



# Legume–barley intercropping stimulates soil N supply and crop yield in the succeeding durum wheat in a rotation under rainfed conditions



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## ABSTRACT

Legume–cereal intercropping is increasingly being appreciated in dryland areas, where severe climatic conditions and intensive agricultural practices, generally dominated by continuous cereal cultivation, determine depletion of soil nutrient resources and decline of soil fertility. This research aimed to assess whether and to what extent a newly introduced legume-based intercropping system is able to ameliorate the biological fertility status of an arable soil in a way that is still noticeable during the succeeding durum wheat cropping season in terms of changes in bacterial community structure, soil C and N pools, and crop yield. A field experiment was carried out under rainfed conditions in Southern Italy on a sandy clay loam soil cultivated with durum wheat following in the rotation a recently established grain legume (pea, faba bean)–barley intercropping. Soil chemical, biochemical and eco-physiological variables together with compositional shifts in the bacterial community structure by LH-PCR fingerprinting were determined at four sampling times during the durum wheat cropping season. Soil fertility was estimated by using a revised version of the biological fertility index. Results showed that even though the microbial biomass was significantly altered, the preceding legume intercrops stimulated C-related functional variables thus leading to an increased release of mineral N, which was larger in crop treatments succeeding pea-based than faba bean-based intercropping. The increased N made available in soil enabled the succeeding durum wheat to achieve an adequate grain yield with a reduced N-fertilizer use. Soil type and environmental conditions rather than crop treatments were major determinants of bacterial community structure. The biological fertility status was not varied, suggesting that in intensively managed rainfed areas long-term crop rotations with intercropped legumes are needed to consistently ameliorate it.

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## 1. Introduction

Intercropping can be referred to as an ancient and traditional cropping system, but it has recently shown a serious potential to contribute to modern and sustainable agriculture. It represents the practice of cultivating two or more crops simultaneously in the same field for a considerable part of their life cycles (Vandermeer, 1989). A major agronomic success of intercropped systems relies on their complementary use of soil resources meaning that inter-specific competition is weaker than intraspecific competition for growing factors (i.e. light, water and nutrients) (Willey, 1979).

Intercropping including legumes was initially practised in tropical agriculture. However, legume-based intercropping is becoming increasingly appreciated also in areas other than the tropics because it is able to provide several agro-ecological services. These are more efficient means for use of environmental resources for plant growth due to a reduced competition for soil N (Hauggaard-Nielsen et al., 2003; Knudsen et al., 2004; Hauggaard-Nielsen and Jensen, 2005), an increased water and nutrient use efficiency (Hauggaard-Nielsen et al., 2009a), a greater yield stability and higher N concentration in cereal grain (Hauggaard-Nielsen et al., 2006, 2009b; Tosti and Guiducci, 2010), reduced nitrous oxide emissions from soil (Pappa et al., 2011), a better control of soil erosion (Inal et al., 2007), and an enhanced weed suppression and pest control (Liebman and Dyck, 1993; Corre-Hellou et al., 2011).

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In a sustainable perspective, the agro-ecological role of intercropped legumes becomes of paramount importance for sustaining the productivity of cropping systems in low-input agricultural systems. This can be particularly appreciated in Mediterranean dry-prone areas where cereal (mainly barley and durum wheat) monoculture leads to a general depletion of soil N, which in turn affects grain yield, nutrient use efficiency and maintenance of soil fertility (Cossani et al., 2009). Moreover, severe climatic conditions exacerbate the decline of the organic pool, which represents a slow release nutrient source in arable soils. Thus given the large demand of crops for available N, deficiencies of N are a constant feature of Mediterranean agriculture and N fertilization is invariably required on an annual basis for non-legume crops, especially cereals (Ryan et al., 2008). Therefore, intercropping of legumes and cereals offers the opportunity to provide an input of biologically fixed N into the agro-ecosystem thus avoiding an excessive use of mineral N-fertilizer (Bedoussac and Justes, 2010). In previous field experiments recently introduced pea-barley intercropping systems resulted in increased yield stability as compared to grain legume monoculture (Hauggaard-Nielsen et al., 2009a). However, during the succeeding winter wheat cropping season contrasting responses were observed in terms of both grain yield and biomass production; while a general depletion of soil mineral N not dependent on the preceding crop was also found (Hauggaard-Nielsen et al., 2009a). A field experiment was therefore established with the intention of gaining an in-depth knowledge of soil fertility responses on an arable soil that had been cultivated with winter wheat for as long as one decade since the start of a crop rotation including a newly introduced legume-based intercropping system. In particular, this study focused on assessing if changes in functional and structural soil microbial community responses were taking place during the cultivation of durum wheat succeeding legumes–barley intercropping. Intercropped faba bean was introduced in the field experimental design to evaluate its potential role as a substitute for grain pea, a crop commonly included in leguminous intercropping systems in semi-arid climates.

Changes in soil fertility in response to differing management systems are currently assessed by monitoring a wide range of soil physico-chemical and biological properties, which can also be combined in multiparametric indices (Bastida et al., 2008). One of these is the index of soil biological fertility (IBF), whose use has been suggested in Mediterranean agricultural soils, and provides an estimate of the fertility status of a soil as linked to C dynamics (Benedetti et al., 2006). However, critical revision of the index is still needed so as to avoid overestimation of the soil fertility status (Tortorella et al., 2013).

Given these premises, the specific purposes of the research were: (i) to assess any lasting effect of grain legumes–barley intercropping on C and N dynamics and on residual N made available in soil to the succeeding durum wheat for biomass production and grain yield; (ii) to validate the use of a modified class limits range for estimating the index of biological fertility (IBF); (iii) to verify whether compositional changes had occurred in the genetic structure of soil bacterial community as determined by the newly introduced intercropping system. To achieve these aims, a number of soil chemical (pH,  $EC_{1:2}$ ,  $C_{org}$ ,  $N_t$ ,  $NH_4^+-N$ ,  $NO_3^- -N$ , exchangeable organic N, total soluble N), biochemical (MBC, MBN,  $R_{bas}$ ,  $C_0$ , PMN) and microbial (MBC/ $C_{org}$ ,  $qM$ ,  $qCO_2$ ,  $qCO_2/C_{org}$ ) variables were measured during the durum wheat cropping season. The synthetic index of biological fertility of soil was calculated by considering the following C-related variables:  $C_{org}$ ,  $R_{bas}$ , MBC,  $qCO_2$ ,  $qM$ . Compositional shifts in the bacterial community structure due to the cropping systems were investigated by community fingerprinting based on length-heterogeneity PCR (LH-PCR) of PCR-amplified 16S rRNA gene fragments from soil-extracted bacterial

DNA. The hypotheses assumed for this study were: in a rotation the recently established grain legume-based intercropping systems can stimulate soil C and N cycling in such a way that the additional N flow made available to the succeeding durum wheat is increased (H1); pea-based and faba bean-based intercropping systems provide differing effects on soil nutrient dynamics (H2); legume-based intercropping systems can effectively contribute to the restoration of the soil biological fertility status in rainfed agriculture (H3); together with the microbial activities also the bacterial community structure is markedly altered by the introduction of legume-based intercropping with cereals (H4).

## 2. Material and methods

### 2.1. Plant material and crop density

The plant species studied were grain pea (*Pisum sativum* L. cv Hardy), faba bean (*Vicia faba* L. cv Sikelia), six-row barley (*Hordeum vulgare* L. cv Aldebaran) and durum wheat (*Triticum turgidum* L. ssp. durum cv Virgilio). Sowing densities for full sole crops were 90 (grain pea), 40 (faba bean), 300 (six-row barley), and 350 (durum wheat) plants  $m^{-2}$ . Grain legumes and barley were intercropped (IC) in alternate rows (16 cm apart) to provide either a 100:50 additive design (100 and 50% of sole crop density for grain legume and barley respectively), or a 50:50 replacement design (both species at 50% of respective sole crop density).

### 2.2. Study site, experimental set-up and crop management

The field plots with legume–barley intercrops (IC) in a two-year rotation with durum wheat (DW) were established at the agricultural experimental center of the Regional Agency for Agriculture “ARSSA” (San Marco Argentano, Cosenza, Italy; 39°38'N, 16°13'E, 100 m above the sea level) on an arable soil that had been continuously cultivated with durum wheat for 10 years since the current experiment started in the 2010/2011 cropping season. The study area shows a typical Mediterranean climate, characterized by mild and rainy winters, relatively warm and dry summers and, generally, extended periods of sunshine throughout most of the year. Historical climatic data (averages over the 1995–2009 period) show that mean annual rainfall and air temperature for the area are, respectively, 709 mm and 16.1 °C. The coldest month is February (mean temperature 3.0 °C) and the hottest one is August (mean temperature 33.4 °C). Soil thermal and moisture regimes are thermic and xeric, respectively. The soil evolves over the alluvial deposits from the nearest river Follone and is classified as Haplic Cambisol (Calcaric, Eutric) (IUSS Working Group WRB, 2006), or as Fluventic Haploxerept, coarse silty, mixed, thermic (Soil Survey Staff, 2010). Soil depth is generally greater than 120 cm and the available water holding capacity (AWC, available moisture between field capacity and wilting point) equals 20.0 cm. The soil is a sandy clay loam with the following characteristics: sand 55 ± 4%, silt 24 ± 5%, clay 21 ± 1%, bulk density 1.42 ± 0.14  $g\ cm^{-3}$ ;  $pH_{CaCl_2}$  7.67 ± 0.05,  $EC_{1:2}$  0.21 ± 0.03  $dS\ m^{-1}$ ,  $C_{org}$  9.81 ± 0.25  $g\ kg^{-1}$ ,  $N_t$  0.95 ± 0.04  $g\ kg^{-1}$ , C/N 10.33 ± 0.51,  $C_{HA}$  1.22 ± 0.25  $g\ kg^{-1}$ ,  $C_{FA}$  0.51 ± 0.18  $g\ kg^{-1}$ , CEC 26.2 ± 1.0  $cmol_{(+)}\ kg^{-1}$ , total  $CaCO_3$  18.0 ± 0.5  $g\ kg^{-1}$ , active  $CaCO_3$  13.5 ± 0.4  $g\ kg^{-1}$ , Olsen-P 12.5 ± 1.6  $mg\ kg^{-1}$ ,  $NH_4^+-N$  17.0 ± 0.4  $mg\ kg^{-1}$ ,  $NO_3^- -N$  6.1 ± 0.5  $mg\ kg^{-1}$ , DTPA-extractable Fe 7.6 ± 0.1  $mg\ kg^{-1}$ , DTPA-extractable Mn 7.1 ± 0.1  $mg\ kg^{-1}$ , DTPA-extractable Cu 1.6 ± 0.1  $mg\ kg^{-1}$ , DTPA-extractable Zn 0.9 ± 0.1  $mg\ kg^{-1}$ .

In the 2010/2011 cropping season, twenty-seven field plots (3 × 10 m each) were arranged in a randomized complete block design, with three replications, to compare the following nine IC treatments: grain legumes (pea, faba bean) and barley grown as

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