Nitrogen Fertilization Strategies for Organic Wheat Production: Crop Yield and Nitrate Leaching

Giacomo Tosti,* Michela Farneselli, Paolo Benincasa, and Marcello Guiducci

ABSTRACT
Nitrogen fertility management represents a crucial aspect for common wheat (Triticum aestivum L.) production, particularly when we deal with organic agriculture. This study was conducted to determine the effect of five N fertilization strategies on yield, grain protein content, and N leaching risk. In a 3-yr field experiment, a faba bean/wheat temporary intercropping (TIC) and four fertilization treatments with extra-farm N sources were compared. Extra-farm N sources were represented by blood meal (BM) and roasted leather (RL) (broadcast all-at-once at seeding or split into one-half at seeding and one-half at tillering in a side-dressing application). Unfertilized and mineral fertilized controls were included. The effect of the legume on TIC wheat N uptake was always positive while dry weight accumulation and yield were generally poorly affected. Regardless of the broadcasting method, BM treatments generally showed a slightly higher yield and grain protein content compared to wheat fertilized with roasted leather (this was also confirmed by fertilizer release efficiency). The risk of N leaching was maximum at the onset of drainage (i.e., during the first phase of crop growth), so using organic fertilizers at preceding stage appeared to be a very risky practice, especially if quick-N-releasing ones are used. Splitting the organic fertilizer rate avoided a large amount of mineralized N to be leached in the watershed during the critical stage of drainage onset. The TIC was the best option in terms of environmental preservation, and assured a constantly higher grain protein content compared to the other organic fertilization treatments.

*Corresponding author (giacomo.tosti@gmail.com).
Dep. of Agricultural, Food and Environmental Sciences, Univ. of Perugia, Borgo XX Giugno, 74; 06121 Perugia, Italy. *Corresponding author (giacomo.tosti@gmail.com).

Abbreviations: BM, blood meal; ETc, crop evapotranspiration; NS, normal row spacing; RL, roasted leather; TIC, temporary intercropping; WS, wide row spacing.

Common wheat is the most important crop for human nutrition in Europe and in other temperate regions worldwide (FAOSTAT, 2014). At present, in addition to the needs of enhanced sustainability and reduced levels of external inputs, wheat production is also facing the threat of an increased occurrence and magnitude of adverse and extreme agro-climatic events (Asseng et al., 2015; Trnka et al., 2014). The implementation of solid agrobiodiversity strategies (Costanza and Bárberi, 2014), together with the recoupling of global biochemical C and nutrient cycles (Drinkwater and Snapp, 2007) in such a framework have the potential to improve the wheat production process in both organic and conventional, low input systems. The holistic approach to the entire cropping system management certainly represents the most informative one (Mäder et al., 2002). Nevertheless, detailed field studies on specific aspects of crop management remain essential for the fine tuning of concrete guidelines for farmers and policymakers (Bengtsson et al., 2005).

Among the several aspects of organic wheat production, N management is crucial (Bilsborrow et al., 2013), particularly for autumn-sown wheat in the Mediterranean area, as low organic matter content in the soil, low temperatures, and high autumn–spring rainfall can cause very low levels of available mineral N in the soil during most of the crop cycle (Fagnano et al., 2012). In conventional agriculture, the use of split application of mineral N fertilizers has been shown to increase fertilization efficiency and the harvest index of wheat (Blandino et al., 2015; Fuertes-Mendizábal et al., 2010). In organic farming, side dressing fertilization is not commonly used and the management of N fertilization is normally based on: (i) increasing the presence of legumes in the crop rotation (Thomsen et al., 2013), (ii) broadcasting organic compounds before sowing (Mazzoncini et al., 2015; Petersen et al., 2013) and (iii) adopting cereal-legume intercropping (Pelzer et al., 2012). An improvement in the use of legume crops represents the most promising option for the achievement of farm N self-sufficiency (Amosse et al., 2014; Farneselli et al., 2013). However, when an organic source of extra-farm N (i.e., agroindustry by-products) is easily available, then it may be both convenient and environmentally sound to rely on that source (Drinkwater and Snapp, 2007; Entry et al., 1997).

Every year, millions of tonnes of animal by-products are produced worldwide; for instance, the rendering industry...
processes nearly 15 Tg per year in the European Union (Swisher, 2006). Animal by-products are protein-rich materials and, therefore, offer a high potential as N-fertilizers (Mondini et al., 2008). Some of these residues are commercially available as certified organic fertilizers and their application as soil amendments is promoted as a beneficial agricultural practice. Blood meal and RL represent two of the main by-products derived from slaughtering and leather-making industrial processes, respectively. Both by-products are allowed to be used in the EU as fertilizers and soil conditioners in organic farming (Commission Regulation (EC) No. 889/2008).

Blood meal derives from the drying of whole blood from slaughtered animals (Dell’Abate et al., 2003). Roasted leather is generated when the hides and skins are scudded in the pre-tanning process and dehydrated; both compounds contain low and high molecular weight proteins and can be considered as a rich source of C and N for the production of organic fertilizers (Ravindran et al., 2013). These compounds are very different in terms of composition and N release rates, as BM is quite fast (Agehara and Warncke, 2005) and RL quite slow (Ravindran et al., 2013). Due to its rapid mineralization rate, BM could be profitably used for side-dressing fertilization in an organic system.

As stated, in addition to organic fertilization, cereal-legume intercropping is one of the agroecosystem strategies most investigated and adopted for wheat production (Costanzo and Barbieri, 2014; Pelzer et al., 2012). In a previous study, Tosti and Guiducci (2010) pointed out the advantages of cereal–legume TIC for durum wheat production in the Mediterranean environment. In the winter cereal–legume TIC, the legume is killed and incorporated into the soil when the cereal begins to shoot; thus, it is used with the sole purpose of improving N availability for the cereal. Cereal–legume TIC can represent a suitable technique to overcome some disadvantages of traditional intercropping, such as the need to keep the grains separated (especially for non-animal use), the difficulties in synchronizing the companion species cycles, and the consequent problems in the mechanization of harvesting operations.

More research is needed not only to evaluate BM, RL, and TIC as organic N sources for winter wheat fertilization, but also to consider the best timing for their broadcasting (or management) and the related environmental risk (Hartz and Johnstone, 2006).

The aim of this experiment was to compare five N fertilization strategies, suitable for organic wheat production (four fertilization treatments with extra-farm N sources and one temporary intercropping treatment), and to evaluate their effects on winter wheat N uptake, grain yield and quality, and N leaching in a Mediterranean environment.

**MATERIALS AND METHODS**

**Experimental Site**

Field experiments were performed in three consecutive years (2009–2010, 2010–2011, and 2011–2012) at the Experimental Station of the Department of Agricultural, Food and Environmental Sciences of the University of Perugia (Papiano, central Italy, 43° N, 165 m a.s.l.). The fields chosen for the experimental trial were homogeneous and the same preceding crop (*Helianthus annuus* L.) was adopted to minimize the differences among years. The soil was a clay-loam vertisol (Fluvic Haplustept), homogeneous (bulk density = 1.4 Mg m⁻³) in the top 0.9 m, with 46% silt, 34% clay, 20% sand, 1.2% organic matter (TOC = 9.3 g kg⁻¹ and total N = 0.82 g kg⁻¹), high contents of extractable P (29.9 mg kg⁻¹) and exchangeable K (258 mg kg⁻¹), and pH₄H₂O = 7.8.

Soil water content at field capacity (θₑ = m⁻³) and at permanent wilting point (θₑWP = m⁻³) were 43.8 and 22.0%, respectively. This soil type is commonly found in the alluvial plains and low hills of central and southern Italy.

Weather data were obtained from an automatic meteorological station inside the experimental site during the whole growing season.

**Treatments and Crop Management**

Each year, 11 treatments were tested in a randomized block design with three replicates: four organic fertilizer treatments, one faba bean/wheat temporary intercropping, two unfertilized controls, and four mineral fertilized controls (Table 1).

Common wheat (*Triticum aestivum* L. ‘Aubusson’) was grown at normal row spacing (0.250 m, normal row spacing [NS]) and fertilized with dry BM (fast mineralization: N = 14.5% and C/N = 3.03) or RL (slow mineralization: N = 11.0% and C/N = 6.39) in a single application (just before seeding, by incorporating the fertilizers into the top soil) at the rate of 80 kg N ha⁻¹ (BM₈₀ and RL₈₀, respectively) or in two applications (one-half dose just before seeding and one-half at tillering). In the split application treatments, to have a prompt response by the crop, the second application was broadcast using only BM, as it has a faster mineralization than RL. Thus, the treatments with side dress fertilization were labeled as RL₄₀/BM₄₀ (40 kg N ha⁻¹ as RL at pre-seeding and 40 kg N ha⁻¹ as BM at tillering) and BM₄₀/BM₄₀ (40 kg N ha⁻¹ as BM at both pre-seeding and tillering).

In addition to the organic fertilization treatments, wheat was sown at wide row spacing (0.375 m, wide row spacing [WS]) in TIC with faba bean (*Vicia faba* L. var. *minor* Beck. ‘Scuro di Torre Lama’). Wheat and faba bean were simultaneously sown, using a plot driller on 30 Oct. 2009, 15 Nov. 2010, and 3 Nov. 2011. The adopted seed rate was 400 kernels m⁻² for wheat and 80 seeds m⁻² for faba bean. In TIC, the intercropping composition was based on the additive principle (Snaydon, 1991), with faba bean seeds placed in the middle of the wheat inter-row space. At the beginning of the legume flowering (BBCH-scale: stage 50; Lancashire et al., 1991), faba bean plants were incorporated into the top soil (=0.15-m depth) by split rotary hoeing (23 Mar. 2010, 5 Apr. 2011, and 28 Mar. 2012). The wheat was beginning to shoot (Zadoks’s scale: stage 32; Zadoks et al., 1974) and no damage was caused to the wheat plants by this operation.

Six control treatments were included in the experimental layout: two unfertilized controls, where wheat was sown at normal (N₀NS) and wide row spacing (N₀WS), and four mineral fertilized controls, where N was applied as urea in two applications (one-half dose at tillering and one-half at shooting) at the dose of 80 kg N ha⁻¹ at normal (N₈₀NS) and wide (N₈₀WS) row spacing and at the non-N-limiting dose of 160 kg N ha⁻¹ at normal (N₁₆₀NS) and wide (N₁₆₀WS) row spacing.

In all the experimental years, wheat was harvested at the end of spring (2 July 2010, 23 June 2011, and 23 June 2012) using a plot combine.
Each plot measured 36 m² and buffer plots were arranged between treatments to avoid any edge effect. Phosphorus and K were applied as basal fertilization (as phosphoric anhydride and potassium sulfate, respectively) at seedbed preparation (90 kg ha⁻¹ of P₂O₅ and 90 kg ha⁻¹ of K₂O).

**Plant Sampling and Analyses**

Aboveground biomass and N accumulation of the crops was determined: at the time of faba bean incorporation into the soil, at wheat anthesis (15 May 2010, 10 May 2011, and 10 May 2012) and at wheat maturity (1 July 2010, 22 June 2011, and 21 June 2012). At each sampling, the aboveground biomass of the crops was hand harvested at ground level from an area of 1.35 m² in WS and 0.90 m² in NS, and it was separated by species at the time of faba bean incorporation into the soil. At wheat maturity, vegetative parts and grains of wheat were kept separated to determine grain yield and grain protein content. All samples were oven dried at 85°C until constant in weight and the dry material was then ground to a fine powder for N analysis. The organic N content was measured on Kjeldahl digests, prepared according to Isaac and Johnson (1976) using an auto analyzer (Flowsys Systea, Rome, Italy). The grain protein content of wheat was calculated on dry matter basis as N% × 5.7.

**Nitrate-Nitrogen Leaching and Soil Mineral Nitrogen**

Every year, two lysimeters consisting of porous, ceramic cups (32-mm external diameter by 95-mm length) were installed (Curley et al., 2010) in each plot (with the exclusion of treatments N80NS and N80WS) at a depth of 0.9 m. The cups were installed just after sowing by drilling the soil vertically at a depth of approximately 1.0 m. The excavated topsoil and lower subsoil were kept separate. Before placing the porous cup, thick slurry prepared from the lower subsoil was poured into the hole. The repacked soil was then added and consolidated with care to avoid preferential water flow. The ceramic cups (SDEC, Reignac-Sur-Indre, France) were joined to a capillary tube, long enough to emerge from the soil surface and sealed at the end by an iron clamp. Samples of the soil solution at 0.9 m were taken using a portable vacuum pump, and then transferred to a storage pot. The NO₃⁻–N concentration in the soil solution was determined by an ion-specific electrode meter (Cardy, Spectrum Technologies, Inc., Plainfield, IL), calibrated at the beginning of each measurement, set by using standard solutions provided with the testing kits (Farneselli et al., 2015). According to the method proposed by Gabriel et al. (2012), NO₃–N concentration data were considered only when soil solution could be successfully extracted from all lysimeters. This occurred after some rainfall restored soil moisture soon after sowing and later on, only after major rainfall (i.e., ≥20 mm).

A simplified model was adopted to estimate the drainage volumes (soil parameters are reported in Table 2). Crop evapotranspiration (ETc, mm) was calculated following the double coefficient procedure (Allen et al., 1998). To estimate the drainage below a depth of 1 m, the water balance of the

<table>
<thead>
<tr>
<th>Treatment code†</th>
<th>Row spacing</th>
<th>Applied N rate kg ha⁻¹</th>
<th>Fertilizer and application timing</th>
</tr>
</thead>
<tbody>
<tr>
<td>BM₄₀</td>
<td>Normal (0.250 m)</td>
<td>80</td>
<td>Blood meal at sowing</td>
</tr>
<tr>
<td>BM₈₀/BM₄₀</td>
<td></td>
<td>80</td>
<td>50% at tillering as blood meal and 50% at wheat shooting as blood meal</td>
</tr>
<tr>
<td>RL₈₀</td>
<td></td>
<td>80</td>
<td>Roasted leather at sowing</td>
</tr>
<tr>
<td>RL₄₀/BM₄₀</td>
<td></td>
<td>80</td>
<td>50% at tillering as roasted leather and 50% at wheat shooting as blood meal</td>
</tr>
<tr>
<td>N₀₉₅</td>
<td></td>
<td>0</td>
<td>Unfertilized control</td>
</tr>
<tr>
<td>N₀₈₅</td>
<td></td>
<td>80</td>
<td>50% at tillering and 50% at wheat shooting as urea</td>
</tr>
<tr>
<td>N₁₆₀₉₅</td>
<td></td>
<td>160</td>
<td>N assimilated by faba bean, the legume was incorporated into the soil at wheat shooting</td>
</tr>
<tr>
<td>N₀₉₅WS</td>
<td>Wide (0.375 m)</td>
<td>0</td>
<td>Unfertilized control</td>
</tr>
<tr>
<td>N₀₈₅WS</td>
<td></td>
<td>80</td>
<td>50% at tillering and 50% at wheat shooting as urea</td>
</tr>
<tr>
<td>N₁₆₀₉₅WS</td>
<td></td>
<td>160</td>
<td>50% at tillering and 50% at wheat shooting as urea</td>
</tr>
</tbody>
</table>

† BM, blood meal; RL, roasted leather; NS, normal row spacing; TIC, temporary intercropping; WS, wide row spacing.
shallow soil layer (1-m depth) was calculated by summing the daily difference between precipitation (P, mm) and ETc to the measured soil water content at the crop sowing date. When the layer under examination reached field capacity (and until field capacity was maintained) the rainfall events were considered equal to the drainage volume below a depth of 0.9 m. As proposed by Gabriel et al. (2012), the NO₃⁻N leached over time intervals between soil solution samplings was calculated as the product of mean NO₃⁻N concentration in the soil solution and the daily drainage obtained for the sampling interval.

Soil samples for the analysis of mineral nitrogen content (Nmin) were taken at sowing (i.e., just before the distribution of the organic fertilizers) and at wheat maturity. Samples were taken using a soil piston auger in two locations per plot, at intervals of 0.3 m in depth up to a depth of 0.9 m. The two subsamples within each soil layer were bulked and frozen until the time of analysis, and then defrosted to take subsamples of 30-g fresh weight and obtain extracts in 1 M KCl (soil/solution ratio 1:2). Soil extracts were filtered and analyzed for nitrate and ammonium in accordance with the methods of Best (1976) and Crooke and Simpson (1971), respectively. The Nmin was determined as the sum of NO₃⁻N and NH₄⁺-N content.

**Apparent Nitrogen Balance and Nitrogen Release Efficiency in the Organic Fertilization Strategies**

A simplified apparent N balance (Hartmann et al., 2014) was calculated for each year, and organic fertilization treatment as the difference between N inputs (IN) and N outputs (OUT) was as follows:

\[ \text{IN} - \text{OUT} = (N_S + \text{ANM} + N_F) - (N_H + N_L + N_U) \]  

where \( N_S \) and \( N_H \) are soil mineral N contents (0–0.9 m) at sowing and harvest, respectively, ANM is the apparent net soil N mineralization (base N mineralization), \( N_F \) is the N released by the considered organic fertilizer, \( N_L \) is the total amount of N taken up by wheat at harvest and \( N_U \) is the N lost through the nitrate leaching process during the entire crop growing cycle. All values are expressed as kg Nmin ha⁻¹.

The ANM and \( N_F \) were estimated considering IN = OUT in Eq. [1]: ANM was calculated in the unfertilized (i.e., where \( N_F = 0 \)) control treatments (\( N_0N_S \) and \( N_0W_S \)) and it was used for estimating the base N mineralization (Cabrera and Kissel, 1988; Zhao et al., 2006) as:

\[ \text{ANM} = (N_U + N_H) - (N_L + N_S) \]  

In treatments where wheat was grown in pure stand, \( N_F \) was calculated as:

\[ N_F = (N_H + N_U + N_L) - (N_S + \text{ANM}) \]  

Since a portion of mineral N in TIC is absorbed by legume plants during the co-growth period, the portion of N derived from atmosphere via symbiotic fixation (%Ndfa) was considered equal to 90% of the total N accumulation in the faba bean aboveground biomass, according to the findings reported by Antichi (2013) for the same experimental years and similar pedoclimatic conditions. Therefore, the mineral N absorbed by legume plants in TIC (i.e., 10% of total N accumulation) was subtracted from ANM, and \( N_F \) was calculated as:

\[ N_F = (N_H + N_U + N_L) - [N_S + (\text{ANM} - 0.1 \times N_{Ufb})] \]  

where \( N_{Ufb} \) represents the total amount of N accumulated in the faba bean biomass at soil incorporation.

Afterward, the nitrogen release efficiency (NRE%) of each organic fertilization strategy was simply calculated as the ratio between \( N_F \) and the ex-novo N supplied (\( N_{supp} \)) with organic fertilizer (80 kg N ha⁻¹) or the incorporation of faba bean soil (i.e., 90% of the total N in faba bean biomass) as follows:

\[ \text{NRE\%} = N_F/N_{supp} \]
Data Analysis

All experimental data were subjected to an ANOVA. Due to the lack of variance homogeneity, each year was analyzed separately and the standard error of the mean (SEM) was used as the variability index for each year.

All the statistical analyses (Bartlett’s test included) were performed by using the software R (R Core Team, 2014).

RESULTS

Weather Conditions and Soil Mineral Nitrogen

In the first 2 yr, the total rainfall during the wheat cycle was high (758 mm in 2009–2010 and 673 mm in 2010–2011), whereas the last year was drier (377 mm) (Fig. 1). The temperature regime was similar in the first 2 yr and was in line with the 30-yr average, whereas the third year was consistently colder (particularly from October to February). However, wheat and faba bean plants grew regularly and never showed any frost damage.

Soil N\(_{\text{min}}\) observed at sowing was rather low in the first 2 yr (72 kg ha\(^{-1}\) in 2009–2010 and 56 kg ha\(^{-1}\) in 2010–2011), whereas it was high in the third year (117 kg ha\(^{-1}\), Fig. 2). The N\(_{\text{min}}\) values recorded at wheat harvest were again the highest in 2011–2012, whereas no treatment showed a clear tendency across the years, except for a certain higher N\(_{\text{min}}\) observed in TIC (Fig. 2).

Faba Bean and Wheat Competition in Temporary Intercropping

At the time of faba bean incorporation into the soil, dry biomass and N accumulation of TIC wheat were quite low and similar in 2010 and 2011 (1.35 Mg ha\(^{-1}\) and 27 kg N ha\(^{-1}\), on average) whereas in 2012, they were higher, reaching 2.4 Mg ha\(^{-1}\) and 62 kg N ha\(^{-1}\); the faba bean dry biomass and N accumulation showed decreasing values across all 3 yr (Fig. 3).

Total N accumulation in the faba bean aboveground biomass was equal to 89, 55, 44 kg ha\(^{-1}\) in 2010, 2011, and 2012, respectively (Fig. 3). Assuming that 90% of the total N accumulated in the legume biomass derived from the atmosphere via symbiotic fixation (Antichi, 2013), those values corresponded to an N supply ex-novo of 80, 50, and 39 kg ha\(^{-1}\) in 2010, 2011, and 2012, respectively. The comparison of wheat performance in TIC vs. unfertilized control (N\(_{0\text{WS}}\)) showed an advantage for wheat in 2010 (+5% dry biomass, +28% N uptake), and a disadvantage in 2011 (~20% dry biomass, ~8% N uptake). In 2012, TIC wheat biomass was slightly reduced (~2%), but N accumulation improved (+13%) compared to N\(_{0\text{WS}}\).

Wheat Nitrogen Uptake

Wheat N uptake was influenced by treatments in all 3 yr (Fig. 4). In 2012, the N uptake recorded in all treatments was considerably higher compared to the other years. Whereas in 2010, RL\(_{80}\) and BM\(_{80}\) had an equivalent effect on wheat N nutrition, in 2011 and 2012 the effect of BM\(_{80}\) was more positive than RL\(_{80}\).

The results of the two broadcasting methods varied over the 3 yr. In 2010, splitting the N dose had a positive effect, particularly on BM. Vice-versa, in 2011 fertilization in a single application at pre-seeding gave a better wheat performance in terms of N uptake. In 2012, no difference was observed between the two broadcasting methods.

In 2010, the N uptake observed in TIC wheat was not significantly different (\(P < 0.05\)) to treatments that received the side-dress fertilization and to N\(_{80\text{WS}}\). In 2011, TIC wheat N uptake was low and similar to RL\(_{40}/BM_{40}\), and, in 2012, it was the lowest compared to the other organic, fertilized treatments.

Yield and Grain Protein Concentration

Wheat yield and grain protein concentration were influenced by treatments in all 3 yr (Table 3). Among the organic fertilizers, BM showed a better effect on yield compared to RL. Splitting the organic fertilization had a positive effect on yield only in 2010 and, in particular, the most evident effect was observed in BM treatments that generally showed a slightly higher yield compared to RL in all 3 yr. The TIC yield was similar to BM\(_{80}\) in 2010 (+31% as compared to N\(_{0\text{WS}}\)), whereas in 2011 and 2012, it was significantly lower compared...
Fig. 3. Dry biomass (Mg ha⁻¹) and N (kg ha⁻¹) accumulated by wheat (dark columns) and faba bean (light columns) in temporary intercropping, recorded at faba bean soil incorporation in the 3 yr of the experiment. Percentages reported above the dark columns represent the variation in temporary intercropped wheat (TIC) as compared to the unfertilized, wide row spacing, control treatment (N₀WS) observed at the same time. Bars indicate ± 1 SE.

Fig. 4. Nitrogen uptake of wheat recorded at shooting, anthesis and maturity in the 3 yr of the experiment. Bars indicate ± 1 SE.
to the other organic fertilization treatments, and similar (2011) or slightly higher (2012) than in N0WS.

Grain protein concentration was poorly affected by splitting the N rate (Table 3); only in 2011, BM80 showed a significant improvement of this parameter compared to BM40/BM40. The BM80 generally showed a slightly higher grain protein content than RL 80 (especially in 2011 and 2012). The TIC allowed a major improvement in wheat grain protein concentration in all 3 yr (this positive effect was less relevant in 2012).

### Nitrogen Release Efficiency

The NRE% values were similar across the 3 yr (Fig. 5). Among the organic fertilization strategies, the use of BM and TIC showed the highest values, whereas RL was constantly lower compared to the other treatments. In 2010, extra-farm organic fertilizers showed similar NRE values (56%, on average), whereas the release efficiency of N supplied by the mineralization of faba bean in TIC reached 83%. In 2011, NRE% was rather low in all treatments (except for TIC and BM80), and, only in this year, did splitting the N dose generate an NRE% reduction. In the third year, BM40/BM40, BM80, and TIC confirmed they were more efficient in terms of N release compared to RL80 and RL40/BM40.

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**Table 3.** Grain yield (Mg ha⁻¹, dry matter) and grain protein content (N % × 5.7) recorded at wheat harvest in the 3 yr of the experiment.

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† BM, blood meal; RL, roasted leather; NS, normal row spacing; TIC, temporary intercropping; WS, wide row spacing.

‡ SEM: Standard error of the mean.

F test significant at P £ 0.05.  
** F test significant at P £ 0.01.  
*** F test significant at P £ 0.001.

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Fig. 5. Proportion of N released on total amount supplied (N release efficiency, %) by the organic fertilization strategies at wheat maturity in the 3 yr of the experiment. Bars indicate ± 1 SE. See section Apparent Nitrogen Balance and Nitrogen Release Efficiency in the Organic Fertilization Strategies within the Materials and Methods section for further details.
Nitrate-Nitrogen Concentration in the Drainage and Overall Cumulated Nitrate-Nitrogen Leaching

Rainfall during the crop cycle caused drainage below a soil depth of 0.9 m only in 2009–2010 and 2010–2011 (Fig. 6). Overall drainage was higher in 2009–2010 than in 2010–2011, but the 2 yr were also different in terms of drainage dynamics: drainage in 2009–2010 started 1 mo later compared to 2010–2011 (i.e., beginning of January vs. beginning of December), but it was faster (i.e., it was high from the initial phase).

The NO₃–N concentration in the soil solution at a depth of 0.9 m showed a decreasing trend for all treatments in both years (Fig. 6). The NO₃–N concentrations observed in BM₈₀ were generally higher than those in RL₈₀, especially during the initial part of the drainage. Splitting the N dose reduced the NO₃–N concentration in the soil solution until side-dress fertilization with BM. Afterward, the NO₃–N concentration in BM₄₀/BM₄₀ and RL₄₀/BM₄₀ increased, reaching levels similar (2009–2010) or even higher (2010–2011) than those recorded in pre-seeding fertilization treatments (BM₈₀ and RL₈₀). Splitting the

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**Fig. 6.** Cumulated drainage (mm) below a depth of 0.9 m (solid line) and NO₃–N concentration (mg L⁻¹) in the soil solution at a depth of 0.9 m during the 3 yr of the experiment. Arrows indicate: side-dress organic fertilization (SD), first mineral fertilization (MF1), faba bean incorporation into the soil (FBI) and second mineral fertilization (MF2). Bars indicate ± 1 SE. Note that in 2012 no drainage was observed.
N rate reduced the NO₃⁻-N concentration in the soil solution. However, the two fertilizers showed a different behavior: the reduction in RL₄₀/BM₄₀ was limited to the initial phase of drainage where it was evident, whereas BM₄₀/BM₄₀ showed lower values than BM₂₀ for a longer period.

During the first phase of drainage, all the extra-farm organic fertilizers promoted higher NO₃⁻-N concentrations compared to the unfertilized controls (at both row distances). The effect of mineral fertilization on NO₃⁻-N concentration varied according to row spacing and year: values in N₁₆₀_NS were similar to those observed in the unfertilized control in the first year, whereas in the second year, a gradual increase was recorded after the first fertilizer distribution. N₁₆₀_WS increased NO₃⁻-N concentration to a greater extent than N₁₆₀_NS in both years.

From the first sampling date, NO₃⁻-N concentrations observed in TIC were not significantly different (2010) or even lower (2011) than those observed in the unfertilized controls.

The amount of NO₃⁻-N leached was similar in 2010 and 2011 (30.5 kg NO₃⁻-N ha⁻¹, on average) and the effect of treatments was rather similar in these 2 yr (Table 4).

As expected from the drainage dynamics (Fig. 6), the majority of the total amount of NO₃⁻-N was leached before the date of faba bean incorporation into the soil, without any significant difference between the treatments (ranging from 83% in 2010 to 99% in 2011, on average). As observed for the NO₃⁻-N concentrations, the amount of NO₃⁻-N leached in BM₈₀ was higher than RL₈₀ (Table 4). Treatments where side dress fertilization was applied were able to better reduce the amount of NO₃⁻-N leached below a depth of 0.9 m compared to treatments with all-at-once fertilization at pre-seeding stage. The effect of row spacing on the NO₃⁻-N leaching was generally negligible. Only in 2011, did wide row spacing cause a slight increase in leaching in the fertilized control treatments, compared to normal row spacing. The TIC mitigated NO₃⁻-N leaching both during the co-growth phase and after faba bean incorporation into the soil. The TIC showed the lowest amounts of leached NO₃⁻-N of all the fertilized treatments, reaching values which were not statistically different from the unfertilized controls.

### DISCUSSION

**Competitive Interaction between Faba Bean and Wheat**

Not only the highly variable rainfall and temperature regime (Fig. 1), but also the variable Nₘᵢ₇ at sowing (Fig. 2) across years, led to very different interaction dynamics between wheat and faba bean.

The biomass and N accumulation of faba bean dropped throughout the 3 yr (Fig. 3) because Nₘᵢ₇ at sowing and the rainfall regime during co-growth period gave an increasing competitive advantage to common wheat (from the first to the third year), that represented the main factor affecting the faba bean performance. However, common wheat did not show a complementary linear increase across years as its dry weight and N accumulation were more influenced by the amount of Nₘᵢ₇ at sowing, rather than by faba bean competition. As reported by many authors, Nₘᵢ₇ at sowing and the rainfall regime during co-growth period are two of the main factors affecting the output of the interaction between legume and non-legume plants in crop mixtures (Bedoussac and Justes, 2010a; Tribouillois et al., 2015). Our data confirm that the N accumulation of faba bean was strongly impaired by the high Nₘᵢ₇ content at sowing (Fig. 2 and 3). Faba bean exerted a buffer effect for TIC wheat N nutrition. When the initial conditions are unfavorable for wheat (as occurred in 2009–2010 or 2010–2011), then faba bean growth (and N assimilation) could not be limited by the competitive cereal effect (Klimek-Kopyra et al., 2013) and high amounts of N could be supplied to the cereal by incorporating legume into the soil. Thus, not only the chances of a fast growth and recovery of the TIC wheat are established, but also the conditions for a positive improvement of grain quality. When the initial conditions are actually favorable for wheat (as occurred in 2012), then faba bean growth and its competitive effect on wheat is reduced. Under such circumstances, from the very first growing phase, TIC wheat can benefit from the well-known

<table>
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<th>Row spacing</th>
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<th>2010</th>
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<td>Percent at FB soil incorporation</td>
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F test significant at P £ 0.05.

† BM, blood meal; RL, roasted leather; NS, normal row spacing; TIC, temporary intercropping; WS, wide row spacing.

‡ SEM, Standard error of the mean.
early stage advantages (Benincasa et al., 2012; Fayaud et al., 2014), whereas faba bean, impaired by a competitive cereal effect, will be pushed to take N for its growth from the atmosphere (Jensen, 1996), and such N will become available once again for the cereal after legume incorporation into the soil (i.e., green manure effect).

As observed in several other intercropping studies (Ghaley et al., 2005), the low soil mineral N level emphasizes the beneficial effect of cereal–legume intercropping. Our study confirms the positive effect of the legume on TIC wheat N uptake, which was always more than proportional compared to dry weight accumulation (Fig. 3).

**Effect of the Nitrogen Fertilization Strategies on Wheat N Uptake**

The effect of organic fertilization strategies on wheat N uptake depended strictly on the year, as a consequence of the observed variability in the availability of the mineral N in the soil at sowing and on drainage timing and intensity (Fig. 2 and 6). In the autumn–winter period, the mineralization rate is reduced by low temperatures and soil oxygen concentration. However, many experiments have shown that mineral N is also released in cold soils (Magid et al., 2001), even though soil temperature is below 5°C.

When N leaching was present, the organic fertilization strategies gave different results in terms of wheat N uptake, and differences were detected both between the two extra-farm, organic fertilizers and between broadcasting methods. In 2010, an intense drainage event was observed during the first decade of January (starting 67 d after sowing, DAS), so it is reasonable to assume that a consistent part of mineralized N from RL80 and BM80 was leached downward and lost: splitting the N dose showed, in fact, a good result on wheat N uptake in that year (Fig. 4 and 6). In 2011, on the other hand, drainage started earlier (from 25 DAS) and it was more gradual compared to the previous year. This made fertilization in a single application at pre-seeding more efficient than the side-dressing fertilization (Fig. 4 and 6). In the absence of drainage (i.e., in 2012), the difference in terms of wheat N uptake between broadcasting methods was negligible, and BM was better than RL (Fig. 4). Nitrogen uptake observed in TIC wheat was similar to that of RL80/BM80, except for 2012, when it was lower than the mean N assimilation observed in all the other organic fertilized treatments. Such behavior was strictly related to the variable amount of N supplied by faba bean biomass (Fig. 3), which accounted for 99, 62, and 49% of the amount supplied with the dry-furrow periods and is leached out of the soil profile, when high NO$_3$–N concentrations can frequently be associated with high drainage volumes (and the early growth stage of winter wheat), as in 2010 (Fig. 6). This situation is highly variable across years and represents a fairly common condition in the Mediterranean regions, in which soil N$_\text{min}$ accumulates during the dry furrow periods and is leached out of the soil profile, when a heavy rainy season occurs (Gabriel et al., 2012). Thus, using organic fertilizers at pre-seeding stage appears to be a very risky practice, especially if quick-N-releasing ones are used. Splitting the organic fertilizer rate prevents important N leaching during the initial stages, when the crop is not capable of a major N fixation (Porter, 2006). Considering the yield achieved with the extra-farm organic N sources, only in 2011, a small increase was observed for both RL$_{80}$ and BM$_{80}$ compared to their respective split applications (Table 3), but such a small yield improvement corresponded to a high increment of N lost in the watershed (Table 4).

The TIC ensured a constantly higher grain protein content compared to the other organic fertilization treatments. Grain protein content represents a fundamental aspect of grain quality (Troccoli et al., 2000). As pointed out by Bedoussac and Justes (2010b), the effect of dilution is very important when dealing with intercropping. However, the grain quality observed in TIC wheat was constantly at a high level, although the yield resulted significantly lower than that of the organic fertilized treatments in 2 (2011 and 2012) of the 3 yr (i.e., when the N supplied by faba bean biomass was consistently below 80 kg N ha$^{-1}$, Fig. 3 and Table 4). Considering the general low quality of organic wheat grain, it is quite usual for the farmers to gain a substantial increase of the selling price for slight improvement of the grain protein content (Mazzoncini et al., 2015), so even a small increase should not be disregarded in terms of net income for the farmers.

**Effect of the Nitrogen Fertilization Strategies on Nitrate-Nitrogen Leaching**

In the Mediterranean region of Europe, increases in the frequency of extreme climate events (such as intense rainfalls) expected during the initial stages of winter crops are likely to reduce yield (Asseng et al., 2015). Our results clearly show that the risk of N leaching is maximum at the onset of drainage, when high NO$_3$–N concentrations can frequently be associated with high drainage volumes (and the early growth stage of winter wheat), as in 2010 (Fig. 6). This situation is highly variable across years and represents a fairly common condition in the Mediterranean regions, in which soil N$_\text{min}$ accumulates during the dry furrow periods and is leached out of the soil profile, when a heavy rainy season occurs (Gabriel et al., 2012). Thus, using organic fertilizers at pre-seeding stage appears to be a very risky practice, especially if quick-N-releasing ones are used. Splitting the organic fertilizer rate prevents important N leaching during the initial stages, when the crop is not capable of a major N uptake from the soil (Fuertes-Mendizábal et al., 2010). In our experiment, the N lost after the side-dressing operations, even though significant in terms of concentration (Fig. 6), resulted negligible due to the usual low drainage volume occurring during this phase (Anderson, 1985).

The TIC was the best option in terms of environmental preservation, and it appeared even more efficient than unfertilized control in reducing NO$_3$–N concentrations in drainage water and, therefore, the overall amount of leached N (Table 4). Our results also confirm a low risk of N losses after
faba bean incorporation into the soil because, at that growth stage, the wheat root system is able to intercept the N released from the mineralizing biomass of faba bean. Even though the soil N$_{\text{min}}$ at harvest in TIC was generally higher when compared to the other organic treatments (Fig. 2), the actual effect of such a surplus on the risk of N leaching appears negligible, especially if compared to the values observed in the unfertilized controls.

CONCLUSIONS

The contrasting climatic conditions in the 3 yr of the experiment allowed robust conclusions to be drawn from our findings. Blood meal was the best organic fertilizer for common wheat N nutrition and grain yield, but it also implied the greatest potential risk in terms of N leaching. On the other hand, RL showed an N release that was too slow, and therefore it appeared to be an inefficient source of N for organic common wheat production. Splitting the rate of organic fertilizers resulted an effective technique as, without jeopardizing grain yield, it limited the N losses by reducing the leaching risk at the time of drainage onset (i.e., when the risk is highest).

Temporary intercropping with faba bean confirmed it was a promising technique to improve the quality of organic wheat in Mediterranean areas. Faba bean biomass incorporation into the soil, when wheat begins to shoot, supplied the wheat with the relevant amount of N: fairly high amounts in years with low soil N fertility (i.e., when wheat is a weak competitor), moderate-low amounts in years with high soil N fertility (i.e., when wheat is a strong competitor). The greatest part of the organic N supplied by faba bean was released (average NRE% = 69%) and used by wheat during the late growing cycle. Thus, the temporary intercropping between wheat and faba bean represents a strategy which maintains a positive effect on both production quality (wheat protein concentration) and the environment (reduction of N leaching losses). The effects of temporary intercropping on wheat yield are still marginal and the technique needs to be better investigated, especially by considering factors such as: choice of legume/wheat species and varieties, timing/techniques of legume biomass incorporation, and/or the possibility of coupling the faba bean incorporation into the soil with the application of a fast mineralizing organic compound (e.g., BM).

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