition. Land equivalent ratios for dry matter yield (LER<sub>DM</sub>) and protein yield (LER<sub>CPY</sub>) were affected by growing seasons. LER<sub>DM</sub> was < 1.00 (0.88) in 2012–13 growing season indicating that intercropping was not favored. This season was characterized by lack of precipitation during December 2012 and January 2013, high precipitation in February (> 62% of season's water input) and mild temperatures during winter (November to February). The very early, moderately tall oat cultivar Flega had the highest LER values, regardless of the companion pea cultivar. This was an indication that Flega was favored by growing in intercrops, however, Flega's mixtures were not among the high-yielding mixtures.

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## 1. Introduction

Winter cereals and legumes are widely grown as forages for animal nutrition with each exhibiting several advantages and disadvantages. Among winter cereals, oats (*Avena* spp. L.) produce high forage yield of high nutritive value under various climatic conditions (Geren, 2014; Kim et al., 2014). Field peas [*Pisum sativum* subsp. *arvense* (L.) Asch.] produce high quality forage in a sustainable way since they acquire part of the needed nitrogen (N) via symbiotic N<sub>2</sub>-fixation and may enrich the soil with N transferred from the plant (Evers, 2011).

Growing cereals and legumes in intercropping systems is a way to deliver “sustainable intensification” (Brooker et al., 2015), especially in low-productivity and low-input agroecosystems (Dordas and Lithourgidis, 2011).

Winter cereal-legume intercrops and specifically pea-oat mixtures have been reported to produce higher forage yields (Kwabiah, 2004), but of lower nutritive value compared to peas grown in pure stands (Mustafa and Seguin, 2004). Productivity of intercrops is usually limited by soil nutrients, mainly N and secondarily phosphorus (Voisin et al., 2002; Hauggaard-Nielsen et al., 2009; Roscher et al., 2011), soil water availability (Chapagain and Riseman, 2015) and by light when the other factors are non-limiting (Bedoussac and Justes, 2010). A prerequisite for the success of intercrops is that the companion species or genotypes to show complementary use of the limiting resources (Brooker et al., 2015).

Complementarity for N is easy to realize in cereal-legume intercrops since the symbiotic N<sub>2</sub>-fixation ability of legumes differentiates their spatiotemporal N niche leading to resource partitioning (Jensen, 1996). Moreover, facilitation may occur in terms of belowground N transfer between the coexisting species (Frankow-Lindberg and Dahlin, 2013). Despite that N transfer can be mutual, the legume-to-cereal transfer prevails (Pirhofer-Walzl et al., 2012; Carlsson and Huss-Danell, 2014). The mechanisms involved in the belowground N transfer between the intercropped species are the intimate transfer of N, along with P, through mycorrhizal hyphal networks (Johansen and Jensen, 1996; Moyer-Henry et al., 2006) and rhizodeposition, which is the release of N from decomposed belowground parts of legumes (rootlets, root hairs and nodules) and root exudates (Wichern et al., 2007a, 2007b; Thilakarathna et al., 2016). Strong facilitation presumes high levels of N<sub>2</sub>-fixation and N accumulation in legumes, a condition not always attainable in agroecosystems (Peoples et al., 2009). However, when it occurs, it is indicative of high amounts of N deposited belowground (Herridge et al., 2008; Mahieu et al., 2009; Peoples et al., 2012).

In semi-arid environments like the Mediterranean, soil water availability exerts a direct effect on productivity and an indirect one via its regulatory role on soil N availability (Tsialtas et al., 2001a, 2001b). Under these conditions, coexisting species or genotypes should also exhibit complementary sharing of soil water resources in order to persist or to attain high productivity (Tsialtas et al., 2001a). There are contradictory findings on the effect of intercropping on water use efficiency (WUE, the ratio of dry biomass produced to the water consumed to produce it) of coexisting species. Chapagain and Riseman (2015) found that wheat (*Triticum aestivum*) grown in mixtures with beans (*Phaseolus vulgaris* and *Vicia faba*) had higher WUE compared to pure stands. In contrast, ryegrass (*Lolium perenne*) showed lower WUE in intercrops with white clover (*Trifolium repens*) (Høgh-Jensen and Schjoerring, 1997). The abovementioned findings are in line with Tsialtas et al. (2005) who reported various effects of competition on WUE of coexisting grasses and forbs.

Quantification of both N<sub>2</sub>-fixation and N transfer from legumes to non-legumes employs N isotope techniques. Among them, the <sup>15</sup>N natural abundance (δ<sup>15</sup>N) method can reliably be practiced in the field, it is more easily applicable than the <sup>15</sup>N-enrichment methods and causes minimum disturbance to the soil-plant system on which it is applied (Carlsson and Huss-Danell, 2014; Peoples et al., 2015; Chalk et al., 2016). However, while <sup>15</sup>N methodologies are commonly used

worldwide, works on quantification of N<sub>2</sub>-fixation and N transfer in pasture legumes are limited in number for the Mediterranean region (e.g., Tsialtas et al., 2004; Carranca et al., 2015). On the other hand, carbon isotope ratio in shoots of C<sub>3</sub> species (δ<sup>13</sup>C) and the subsequent calculation of carbon isotope discrimination (Δ, a measure of the <sup>13</sup>C/<sup>12</sup>C ratio in plant tissues compared to the air) have widely been used as an indirect, long-term assessment of intrinsic WUE (WUE<sub>i</sub>, the ratio of CO<sub>2</sub> assimilation rate to stomatal conductance). Farquhar and Richards (1984) negatively associated the two parameters since both are related to the ratio of intercellular to ambient CO<sub>2</sub> concentrations (c<sub>i</sub>/c<sub>a</sub>). Thus, Δ values can be used as a reliable, easily applicable tool to assess water partitioning among grassland species under field conditions (Tsialtas et al., 2001a).

While much work has been conducted on the complementarity of intercropped species (e.g., Pirhofer-Walzl et al., 2012; Schipanski and Drinkwater, 2012), testing different genotypes for their complementarity in mixtures is limited (e.g., Hauggaard-Nielsen and Jensen, 2001; Giles et al., 2017). Identification of traits contributing to complementarity, thus leading to high-yielding intercrops, is an indispensable condition for the proliferation of this cropping system since the currently used cultivars have been selected for growth in pure stands (Pappa et al., 2012; Brooker et al., 2015).

By growing three field pea and three oat cultivars in pure stands and interspecific, binary intercrops, we aimed to: 1) evaluate cultivar complementarity for N<sub>2</sub>-fixation, N transfer (from pea to oat) and soil water sharing and 2) identify traits contributing to niche complementarity, which could be used for the selection of both pea and oat cultivars well-adapted to intercropping.

## 2. Materials and methods

### 2.1. Site and experiment set up

Field experiments were conducted at adjacent sites during 2011–2012 and 2012–2013 growing seasons (hereafter 2012 and 2013) in Thermi, northern Greece. The soil was a Typic Xerorthent loam, with pH (1:1 in H<sub>2</sub>O) 7.40, organic matter 1.24%, NO<sub>3</sub>-N 38 mg kg<sup>-1</sup>, Olsen-P 16 mg kg<sup>-1</sup>, K 157 mg kg<sup>-1</sup>, and CaCO<sub>3</sub> 3.5% at 0–30 cm depth. Soil sampling was conducted in November before the establishment of the experiment and analyses followed Sparks (1996). Fig. 1 presents the mean monthly temperature and monthly precipitation of the experimental site during the two growing seasons. In the 2012 growing season, winter temperatures (November to February) were lower, but rainfall was much higher in April and May compared to the respective months in the next season. No precipitation was recorded in December 2012 and January 2013 but in February 2013, the monthly

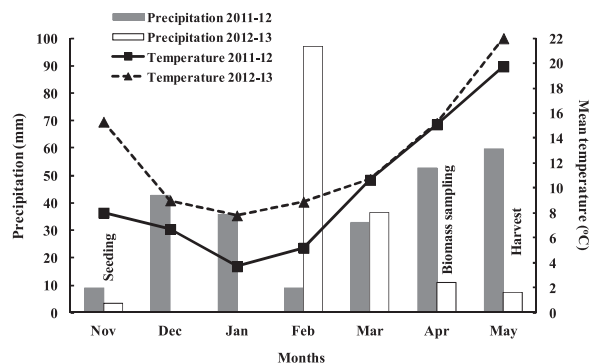


Fig. 1. Monthly mean air temperature and precipitation during the two growing seasons (November to May). Seeding took place after mid November (22 November 2011 and 15 November 2012), biomass sampling for the estimation of N<sub>2</sub>-fixation was conducted the second half of April (30 April 2012 and 17 April 2013) and dry matter yield was measured in May (23 May 2012 and 8 May 2013). In December 2012 and January 2013, precipitation was nil.

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