Università di Pisa

Corso di dottorato congiunto tra Università di Pisa e Scuola
Superiore Sant'Anna in "Scienza delle Produzioni Vegetali"

XXIV Cycle (2009-2011)
S.S.D. AGR/02

# Effect of intercropping on yield and quality of organic durum wheat

*Candidato:* Daniele Antichi

*Coordinatore del Corso:* Chiar.mo Prof. Alberto Pardossi

*Relatori:* Chiar.mo Prof. Marco Mazzoncini
Chiar.mo Prof. Paolo Bàrberi

Settembre 2013

*To Jesus, my Lord*

*... e all'Italia....*

# Acknowledgements

My PhD scholarship was funded by the Scuola Superiore Sant'Anna within the frame of the joint PhD Programme on Crop Science of University of Pisa and Scuola Sant'Anna.
The present research activity was part of the National Research Project NITBIO *Interventi agronomici atti ad ottimizzare la disponibilità di azoto per la produzione di frumenti di qualità in agricoltura biologica*) project (2010-2013), funded by the Italian Ministry of Agriculture (MIPAAF) and coordinated by University of Perugia, in the person of Prof. Marcello Guiducci.

This doctoral dissertation is lovingly dedicated to all the very special people taking part of my life.
First I would like to thank my immediate family members: Stefania, my spouse and my life's companion, and also Vivi e Susi, my little princesses. "*There's so much I wanna say....",* but I can't express all my feelings for you. Simply, "thanks" for all the patience and love in all these months of hard work when I was frequently on edge. You are really part of me and nothing of what I do would be possible without all of you, my dear! *Vi amo tutte, amori miei!*
My thankfulness and love also to all the other relatives "behind the curtain": my beloved mam, Luciana (*ti voglio bene!*), and to my very special parents in law, Nadia and Stefano, who encouraged me and gave me affection every time I needed.

A special thanks to my tutors, Marco and Paolo, who gave me in all these years not only professional support but also sincere friendliness and authentic humanity. I could not express in the right way my feeling of gratitude for you both, thanks for the opportunity you gave me to enter into the world of science and to improve year by year.

A "very warm" thanks to all the staff (Henrik, Dorette, Claus, Ingelis, Ana, Per and oll the others) of the former building 309 of the Biosystems Division of Risø DTU in Roskilde for their precious help and humanity. All of you gave his best to make me feel real warmth when I was there in the cold cold North!!! Thanks a lot to Per, for his very kind and fruitful collaboration for the $^{15}$N analysis of my samples. Above all, my gratitude goes to Henrik, not only my *guru of intercropping and $^{15}$N*, but a very special person and friend, indeed! *Tak!!!*

Thanks also to all the partners of the NITBIO Project (Marcello Guiducci, Giacomo Tosti, Maurizio Perenzin, Fabrizio Quaranta, Maria Grazia D'Egidio, Cristina Cecchini and their colleagues) for their precious advices and kind cooperation.
I would like to thank also the staff of the former Dipartimento di Agronomia (DAGA), and especially to Professor Alessandro Masoni and his team (Prof. Laura Ercoli, Prof. Marco Mariotti, dr. Iduna Arduini, dr. Silvia Pampana), for their appreciable scientific advices, Professor Luciana Angelini and dr. Silvia Tavarini, Filippo, Silvia and all the administrative staff.

And last, but not least, I would like to say a very huge "thanks" as well to all my colleagues and friends who helped me in this work, or simply made my life easy in the worst moments:

- all the staff of "Rottaia": Lorenzo, Romano, Fabrizio, Marco, Mario, Salvatore. Without your "fantasy" and your technical skills, my crops would have not had any chance to grow...;
- all the staff of the CIRAA who participated at the research work: "il laboratorio di Luciano" as a whole (Luciano and his very special workers: Nadia, Rosenda, Serena, who prepared mountains of samples), "la Mandria" (Rosalba, Sabrina, Lucia e Laura, who analyzed all my thousand and thousand samples, and above all who prepared hundreds of small tin capsules...), "la Sperimentazione" (Alino, Marco e Giovanni, "i miei mietitrebbiatori atomici!");
- Roberta and Andrea and their chemical lab;
- all my very special friends and colleagues: Massimo (*a voglia! ce l'ho!)*, Stefano, Federica, Camilla, Paola, Betti, Marco, Antonio, Giulia, Gianluca, Andrea, Ambrogio, Gionata,Giacomo who helped me physically and gave me lot of friendship in these very busy moments. Thanks to all of you!!!
- all my Phd-students colleagues: Domenico, Sandro, Eleonora, Francesca, Raffaella, Chiara, Giovanna. Good luck to all of you!
- Professor Alberto Pardossi: thanks for your encouragement and your infinite patience!

# Summary

Intercropping can be one of the most promising tools for organic farmers for designing more sustainable cropping systems as well as for improving yield quantity and quality of many crops. A particular kind of intercropping is performed by growing together a cash crop (usually a cereal) with a legume cover crop to reduce weed incidence and to supply more nitrogen to the cereal. The latter objective is particularly difficult to achieve by organic farmers, who can rely neither on external chemical inputs nor on cheap and effective organic fertilizers.

A two-years field experiment was carried out at the Rottaia Experimental Station of the Dipartimento di Scienze Agrarie, Alimentari e Agro-ambientali of the University of Pisa, aimed at studying the effect of permanent and temporary intercropping with hairy vetch (*Vicia villosa* Roth), pigeon bean (*Vicia faba* var. minor Beck) and field pea (*Pisum sativum* L.) on grain yield and quality of durum wheat (*Triticum durum* Desf.). In temporary intercropping treatments, the legumes were incorporated into the soil by a rotary hoe at the beginning of stem elongation stage of the cereal. Additionally, five durum wheat pure crops at increasing rate of mineral N fertilization (0-40-80-120-160 kg N ha$^{-1}$ as ammonium nitrate) and the pure stands of the three legumes were included as control treatments.

In both years, legumes were excessively competitive with cereal when permanently intercropped, whilst they were able to support wheat grain yield when incorporated into the soil at the beginning of stem elongation phase of wheat. All the main parameters of wheat grain quality at maturity (protein content, gluten content, SDS, test weight) were significantly increased by intercropping only in the second year. Nitrogen fixation of legumes was assessed by $^{15}$N natural abundance technique in both years. Results revealed a stimulation of symbiotic N-fixation in legumes intercropped with wheat, due to the stronger competitive ability of the cereal for soil mineral nitrogen. Anyway, a significant transfer of N fixed by legumes to wheat was not observed, and neither was a residual effect on the yield of the following maize crop.

A parallel experiment was carried out in lysimeters in order to assess the main differences in terms of N availability among several strategies of N fertilization of durum wheat: no fertilization, mineral fertilization (80 kg N ha$^{-1}$, split in two applications), organic fertilization (80 kg N ha$^{-1}$ as dried blood) applied once or split in two applications of half rate each, temporary intercropping with pigeon bean.

Due to the composition of the soil, rich in sand (86%) and poor in organic matter (0.8%), organic fertilizer and intercropping were not fully mineralized over the growing period of the wheat. Consequently, neither the yield nor the grain quality of the wheat were positively affected by these treatments. In terms of N budget, until the harvest date of wheat all the treatments, except for intercropping, produced a significant N surplus. In the fallow year, only for mineral fertilization a minimum surplus of N was still recorded, whilst organic fertilizers and intercropping showed higher output than input.

Temporary intercropping between wheat and facilitative legumes was concluded to be a promising way to enhance in the short term the grain quality of durum wheat, without neither significant yield losses, nor negative externalities on the environment.

Anyway, some bottlenecks in terms of excessive competition for niches among intercrops, and also from the point of view of mechanization were also highlighted. Permanent intercropping, on the other hand, was shown to have the potential to significantly increase land use efficiency of crops, provided that only low inter-specific and intra-specific competitions take place.

# Riassunto

La tecnica della consociazione rappresenta uno degli strumenti più promettenti a disposizione degli agricoltori biologici per il miglioramento quali-quantitativo delle rese colturali, contribuendo inoltre ad incrementare la loro stabilità nel tempo, nonché l'efficienza d'uso delle risorse dei sistemi colturali. Questi aspetti appaiono particolarmente rilevanti nell'attuale contesto di mitigazione dei cambiamenti climatici, nel quale l'agricoltura è chiamata a recitare un ruolo importante, attraverso il contenimento delle emissioni di gas serra ed un migliore uso delle risorse non rinnovabili. Una tipologia particolare di consociazione, denominata *facilitative intercropping*, si realizza mediante la coltivazione simultanea sulla stessa unità di superficie di una coltura cerealicola da reddito, come ad esempio il frumento, e di una coltura di copertura leguminosa, destinata unicamente a fornire servizi ecologici utili ai fini produttivi del cereale. In particolare, nell'ambito delle produzioni cerealicole organico-biologiche, ampio risalto è dato al contenimento della flora infestante e all'apporto di azoto di provenienza biologica, fissato dalla specie leguminosa mediante l'instaurarsi di una simbiosi radicale con i batteri del genere *Rhizobium* e, quindi, trasferito al cereale. La scarsa disponibilità di azoto in prossimità delle fasi determinanti per la quantità e la quantità delle produzioni, oltre all'eccessivo sviluppo della flora infestante, rappresentano infatti i principali fattori limitanti l'ottenimento di rese granellari soddisfacenti e di alto valore commerciale per il frumento duro coltivato in agricoltura biologica nei nostri ambienti.

Al fine di valutare gli effetti di questa tecnica, tra il 2009 ed il 2011 sono stati condotti due esperimenti in parallelo, realizzati presso la Stazione Sperimentale di Rottaia del Dipartimento di Scienze Agrarie, Alimentari ed Agro-ambientali dell'Università di Pisa.
Nel primo esperimento, condotto in campo su scala parcellare, sono state poste a confronto due diverse strategie di gestione (temporanea, con sovescio in coltura della leguminosa alla levata del cereale *vs* permanente, con il mantenimento della leguminosa fino alla raccolta) della consociazione tra il frumento duro e una delle tre diverse specie di leguminosa da granella esaminate (veccia vellutata, favino e pisello proteico). Complessivamente, le consociazioni temporanee, in particolare quelle con veccia e favino, si sono rivelate in grado di determinare rese superiori per quantità e qualità rispetto a quelle del frumento coltivato in purezza con la medesima disposizione spaziale a file larghe.

Un incremento significativo dei valori del contenuto proteico, del tenore di glutine e dell'SDS è stato osservato solo nel secondo dei due anni, in entrambe le tipologie di consociazione. Le tesi gestite in modo permanente hanno determinato significativi cali produttivi del cereale, dovuti all'insorgere di fenomeni competitivi eccessivi tra le colture consociate. In particolare, il favino ha mostrato la maggiore abilità competitiva nei confronti del frumento, che si è manifestata con un forte ombreggiamento del cereale sin dalla fase di accestimento. L'analisi degli indici di competizione ha permesso di individuare proprio in questa elevata competizione interspecifica durante le prime fasi di sviluppo delle colture il fattore limitante lo sviluppo armonico delle specie consociate. Ciò nonostante, le colture permanenti hanno mostrato i valori più elevati di produzione di biomassa totale delle colture, oltre che i livelli più bassi di presenza delle infestanti, confermando le indicazioni riportate in bibliografia di un miglior uso delle risorse per unità di superficie. La forte competizione tra frumento e leguminose ha prodotto un livello maggiore di fissazione simbiontica dell'azoto atmosferico all'interno delle consociazioni, rispetto alle leguminose coltivate in purezza, confermando l'ipotesi dell'importanza dell'azoto come fattore limitante le rese nei sistemi cerealicoli.

In un secondo esperimento, condotto in vasche lisimetriche, la consociazione temporanea tra frumento duro e favino è stata posta a confronto con la coltura pura di frumento duro, coltivato anch'esso a file larghe e concimato con 80 unità di N proveniente da diverse fonti (nitrato ammonico frazionato in due interventi al 50% della dose ciascuno, sangue secco frazionato in due interventi al 50% della dose ciascuno, sangue secco distribuito in un unico intervento), oltre ad un testimone non fertilizzato. In questo caso, la consociazione non ha prodotto un incremento significativo né delle rese granellari, né della produzione complessiva di biomassa rispetto al testimone. Questo è stato dovuto principalmente alla natura del terreno, caratterizzato da una tessitura sabbiosa e da un ridotto tenore in sostanza organica, fattori che hanno influenzato negativamente la dinamica di mineralizzazione delle matrici organiche apportate al terreno (sia della leguminosa sovesciata, sia del concime a base di sangue secco). Le analisi delle acque di lisciviazione non hanno messo in evidenza particolari differenze tra i trattamenti in termini di presenza di nitrati né durante la permanenza delle colture in campo, né nel periodo in cui il terreno è stato mantenuto incolto a seguito della raccolta del frumento. Tuttavia, la stima del bilancio apparente dell'azoto ha messo in evidenza valori di surplus positivi per le tesi concimate e negativi per la tesi consociata, contribuendo a confermare l'ipotesi di un minor rischio di perdite di azoto per quest’ultima, in condizioni di bassa disponibilità dell’elemento, ma di elevata piovosità.

# Table of Contents

# 1. Introduction

## 1.1 Future challenges for agriculture

Sustainable agriculture has been defined in many different ways (Bàrberi, 2013). Still, basically "sustainable" refers to something which is able to meet all its expectations, without exhausting nor deteriorating the resources which is based on, and hence to last for an indefinite time in the long run. For agriculture, which is mainly deputed to the production of food and also deals with biological systems, expectations are usually equivalent to challenges.

In the next future, the three main challenges that agriculture is demanded to cope with, will be enormous. First, to feed a 9 to 10 billion people foreseen by 2050, by reducing also inequity in the distribution and quality of food over the globe (Godfray et al., 2010). Second, to achieve the feat of food security without compromising environmental resources, not only through the reduction of negative impacts, but also enforcing positive contribution of the sector on the environment (Smith, 2013). And, finally, to sustain the economic viability of farming and enhance the quality of life for farmers and society as a whole, in a context of global crisis of even consolidated socio-economic systems (Ervin et al., 2010).

Furthermore, the mission of the agricultural sector in the coming decades cannot be thought independently from the effects of the so called "*climate change*", which imposes on the question the burden of uncertainty and variability of environmental conditions, making not hundred percent predictable the long-term effects of the different options of land management from the point of view of sustainability.

Climate change has been defined as "*a change in the state of the climate that can be identified (e.g. using statistical tests) by changes in the mean and/or the variability of its properties, and that persists for an extended period, typically decades or longer. It refers to any change in climate over time, whether due to natural variability or as a result of human activity*" (IPCC, 2007). The relationship between agriculture and climate change is dual. On one hand, agriculture is affected by climate change, either positively or negatively. Nevertheless, in most cases it is believed that adverse effects are preponderant: extreme events (flooding, heat waves, hurricanes, storms, etc.), global warming, fluctuant weather conditions, breaking of seasonality, thermal shocks, are all detrimental factors for agricultural production and human well-being, especially for developing countries, where the whole economy still relies on the primary sector. This is why the FAO (2009) strongly claimed that agriculture, first, had to *adapt* to climate change, in order to contrast the immediate risk of food insecurity, by providing new strategies and techniques for improving productivity in areas mostly impacted. Additionally, as climate change is predicted to long last, agriculture should be redesigned in order to improve the resilience of food production to future environmental changes. For this aim, the conservation and protection of agrobiodiversity will be determinant (Bàrberi, 2013; Smith, 2013).

On the other hand, together with other human activities, agriculture plays a significant role also as a cause of climate change itself, mainly through the emission of greenhouse gasses (GHG) in the atmosphere (see report in Appendix 2 for further details). The high relative importance of agriculture over the other economic sectors is mainly due to the high portion of global land devoted to agriculture, which makes it the most important activity in terms of land use and impact on the environment. Nevertheless, agriculture have been also identified as one of the most important components of new global strategies aiming at the *mitigation* of climate change (IPCC, 2007; FAO, 2009), basically through a better management of natural resources, the storage of $CO_2$ in the soil and a low reliance on non-renewable energy inputs.

An effective contrast to climate change, which also would not fail to meet the increasing food demand, could not be addressed by increasing the size of agricultural land, as this would mean an additional loss of ecological services, possibly unsustainable (Smith, 2013). All the proposed options pass through two alternative, or even complementary, strategies. The first one is the so called "*sustainable intensification*", which identifies a process aiming to improve the efficiency of food production without significant changes of the current ''business as usual'' agricultural system, mainly relying upon agro-industry, with the aim to hit the ceiling of crop productivity (Garnett et al., 2013). The other strategy, which may be termed as "*ecological intensification",* implies the rethinking of the whole agricultural sector, with the adoption of short-distance models of food supply chain, a dietary change toward less land-demanding food, and also different socio-economic rules, aiming to the reduction of food wastes, the promotion of biodiversity and the extensification of farming (Smith, 2013). In such a system, food production should be based on agro-ecological principles, with the objective of enhancing at most the ecological services provided by agroecosystems and reducing at least the use of inputs external to the farm.

This latter is the vision generally shared by organic farming movements, as evidenced by the recent campaign launched by IFOAM (http://www.ifoam.org/en/core-advocacy-campaigns/ecological-intensification), and also by FAO (2009), who identified in the organic farming model one of the pillars in the contribution of agriculture to the mitigation of climate change.

Anyway, the reality suggests that also organic farming needs to improve its efficiency in the use of natural resources.

Partially, this is because a consistent portion of farmers who converted from conventional to organic farming did it mainly driven by market reasons and without the consolidation of their technical skills. On the other hand, due also to the high complexity of mechanisms driving the functioning of agroecosystems, very fragmented and incomplete results were delivered by researchers to stakeholders.

This have consequently led them to apply a simplified "*input substitution approach*", i.e. the substitution of less noxious inputs for agrochemicals (Rosset and Altieri, 1997), disregarding the missing integration of ecological services within farming systems. As a result, organic farming as a whole did not fulfill completely the promised eco-efficiency so far, due to a sort of "conventionalization" of farming practices, which brought the same economic and ecological problems faced by conventional farmers to arise (Darnhofer et al., 2010).

Thus, an in-depth comprehension of mechanisms regulating the expression of beneficial ecological services in agroecosystems will be the key factor to redesign crop techniques in order to enhance the efficiency of crop production. In the challenging future on the horizon, such agroecology-based knowledge may be the real determinant of the degree of success of agriculture in the achievement of its multiple objectives.

## 1.2 The need to improve the efficiency of food production: the concrete case of durum wheat in Italy

Italy is the second country in Europe, and the seventh in the world, in terms of the size of land managed organically, which amounted at 1.17 Mha in 2012, i.e. 6.4% of total agricultural area (EUROSTAT, 2012; IFOAM, 2012; SINAB, 2012). Cereals represent 14.4% of total organic area, and also has a high share in the organic market (SINAB, 2012).

Whatever the cultivation system, wheat is steadily grown each year on 2 Mha over the country, with predominance of durum wheat (*Triticum durum* Desf.) in the Centre-South, with about 1.2 Mha, and of common wheat (*Triticum aestivum* L.) in the Centre-North, with about 0.8 Mha (ISTAT, 2012). Durum wheat kernels are milled mostly for production of pasta, but also for traditional breads, in the South. This shows the importance of the crop from an economic point of view, as bread and pasta are two of the main components of the Mediterranean diet.

On average, the grain yield of the crop is higher in the South of Italy (even more than 5.0 t ha$^{-1}$), due to the longer growth cycle and the higher temperatures, than in the Centre (between 3.0 and 4.5 t ha$^{-1}$).

Under organic farming, yield depletion is reported to be in a range of 16 to 40% compared to conventional (Bàrberi and Mazzoncini, 2006; Quaranta et al., 2010; Fagnano et al., 2012), and also grain quality seldom meets the requirements of food industry, leading to increasing import of organic wheat from abroad, and thereby to dramatical economic losses for Italian farmers.

The gap between conventional and organic wheat production may be explained with the concurrence of several factors, with particular emphasis on plant disease severity, weed competition and nitrogen deficiency.

The risk of infection by phytopathogenic fungi, such as species of *Fusarium* genus, responsible of Fusarium head blight, as well as the contamination of kernels by mycotoxins, is well-documented to be not significantly different from conventional wheat (Quaranta et al., 2010). The scarcity of direct means to tackle these pathogens under organic, due to the ban of synthetic fungicides, is indeed quite compensated by indirect measures, such as the choice of resistant varieties, the adoption of low seeding density and wide row distance, the application of moderate rates of N, and also the incorporation of crop residues into the soil.

Weed pressure and N crop deficiency, however, are wide-spread identified as the two main determinant of yield depletion under organic management (Hansen et al., 2000; Barberi, 2002). Weeds are indeed strong competitors for light, water, space and also nutrients, and thereby are capable of strong detrimental effects on crop yields, wheat included (Amossé et al., 2013a). Anyway, cultural methods (e.g. cultivation of cover crops, choice of proper crop sequence, false seedbed technique, narrow rows design, high seed density, spreading varieties), in combination with direct means (above all, flex tine harrowing), look quite effective in weed control in winter cereals.

On the other hand, nitrogen deficiency is considered of primary importance for wheat production, as the element is considered not only the most important determinant for yield level, but also for grain quality, which is strongly correlated with the acceptability of the product on the market, and hence on its sell price (Garrido-Lestache et al., 2005). This is mainly because many rheological parameters of doughs prepared from wheat flours strongly depend on content and type of storage proteins in kernels, and more precisely in the endosperm (Giuliani et al., 2011). The gluten fraction of protein is even more important, as it strongly influences the organoleptic and rheological properties of the dough made from wheat flour. In particular, the ratio of the two main components of gluten, namely glyadine and glutenine, may alter dough elasticity and viscosity (Giuliani et al., 2011). The secondary structure of the protein, i.e. the way that protein link to each other, is an other important quality parameter influenced by N nutrition, as well as by sulphur availability (Gooding et al., 2007).

In Mediterranean climates, wheat usually experiences N shortage for most of its cycle, due to the fact that the annual peaks of rainfall and of soil organic matter mineralization overlap in both autumn and spring. This peculiar combination may cause very low levels of N in the soil during winter and spring (Tosti and Guiducci, 2010), by increasing the risk of losses (e.g. through leaching or denitrification) also for N originally available, resulting from the mineralization of previous crop residues, or from the application of fertilizers, broadcasted before sowing.

Under organic farming this problem cannot be easily solved. Side dressing fertilization with organic N fertilizers is not really effective, due to the slowness and the variability of N release from organic materials, which may cause possible asynchronism between soil N availability and plant N uptake (Tosti and Guiducci, 2010). In addition, the high unit cost of organic fertilizers, and the low trafficability of fields in winter and spring due to the frequent rainfalls and the plant height, make the application of organic fertilizers to wheat after tillering almost not practicable. Thus, a possible solution to increase N availability in the soil may come from the exploitation of agro-ecological mechanisms underlying cropping systems, such as, for instance the introduction in wheat-based organic cropping systems of $N_2$-fixing legumes, which are historically the only biological N source comparable to fertilization, as stated by Peoples et al. (2009a).

Anyway, this option cannot be considered suitable exclusively in the context of organic wheat production. Indeed, also among conventional farmers there is the awareness of the need to reduce mineral fertilizer use. This is primarily due to economic reasons, as the price of mineral fertilizers is increasing year by year, following the trend of oil price and reaching levels almost unaffordable for farmers, especially if compared to the very low market price of wheat grain. Still, the increasingly pressure to reduce the use of non-renewable resources in agriculture, in the context of climate change mitigation, pushed decision-makers to introduce new laws, and consequently new specific schemes of payments. Only farmers willing to enhance the provision of ecological services in their farms, as well as to reduce the use of non-renewable resources, may now receive specific subsidies, or at least conserve their access to regular funding schemes, such as the CAP payments. From this point of view, the substitution with $N_2$-fixing legumes of mineral N fertilizers, reliant on fossil energy and responsible for most GHG emission from agriculture, is fully part of this process (Jensen and Hauggaard-Nielsen, 2003), as it shows the potential to strongly reduce the use of synthetically fixed N in cropping systems, and to provide many other ecological services helpful in the mitigation of climate change (see Appendix 2 for further details).

## 1.3 Agro-ecology into practice: legume-cereal intercropping

Primarily, legumes can be introduced into wheat-based cropping systems as main crops in the sequence, contributing to the spatial and temporal diversification of cropping systems. This choice may benefit wheat yield and quality through the so called “N sparing effect”, which happens when the post-harvest incorporation of legume residues into the soil generates an excess of N potentially available for the following wheat crop (Chu et al., 2004). Anyway, the high levels of rainfall occurring during fall-winter in Mediterranean regions may make this potential benefit ineffective, due to nitrate leaching. Furthermore, crop rotational effect, as well as pre-sowing fertilization, might have only limited effect on grain quality, due to the asynchronism between the peak of N mineralization and the high N-demanding crop stages.

Thus, the most promising strategy to enhance the efficiency of wheat production may pass through the simultaneous growth of legumes and cereals on the same field, which is termed “intercropping”. In the next paragraphs, the general concepts of this technique will be elucidated, with particular focus on cereal-legume intercropping.

### *1.3.1. Intercropping: definitions and classifications*

Historically, intercropping and, more generally, mixed crops have long been recognized as very common practices in agriculture, and especially in developing tropics, where they are widespread also nowadays (Willey, 1979; Hauggaard-Nielsen et al., 2009a). In developed countries, the intensification of agricultural production imposed in the last century by the green revolution caused the abandonment of this traditional technique in favor of mineral fertilizer application. Now it is clear that intercropping has survived the green revolution in the tropics, allowing local populations to maintain sustainable models of ecological agriculture, less reliant on fossil-fuel resources. In a context of climate change mitigation, this technique becomes thus to receive renewed attention from researchers and stakeholders, also from developed countries (Horwith, 1985; Hauggaard-Nielsen et al., 2009a).

Willey (1979) defined intercropping as "*the growing of two (or more) crops* -defined as "*components*" or *"intercrops*" or "*associates*"- *simultaneously on the same area of ground"*. This classical definition of intercropping, given by the author in order to simplify and homogenize the number of different examples of mixed cropping systems reviewed, does not fully explain the main core of intercropping. What mainly characterizes mixed cropping systems is not only the fact that different plants grow in close proximity to each other, but rather that they realize mutual relations which, at the end, may produce for the plant community advantages or disadvantages in a larger extent than simply summing the performance of each single component.

In their wider definition, Caporali et al. (1987) identified with intercropping an association of individual plants with different genotypes, growing in close proximity to each other insofar as to allow the manifestation of agronomical mutual interactions. Interaction means every conditioning that each of the intercrops is able to perform on the other. The success of an intercropping in meeting its expectations strongly depends on the strength and the quality of these interactions, which in turn may be differently expressed depending on the intercropping strategy adopted.

Intercropping may be classified in different typologies in function of several factors, usually overlapping:

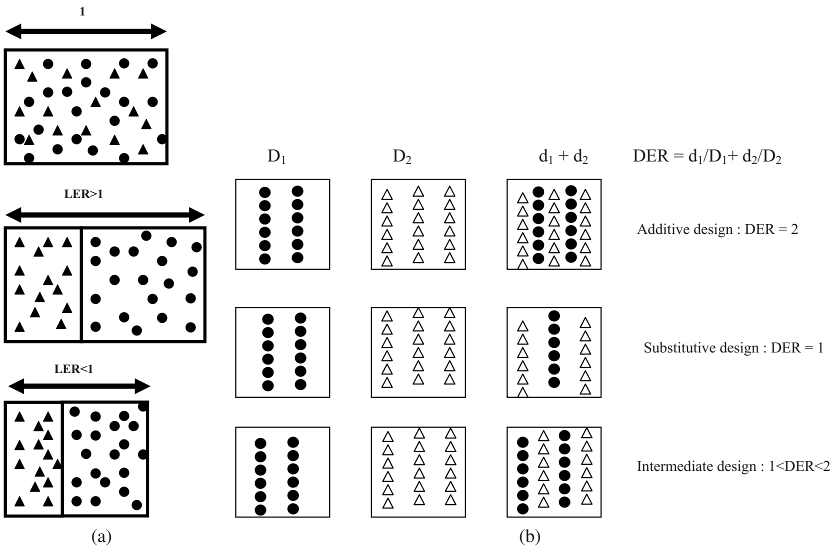
a. growth habit of associates;
b. duration of co-growth between associates;
c. number of components;
d. design;
e. spatial-temporal arrangement of associates;
f. utilization of crop products.

a. Concerning growth habit of components, tree intercropping, herbaceous intercropping and mixed intercropping can be distinguished. Mixed type is particularly widespread in tropical agriculture, where is reported to achieve high land productivity, even in degraded soils (Willey, 1979; Altieri, 1999). Obviously, herbaceous intercropping is the most suitable type for arable crop rotations.
b. The duration of co-growth of components is extremely important for the expression of mutual interactions among intercrops. Fukai and Trenbath (1993) distinguished between intercropping with components of similar or differing growth durations, with

these latter showing the best performances, because of the better exploitation of resources over space and time. Vandermeer (1989) introduced another criterium of interpretation, differentiating intercropping where co-growth lasts for the whole cycle of all the components (the so called "*permanent intercropping*"), from the other kind (the so called "*temporary intercropping*"), where associates coexist only for a limited time. A practical example of temporary intercropping is the "*relay intercropping*", where one of the two components is under-sown in the other already established, in order to reduce interspecific competition in certain periods of the season (Vandermeer, 1989), possibly resulting in growth depletion in at least one of the intercrops.

c. Usually, intercropping involves no more than two or at least three components, due to the increasingly high complexity of management at increasing number of species in the mixture. Anyway, also intercropping with high number of associates are reported in the literature, and especially in the context of agroforestry or fodder crops (Altieri, 1999).
d. The relative contribution of each component to intercropping, expressed by the seed ratio and also the seeding density, defines the design of intercropping (Figure 1).

*Figure 1 - Land and density equivalent ratios. (a) The land equivalent ratio (LER) of a multispecies system is the area needed to produce the same outputs as one unit of land with a pattern of sole cropping; (b) the density equivalent ratio (DER) indicates the crowding of the mixture. The symbols represent the plant population density. From Malézieux et al. (2009)*



In the *replacement* design, the total relative density (100%) is held constant while the relative proportion of each species is varied according to the recommended sole crop density (Hauggaard-Nielsen et al., 2006). The overall relative density exceeds 100% in the *additive* design, however, where seeding density of at least one of the components is kept equal to that recommended for sole cropping, in order to maximize mutual interactions among associates (Fukai and Trenbath, 1993). In replacement designs, the component crops may be potential competitors for the same niches, but they interact positively due to the effect of the particular seeding ratio (i.e. the relative proportion of the seeds of each species on the total seed density of intercrop), chosen in order to minimize competition from the dominant species and to maximize complementarity. On the other hand, the adoption of an additive design implies that the components might compete for different niches, producing more mutual positive interactions than competition (Fukai and Trenbath, 1993).

e. The spatial arrangement of intercrops is related to several aspects of management, such as mechanization (i.e. how to put into practice the desired intercropping in function of the available machines for sowing and harvest) and marketing (utilization of crop products, post-harvest separation of the grain of each components), but also reflects the aims of intercropping. The higher the number of component plants per area unit and the proximity at which they co-growth, the higher will be the strength of their interactions.

   Accordingly, Francis (1986), Ofori and Stern (1987) and Vandermeer (1989) classified intercropping as follows:

   - *mixed intercropping*: when intercrops are grown in close proximity to each other and without a precise spatial arrangement. This strategy is particularly suitable for subsistence agriculture contexts, and for systems with low level of mechanization. Normally, the products of each intercrop are not separated in post-harvest, but sold as a bulk for food or feed;
   - *row intercropping*: when intercrops are displaced on different rows (alternate or coupled), in order to reduce competition and to perform in-crop field operations;
   - *strip intercropping*: when intercrops are grown on separate strips obtained by combining several rows. The aim is to make easier the mechanization of field operations compared to alternate rows, and preserving at the same time the advantages coming from the proximity of the different associates. For these reasons, the width of strips must be not lower than the width of available machines, but also not as high as to reduce at least the contact among associates.

f. In permanent intercropping, the utilization of the crop products of all components is understood, as all the crops co-grow until harvest. Conversely, in temporary intercropping the overlap between the cycles of the components not always allows for the combined harvest of all plant products. For instance, in relay intercropping systems where a fodder legume (e.g. a clover species) is undersown in a winter cereal already established, after the harvest of the cereal the legume might be grown undefinitely for seed production, kept for pasture or ploughed under as green manure (Amossé et al., 2013a). Wojtowski (2006) introduced a new category of intercropping, the so termed "*facilitative intercropping*", which identifies intercropping where one component is not grown for production, but exclusively to facilitate the others. Soil cover or shading against weeds, nutrient supply through root exudates or incorporation of plant biomass into the soil, modification of the soil profile explored by roots, pest-breaking action, are only few examples of this kind of facilitation. For instance, in the relay intercropping experiment carried out by Amossé et al. (2013a) four different forage legumes (*Medicago* spp. and *Trifolium* spp.) were undersown in organic wheat fields, with the aims to provide help for weed control in the cereal crop and to reduce the time between the harvest of the cereal and the sowing date of the legumes. In other field experiments carried out in Italy (Li Destri Nicosia et al., 2005; Carpi et al., 2009; Di Miceli et al., 2009; Tosti and Guiducci, 2010), a grain legume was temporarily intercropped with winter wheat and incorporated into the soil as green manure in early spring, with the aim to increase the yield and enhance the grain quality of the wheat.

When designing a cereal-legume intercropping system, all the above listed options should be evaluated in function of the desired level and typology of interactions among component species, which might significantly affect the ecological efficiency of intercropping.

### *1.3.2. Plant interactions under intercropping*

Interactions among components of intercropping may be extremely complex, and difficult to distinguish one from each other, as they normally co-occur at the same time. For convenience, interactions may be classified as: i) competitive; and ii) non-competitive (Caporali et al., 1987; Fukai and Trenbath, 1993; Midmore, 1993).

A competition occurs when the availability of a natural resource becomes scarcer than required by two or more contending individuals (Ofori and Stern, 1987). Competition for resources is one of the main drivers of the performances of ecological communities, and of cropping systems as well. At a glance, the intensity of competition depends on the number of individuals contending the same resources in a given area unit, regardless of whether they belong to the same or different species. Thus, this mechanism of competition applies both to pure crops and intercropping, with the difference that in intercropping both *intra-species* and *inter-species* competition take place. If inter-specific competition is lower than intra-specific, then intercropping may be more advantageous than sole crops (Willey, 1979; Caporali et al., 1987; Vandermeer, 1992; Fukai and Trenbath, 1993; Midmore, 1993).

As regards to this, Willey (1979) introduced the terms of *mutual inhibition, mutual cooperation* and *compensation* (or *complementarity*) to describe all the possible outcomes of an intercropping. Mutual inhibition happens when all the intercrops perform worse than respective sole crops, whilst mutual cooperation is completely the opposite (i.e. when all components perform better than sole crops). Compensation, which is the case in between (i.e. when an intercrop performs better than sole crops, and the others worse), is maybe the most frequent situation. The more competitive species is termed "*dominant*" and the less "*dominated*" (Willey, 1979; Ofori and Stern, 1987; Fukai and Trenbath, 1993).

Nevertheless, according to the literature, the higher resource use efficiency attributed to intercropping compared to sole crops derives more from its global performance (e.g. total dry matter production of all the intercrops), rather than from an enhancement of its single components. For instance, wheat-legume intercropping was seldom reported to significantly increase the grain yield of wheat compared to wheat pure stands (Li Destri Nicosia et al., 2005; Fan et al., 2006; Szumigalski and Van Acker, 2006; Carpi et al., 2009; Di Miceli et al., 2009; Tosti and Guiducci, 2010; Amossé et al., 2013b), whereas, on the other hand, total grain yield of intercropping (i.e. wheat + legume) normally exceeded that of sole crops.

In any case, the competition of an associate in a mixed system can be affected by inherent properties and extrinsic factors (Fukai and Trenbath, 1993). Inherent is the so called "*competitive ability*" of a species for a given resource, that is the capacity of a species to capture the limiting resource in presence of other contenders. Extrinsic is the whole set of environmental and management factors potentially affecting the amount of a given resource conquered by a species.

On the other side, non competitive interactions between intercrops include all mechanisms put into action by plants to modify their close environment to their benefit.

Harper (1977) classified these mechanisms in two different groups. There are mechanisms aiming to introduce or subtract substances (e.g. nutrients, allelopathic compounds, semiochemicals) into the environment shared with other species, whereas there are others leading to the modification of the status of this environment (e.g. shading, wind breaks, pest breaks).

Clearly, both competitive and non-competitive interactions among the components are worthy to be considered when analyzing the global performance of an intercropping system. The most important interactions involved in a cereal-legume intercropping will be discussed in the following.

### *1.3.3. Competition for resources under cereal-legume intercropping*

Intercropping might be more efficient than sole crops if complementarity for key natural resources is higher than competition. This complementarity may be achieved if the competitive ability of each intercrop is well balanced, and competition for niches differs in space and time among components, due to differences in terms of physiological, morphological or ecological plant traits (Willey, 1979; Caporali et al., 1987; Midmore, 1993).

Competitive interactions among intercrops mainly regard only some important agroecological resources, such as solar radiation, soil nutrients and water.

Solar radiation is a crucial resource for crop productivity, and also differs from the others in terms of availability. According to Willey (1979), light is "*instantaneously available*" and has to be "*instantaneously intercepted*", without any possibility of storage for further utilization.

Intercropping is usually reported to achieve higher efficiency of solar energy use than sole crops (Willey, 1979; Ofori and Stern, 1987; Keating and Carberry, 1993). Anyway, as also pointed out by Keating and Carberry (1993) in their review, the relationship between intercrop complementarity and solar radiation capture is not only a matter of reducing the amount of light hitting the ground, but also of how efficient is the conversion of solar energy in plant biomass. This issue is not straightforward so far, due to the complexity of environmental and managerial (e.g. crop row width, seeding time) factors potentially able to impact on this aspect (Thorsted et al., 2006).

For cereal-legume intercropping, competition for solar radiation may be particularly important in periods of the year when light is limited in availability (e.g. in fall-winter, during the first establishment of the intercrops), or when is fully available and then photosynthetic activity becomes especially important as determinant of plant yield and quality (for winter cereals, this generally occurs in spring, after tillering). Furthermore, avoiding waste of light is, indirectly, a way to keep also weed populations under control in the cropping system, as they might reduce their photosynthetic activity, and hence their growth.

In any situations, spatial complementarity of intercrops may be crucial for a satisfying capture of light. This issue might be addressed if component crops have a complementary leaf architecture, morphology and light use efficiency (Willey, 1979). Generally, intercropping between cereals and legumes with different canopy height and growth habits (e.g. short prostrate legume vs tall upright wheat), different leaf inclination (e.g. vertical for wheat plants vs horizontal for legume plants) and different photosynthetic efficiency (e.g. C4 tall plants vs C3 short plants), might result in a less homogenous canopy, and hence in a higher light interception than pure crops (Willey, 1979). In this case, the overgrowth of the tallest plant should be definitively avoided, in order to keep shading under certain levels, otherwise the shorter associate might fail (Blaser et al., 2011).

To use solar radiation in an even more efficient way, intercrops might also provide light interception (also expressed in terms of Leaf Area Index, LAI, or of intercrop soil cover) for a longer period of time than sole crops do. This might be possible if the duration of cycle of at least one intercrop would exceed that of the others and that of relative sole crops. This might be the case, for instance, of relay intercropping between winter cereals and legume cover crops, which are undersown in the established cereal and kept growing until the next year after the harvest of the cereal, providing a continuous soil cover and, hence, a continuous interception of light (Blaser et al., 2011; Amossé et al., 2013b).

In many studies, cereal-legume intercropping resulted in increased capture of nutrients from the soil, and particularly of nitrogen (Willey, 1979; Horwith, 1985; Chu et al., 2004; Szumigalski and Van Acker, 2006; Bedoussac and Justes, 2009; Hauggaard-Nielsen et al., 2009a; Mariotti et al., 2012), phosphorus (Morris and Garrity, 1993a; Hinsinger et al., 2011; Betencourt et al., 2012), potassium (Morris and Garrity, 1993a), iron (Zuo and Zhang, 2009), zinc (Zuo and Zhang, 2009) and calcium (Li et al., 2004).

With respect to competitive interactions, intercrops may effectively capture these elements when a complementarity of the root systems of the intercrops occurs. Complementarity might be due to: spatial diversification of roots, temporal diversification of peaks of nutrient demand, specific competitive ability for different nutrients (Willey, 1979; Caporali et al., 1987).

Spatial diversification of roots might take place when intercrops are physically separated, like in strip-intercropping, or when their roots explore different soil layers, not leading to the overlapping of the specific depletion zones for the same elements (Vandermeer, 1989; Corre-Hellou et al., 2006; Li et al., 2006). This might be the typical case of a cereal-legume intercropping, with the taproot of the legume exploring only upper soil in a limited extent, and fasciculate roots of the cereal distributing along the soil profile and also in radial sense. In this sense, the cereal exhibits higher below-ground competitive ability for nutrients than legume (Mariotti et al., 2009).

Competition for the same niche element might be avoided also by shifting in time the peaks of plant nutrient uptake in the different intercrops. This might be put into practice by not sowing simultaneously the two intercrops, like in relay-intercropping systems, or by manipulating the growth of one intercrop in order to produce a difference in the phenology of associates (Ofori and Stern, 1987). For instance, early N fertilization may favor the initial growth of the cereal, thereby allowing to avoid the risk of a strong competition for N between plantlets of legume and cereals immediately after emergence (Fukai and Trenbath, 1993).

Finally, the role of affinity for different elements may become important when the growth of each intercrop is limited by different nutrients, leading to a complementary resource capture. In cereal-legume intercropping this usually do not occur, as the crops share the same niches.

Concerning water, a higher use efficiency in intercropping than sole crops was not clearly elucidated so far (Willey, 1979; Morris and Garrity, 1993b; Arslan and Kurdali, 1996; Szumigalski and Van Acker, 2008). This is because of the number of environmental and managerial factors potentially altering the amount of water intercepted by intercrops, such as weather conditions, pest and disease incidence, growth depletion due to other factors than water, irrigation, fertilization, etc. Generally, it is believed that cereal-legume intercropping might increase the amount of water absorption thanks to the exploration of different soil layers by the roots of intercrops. Furthermore, reduction of plant transpiration and soil evaporation through, respectively, shading and soil cover provided by the canopy of intercrops, might be other indirect competitive interactions which may play a role in the relationships between intercropping and water (Willey, 1979).

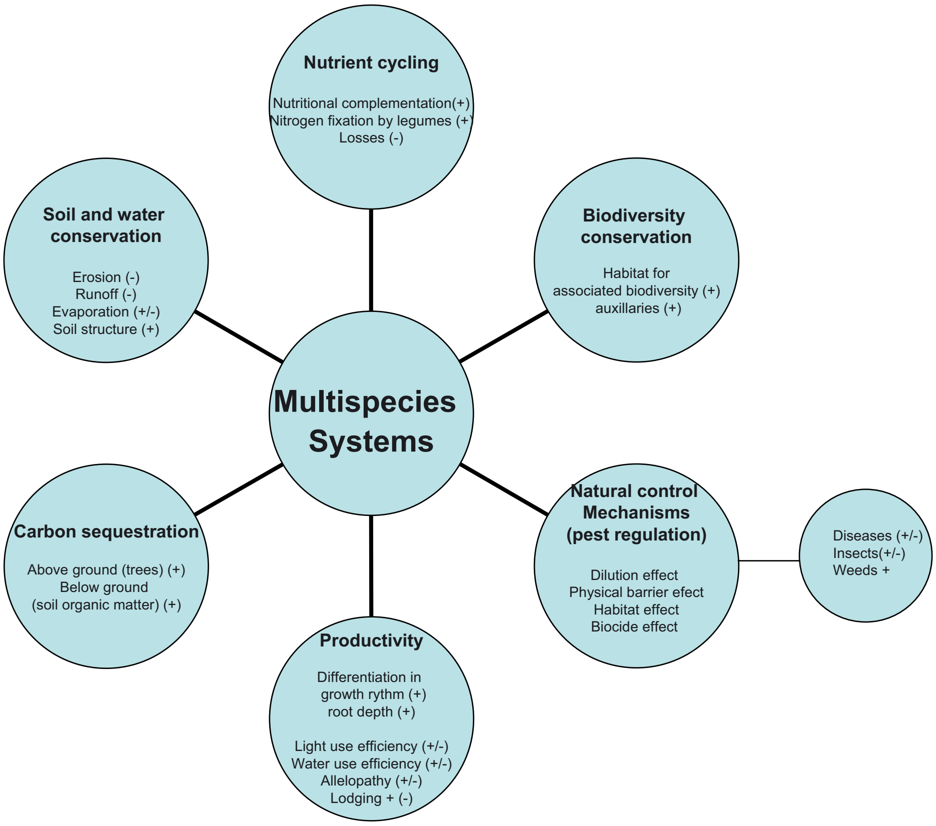
In cereal-legume intercropping grown under dry conditions, competition for water might become crucial only in dry season, such as, for instance, late spring in Mediterranean areas (Ofori and Stern, 1987). In these conditions, when water availability is lower than required by the dominated species (for instance, a late established legume undersown in the cereal), an overgrowth of the dominant species may happen, with negative consequences on the performance of the intercropping as a whole.

### *1.3.4. Non-competitive interactions under cereal-legume intercropping*

Non competitive interactions among intercrops might be extremely relevant for the global performance of intercropping. Basically, this kind of relationships among plant within the community is mainly due to the ecological behavior of the intercrops, and to the way they relate to other components of agroecosystems.

Intercropping is well known to be able to provide several important ecological services, determining noteworthy advantages for intercrops with respect to sole crops (Figure 2).

*Figure 2 - Processes and induced properties in multispecies systems. From Malézieux et al. (2009)*



Above all, the interactions of highest importance for cereal-legume intercropping under organic farming or low-input conditions are those concerning the amelioration of soil fertility through the increased availability of nitrogen and phosphorus.

### a. Non-competitive interactions for N in cereal-legume intercropping

When fertilizer N is limited, symbiotic nitrogen fixation of legumes is the major source of N for cropping systems (Fujita et al., 1992). The amount of N fixed in a season is mainly a function of the combination among legume species, environmental conditions and crop management.

The effect of legume species on the potential level of $N_2$-fixation was deeply investigated in the past, and consequently a plethora of data on this issue are available (Peoples et al., 1995a). Under intercropping, rather than species in itself, what is of high importance is the whole set of plant traits which contribute to define the adaptability of a legume to be grown in a mixture with a given cereal. Fukai and Trenbath (1993), for instance, argued that legumes with spreading or climbing indefinite growth may supply more N to the soil than definite types, due to increased biomass production in condition of exceeding competition from the intercropped cereal.

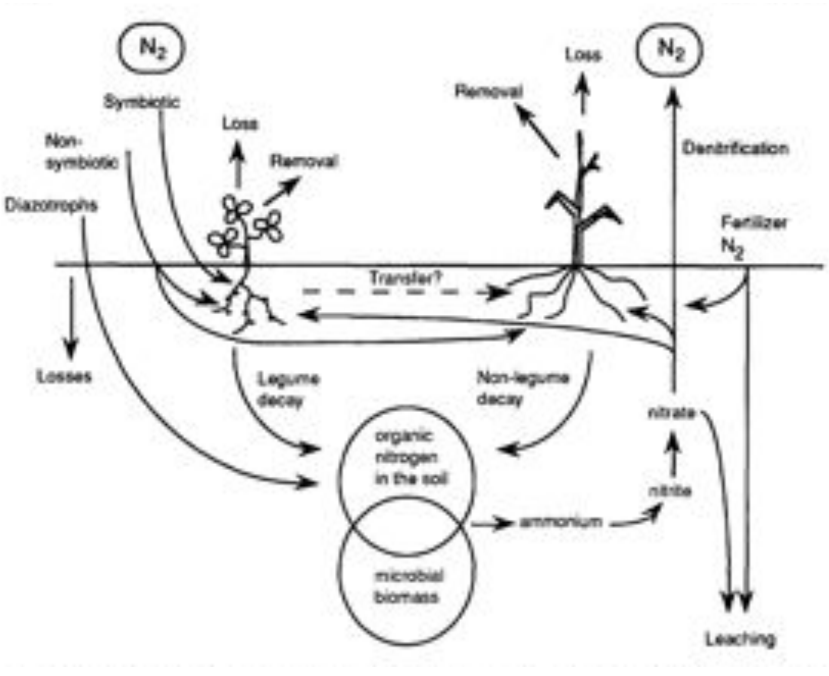
The potential effect of environmental conditions on symbiotic $N_2$-fixation is likely the most important, and its importance will increase even more in the next future, due to the high variability of the status of the atmosphere caused by the climate change. A list of circumstances likely to occur in future scenarios are provided in Appendix 2.

In general terms, all conditions favoring the growth of the legume might positively affect the total amount of N fixed by the crops (Fujita et al., 1992). A harsh environment for legume pest and pathogens, for instance, may reduce the severity of legume damages due to biological agents. On the other hand, adverse conditions for the cereal intercrop may result in higher growth of the companion legume, indirectly increasing also the total amount of $N_2$ fixed from the atmosphere.

Conditions of low availability of soil N usually promotes biological $N_2$-fixation from the atmosphere (Fujita et al., 1992). Still, under intercropping between legumes and non-$N_2$-fixing plants, this general trend implies a number of differing mechanisms. When N becomes scarce in the soil, competitive interactions for the niche immediately occur among intercrops, especially in the specimen of intercropping with additive design (Fukai and Trenbath, 1993). In these conditions, the level of soil N interception is mainly determined by the growth and the depth of penetration of root systems of intercrops (Corre-Hellou et al., 2007). Cereals obviously have higher ability than legume to intercept N in deep soil layers, due to the different root structure and development. Intercropping might also stimulate the deepening of cereal roots, due to the overlapping between the root depletion zones for N of the two intercrops (Hauggaard-Nielsen et al., 2001b). The exhaustion of N in upper soil layers might force legumes to invest more energy in atmospheric $N_2$-fixation. Indeed, a significant increase in the rate of N fixation in legumes intercropped with cereals compared to pure stands was observed by many authors (Jensen, 1996; Kurdali et al., 1996; Hauggaard-Nielsen et al., 2001a, 2003; Fan et al., 2006; Hauggaard-Nielsen et al., 2008; Bedoussac and Justes, 2009; Hauggaard-Nielsen et al., 2009a). This evidence should be also related to the intensity of growth depletion caused by cereals on legumes, as high rate of N derived from the atmosphere may be due to a lower dry matter production of intercropped legumes and a conversely higher concentration of N in plant tissues (Naudin et al., 2010).

A further contribution of the environment on the effect of symbiotic $N_2$-fixation of legumes regards the regulation of the fate of fixed N in the soil. Stern (1993) summarized in his work all the complex mechanisms which might take place under cereal-legume intercropping (Figure 3).

*Figure 3 - Main nitrogen pathways in a legume/non-legume intercrop system. The length or size of arrow is not an indication of its relative importance. From (Stern, 1993)*



As shown in the figure, fixed N may encountered many ways of loss in the soil. The desirable achievement of a cereal-legume intercropping should be that N fixed from the atmosphere would stayed in the system, either by contributing to the increase of organic N pool, in a long-run perspective, or even better by directly transferring to the cereal root zone. This process, termed "*N transfer*", was demonstrated to occur in several experiments of intercropping between cereals and legumes (Brophy et al., 1987; Stern, 1993), but always with high variability depending on peculiar environmental and managerial conditions. This is why many other papers did not report a significant direct transfer of N from legume to intercropped cereal (Ofori and Stern, 1987; Jensen, 1996; Kurdali et al., 1996), drawing the conclusion that facilitation of cereals under intercropping with legumes would have been derived basically by the increased competitive ability of roots to capture N from the soil (Fujita et al., 1992; Bedoussac and Justes, 2009).

The reasons behind this uncertainty might be found in the number of factors likely to interfere with N transfer in the soil. When N reaches the soil through the falling of plant portions (e.g. senescent leaves or branches), which may occur in the case of pest or disease attack or under drought conditions, the availability of N for the cereal component might be conditioned by the dynamics of mineralization of the plant materials, determined by the metabolism of detritivore organisms. As elucidated by San-nai and Ming-pu (2000), N transfer may happen also directly through root exudates in three different ways: i) nitrogen passes in soluble form from the donor legume root into the soil solution, moves by diffusion or/and mass flow to the receiver root and is taken up by the latter; ii) nitrogen passes into the soil solution as before, but then is taken up and transported by mycorrhizal hyphae attached to the receiver roots; iii) if mycorrhizal hyphae form bridges between the two root systems, the nitrogen could pass into the fungus within the donor root and be transported into the receiver root without ever being in the soil solution. All the intermediate actors might be modified by a number of factors (Fujita et al., 1992), even by earthworms activity, as shown by (Schmidt and Curry, 1999), or by the interception of fixed N by weeds (Bulson et al., 1997).

When a significative portion of N fixed by legumes is not intercepted by cereals, thus a N loss may occur through leaching of nitrates or denitrification, leading to a negative impact on the environment, as well as on the economy of intercropping (Fujita et al., 1992; Hauggaard-Nielsen et al., 2003; Pappa et al., 2011). For these reasons, the proper choice of the position of intercropping in the whole crop sequence might be crucial, in order to avoid significant N losses (Launay et al., 2009).

The role of crop management in regulation of biological $N_2$-fixation of legumes is as much important as environment, being able to manipulate, positively or negatively, the efficiency of the process. For instance, Fujita (1992) and Midmore (1993) reported several managerial options of intercropping potentially able to modulate this process. Besides the choice of intercrop species, which was discussed above, also the choice of intercropping design and of spatial arrangement of rows might play a role. According to Ofori and Stern (1987), the closest the proximity between intercrops, the highest will be the growth depletion of the legume, due to the shading of its canopy caused by the non-legume species. At the end, this might affect the amount of fixed N entering the system.

In conditions of predominance of cereals in early stages, an earlier sowing of the legumes respect to cereals might enhance crop growth and, consequently, increase the level of $N_2$-fixation (Fujita et al., 1992). Conversely, a late sowing of legumes, like in relay intercropping systems, might result in legume growth depletion due to shading and competition for soil water and nutrients (Amossé et al., 2013b).

The choice of the strategy of intercropping concerned to duration of co-growth is an other important managerial option. Limiting co-growth as in the case of a temporary intercropping, might reduce at least the competition between intercrops even in additive design, and enhance the contribution of $N_2$ fixed by the legume in the N nutrition of the companion cereal (Li Destri Nicosia et al., 2005; Carpi et al., 2009; Di Miceli et al., 2009; Tosti and Guiducci, 2010).

Additionally, the effect of combined-N addition to the system was also demonstrated to have a positive effect on the global resource use efficiency of cereal-legume intercropping tested under low-input management (Ofori and Stern, 1987; Jensen, 1996; Andersen et al., 2005; Ghaley et al., 2005; Bedoussac and Justes, 2009; Naudin et al., 2010; Mariotti et al., 2012; Pelzer et al., 2012). Still, in all these papers N fertilization increased the total dry matter produced by intercropping through an increased proportion of the cereals in the mixture, which might be wished when legumes dominate cereals. The side effect of mineral N on biological $N_2$-fixation was then found detrimental only at exceeding N rates, i.e. when the less intense competition for soil N did not compensate for the lower presence of legumes in the mixture (Andersen et al., 2005; Naudin et al., 2010; Mariotti et al., 2012).

### b. Non-competitive interactions for P in cereal-legume intercropping

Phosphorus is an other soil nutrient extremely important for crop growth, and seldom available for plants due to phenomena of immobilization linked to abnormal soil pH (Li et al., 2003). Cereal-legume intercropping is reported to improve the efficiency in P use compared to sole crops (Morris and Garrity, 1993a; Li et al., 2003; Hinsinger et al., 2011; Betencourt et al., 2012), through both direct or indirect modification of the root zone (Figure 4).

Among direct effects on P solubilization, the release of phosphatase through legume root exudates is of major importance (Hinsinger et al., 2011).

Indirect measures regard, first, modification of soil pH. Legumes are, indeed, reported to release acid root exudates into the soil, which may increase the solubility of P in alkaline soils (Li et al., 2003). Conversely, in acid soils a facilitation may come also from the effect of cereals, whose roots may increase soil pH, as reported by Betencourt et al. (2012).

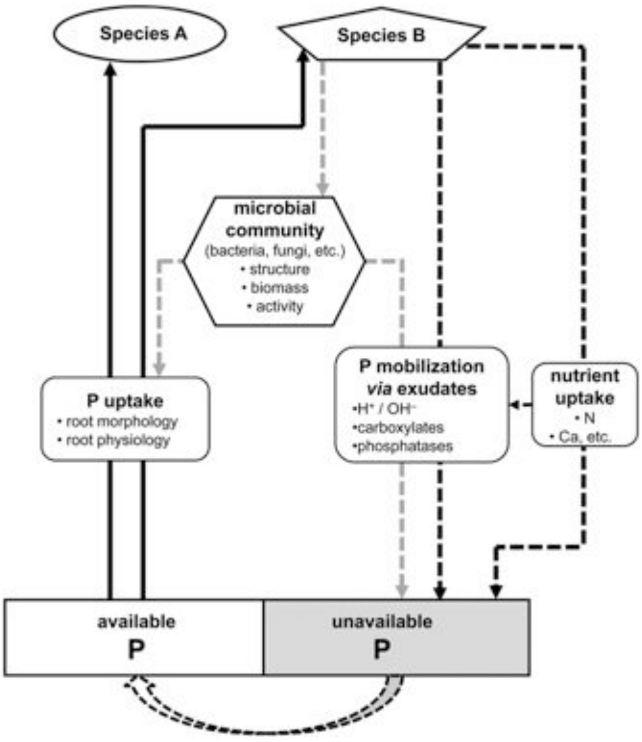
Interestingly, the same effect of alkalization might happen also due to the higher amount of calcium taken up by legumes compared to cereals. As Ca uptake and P availability are inversely correlated (Ca is reported to increase the adsorption of phosphates onto clay particles), a higher Ca uptake in legumes can result in a higher phosphate availability for the companion cereal (Hinsinger et al., 2011).

Also microbially mediated non-competitive interactions among intercrops and rhizosphere might play an important role in P mobilization.

Arbuscolar mycorrhizal fungi (AMF) are reported to realize mutualistic symbioses with roots of several cereals and legumes, and hence under intercropping ameliorating soil habitability for AMF an increased availability of P can be observed, due to the solubilization exerted by fungal hyphae on immobilized soil P. Besides phosphorus, AMF hyphal network seem also to act as bridges among different plant roots for transfer of N (San-nai and Ming-pu, 2000).

The exudation of chemicals, together with the modification of rhizosphere, acted by plants might also affect microbial population biomass, composition and activity, and thereby altering the availability of phosphorus under mixed plant systems (Hinsinger et al., 2011). Anyway, this complex of mechanisms is still far from clarification.

*Figure 4 - Root-induced (direct) and microbially mediated (indirect) positive interactions (facilitation) altering P availability in the rhizosphere of two intercropped species. Dotted arrows indicate how species B can mobilize P that is initially not available to species A, either directly (black arrows) or indirectly via soil microorganisms (gray arrows) such as bacteria and fungi, mycorrhizal or not. These processes result in increases in the size of the available P pool at the expense of the unavailable pool (indicated by the curved arrow). Solid black arrows indicate P uptake by the two species from the available P pool. From (Hinsinger et al., 2011)*



### c. Other non-competitive interactions in cereal-legume intercropping

Many papers demonstrated a lower incidence of pests and diseases in intercrops than sole crops (Perrin and Phillips, 1978; Hooks and Johnson, 2006; Hauggaard-Nielsen et al., 2008; Pelzer et al., 2012). Pridham and Entz (2008) reported for instance a lower incidence of wheat diseases in a wheat-pea intercropping than sole wheat.

Trenbath (1993) elucidated the three main mechanisms through which intercropping can lead to pest and disease attack escape. These included: i) the associates caused plant of the attacked component to be less good hosts (e.g. by reducing their growth through shading and other competitive interactions); ii) associates interacted directly with attacker (e.g. trap crops); iii) intercropping favor natural enemies of the attacker by modifying the surrounding environment (e.g. by providing food or shelter to enemies).

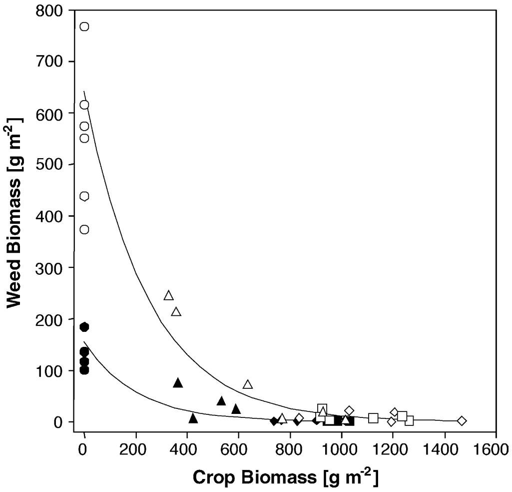
A particular kind of intercropping, including different varieties or populations of the same species (i.e. the cereal or/and the legume), was tested with the specific aim to keep under thresholds the damages caused by fungal diseases on cereals and legumes. The rationale of this type of intercropping is to increase the biodiversity of the cropping system not only *among* species, but also *within* species. Obviously, the optimum would be to mix genotypes of the same species with differing complementary traits, such as resistance to different pathogens, differing morphology (tall and short plants) and phenology (early maturing genotypes and late maturing genotypes). This might allow to achieve a good compensation of the detrimental effect on crop yields due to the use of resistant but less productive genotypes (Finckh et al., 2000).

Concerning soil-borne diseases, the suppressive effect produced by intercropping is documented to take place also through the release of biocidal compounds (Hauggaard-Nielsen and Jensen, 2005), or indirectly by stimulation of other microbial populations, such as arbuscolar mycorrhizal fungi (AMF) (San-nai and Ming-pu, 2000; Li et al., 2009).

An important target to address for cereal-legume intercropping is weed control, as shown by the plenty of papers dealing with this issue (Bulson et al., 1997; Hauggaard-Nielsen et al., 2001a; Poggio, 2005; Hauggaard-Nielsen et al., 2006; Hauggaard-Nielsen et al., 2008; Pridham and Entz, 2008; Corre-Hellou et al., 2011). Intercropping can interfere with weed growth through competitive mechanisms, including shading, capture of water and nutrient uptake, which at the end might increase the dry matter production of intercrops and, reversely, reduce that of weeds (Liebman and Dyck, 1993) (Figure 5).

Still, also non-competitive interactions can play a role. The release of allelopathic substances into the soil through root exudates or incorporation of residues of an intercrop may reduce the germination of weed seeds or reduce the growth of weed seedlings (Fernàndez-Aparicio et al., 2007). Allelopathic effect was demonstrated not only for cereals, but also for several legume species, such as hairy vetch (*Vicia villosa* Roth.) (Hill et al., 2007). Furthermore, the modification of the proximate environment , through, for instance, nutrient uptake, water capture, modification of relative humidity of air, might lead to the change of the composition of weed populations and also of their dynamics in the long-run (Poggio, 2005).

*Figure 5 - Relationship between weed and crop biomass at crop maturity in the experiments carried out by Poggio et al. (2005). Symbols: no crop (circles), pea (triangles), barley (squares), mixture (diamonds); empty symbols correspond to Buenos Aires experiments and solid symbols correspond to Rojas field experiment.*



### 1.4. Aims of the research

The aims of this research were to test the effects of facilitative intercropping between durum wheat and grain legumes on the yield and quality of wheat grain, in the context of organic farming and low-input conditions (without addition of mineral N fertilizers). Different options of intercropping were evaluated in a field experiment, including different species of legumes and differing duration of co-growth (temporal vs permanent intercropping). The two strategies of intercropping were evaluated in order to assess potential differences in resource use efficiency between them. An additive design of intercropping was chosen, whatever the treatment, with the aim to emphasize interactions among associates. The spatial arrangement (alternate rows) of the temporary intercropping was designed in order to allow the mechanical termination of the legume between the rows of the wheat. The same arrangement was adopted also for permanent intercropping treatments in order to allow comparison between the two strategies.

Besides the effect on the productivity and the mineral nutrition of the main crop, i.e. wheat, where the main focus was on, also additional parameters of resource use efficiency of the intercropping as a whole were considered.

Mutual interactions among components were also analyzed by mean of the measurement of crop growth over time, the analysis of competition indexes, and the estimation of symbiotic $N_2$-fixation in legumes.

Side effects of intercropping on the cropping systems (weed suppression, effect on residual soil fertility) were also evaluated to complete the analysis.

In order to assess the dynamic of N release in temporary intercropping, a parallel experiment in lysimeters was set up, where intercropping was compared with wheat sole crop unfertilized or fertilized with mineral and organic N fertilizers, in order to underline potential differences in N mineralization and N budget.

# 2. Materials and Methods

In the period 2009-2011 two parallel experiments, one in open field, one in lysimeters, were carried out at the Rottaia Experimental Station of the Dipartimento di Scienze Agrarie, Alimentari e Agro-ambientali of the University of Pisa, San Piero a Grado, Italy (43°40’ N; 10°18’ E) in the framework of the NITBIO (*Interventi agronomici atti ad ottimizzare la disponibilità di azoto per la produzione di frumenti di qualità in agricoltura biologica*) project (2010-2013), funded by the Italian Ministry of Agriculture (MIPAAF). Details about each experiment are described below.

## 2.1 Field experiment

### *2.1.1.Site characteristics, weather trend, experimental design, crop management*

A field experiment was set up in 2009/10, and replicated in 2010/11 in an adjacent field, in order to study the effects of intercropping with grain legumes on yield, biomass production, grain quality, growth dynamics and mineral nutrition of durum wheat (*Triticum durum* Desf.), grown under field conditions.

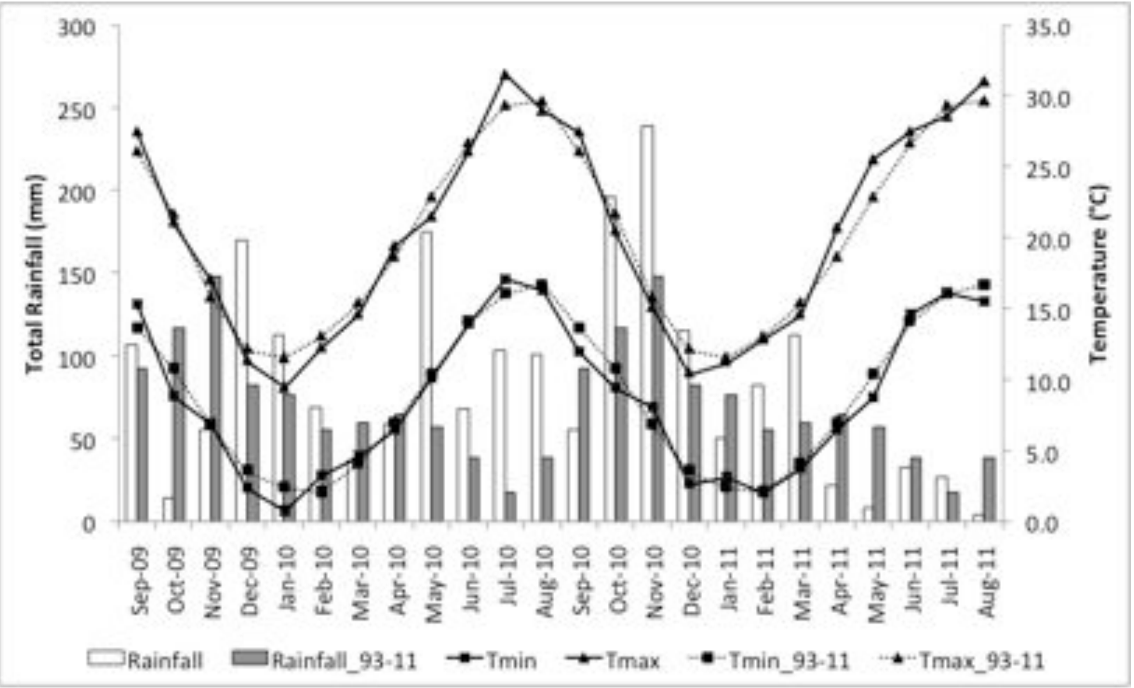
Climatic conditions of the site are representative of Mediterranean coastal areas, with a yearly average rainfall and temperature of 844 mm and 15°C, respectively. Rainfall are mostly concentrated in autumn and early spring, with very dry conditions during the summer. During the cold season, temperatures rarely reach very low values (the lowest mean minimum temperature on a monthly base is 2.1°C, recorded in February), whilst in the summer they can be very high (the highest monthly mean maximum temperature is 29.7°C, recorded in August).

Weather trends recorded in 2009/10 and 2010/11 at the weather station of the experimental station are depicted in Figure 6.

As shown in the chart, the two years of the experiment were completely different from the point of view of the precipitations. In 2009/10, rainfalls mostly concentrated in winter and late spring, whilst in the second year they covered all the fall-winter season and suddenly stopped in spring. The weather consequently affected timeliness of soil tillage and other field operations, first of all the seeding date. In the first season, the crops were drilled in autumn, in the presence of a very dry and cloddy soil; on the contrary, in the second year the soil remained too wet until February due to the very frequent rainfalls occurred from October.

On the other hand, temperatures were pretty in line with long term trends, except for some negative variations from the mean values in 2009/10 and positive variations in 2010/11 (Fig. 6).

*Figure 6 - Monthly rainfall (mm), maximum and minimum temperatures (°C) recorded in 2009/10 and 2010/11 at the experimental station. Also long term (1993-2011) patterns are depicted.*



The fields where the experiment was carried out in the two years had the same crop rotation, with fallow as preceding crop. The main characteristics of the soil in the two experimental fields were determined before the establishment of the experiment, in September 2009 and September 2010, respectively. For each field, 5 soil cores were randomly collected at 30 cm of depth with a 5.7 cm soil probe. Soil samples were then put into plastic bags properly labeled and stored in a cold room at 4°C for few days, before analysis.

Results of soil characterization and applied methodology are reported in Table 1.

The soil interested by the experiment in both years was a Typic Xerofluvent loamy soil, with a medium content of soil organic matter and nutrients, pH was lightly alkaline, and the risk of salinity was pretty low.

**Table 1 -** Main soil characteristics measured in the 0-30 cm soil layer before the beginning of the experiment in 2009/10 and in 2010/11. n.a. is not available

| Parameter | 2009/10 | 2010/11 | Measure Unit |
|---|---|---|---|
| Clay (<2 µm)[1] | 272.36 | 288.36 | g $kg^{-1}$ |
| Silt (2-50 µm)[1] | 297.48 | 379.10 | g $kg^{-1}$ |
| Sand (50-2000 µm)[1] | 430.16 | 332.54 | g $kg^{-1}$ |
| Soil Organic Matter[2] | 22.08 | 21.78 | g $kg^{-1}$ |
| pH[3] | 7.91 | 8.20 | - |
| Total N[4] | 1.07 | 1.11 | g $kg^{-1}$ |
| Extractable P[5] | 18.65 | 3.64 | mg $kg^{-1}$ |
| Exchangeable K[6] | 192.02 | n.a. | mg $kg^{-1}$ |
| Total limestone [7] | 10.19 | n.a. | g $kg^{-1}$ |
| Electrical Conductivity[8] | 232.16 | 74.72 | µS $cm^{-1}$ |
| Cation Exchange Capacity[9] | 25.68 | n.a. | meq 100 $g^{-1}$ |

[1] *USDA Method (Gee and Bauder, 1986)*
[2] *Walkley–Black method (Nelson and Sommers, 1982)*
[3] *pH meter using an extract of a 1 to 2.5 dilution of soil with water (McLean, 1982)*
[4] *Kjeldahl method (Bremner and Mulvaney, 1982)*
[5] *Olsen method (Olsen and Sommers, 1982)*
[6] *(Mehlich, 1984)*
[7] *(Nelson, 1982)*
[8] *Potentiometric method (Violante and Adamo, 2000)*
[9] *Barium chloride-compulsive exchange method (Rhoades, 1982)*

In both years the experimental field was ploughed at 30 cm of depth, then harrowed by rotary harrow and rolled. When the seedbed had been prepared, the fields were subdivided in 42 plots of 7.2 m width and 7 m length each. On these plots 14 different treatments were compared under a Completely Randomized Block (CRB) design (Gomez and Gomez, 1984) with 3 replications (Fig. 7). Treatments were the following:

- HV-DW PIC: permanent intercropping between durum wheat (cv. Claudio) and hairy vetch (*Vicia villosa* Roth cv. Capello);
- FP-DW PIC: permanent intercropping between durum wheat (cv. Claudio) and field pea (*Pisum sativum* L. cv. Corallo);

- PB-DW PIC: permanent intercropping between durum wheat (cv. Claudio) and pigeon bean (*Vicia faba* var. minor Beck cv. Torre Lama Scuro);
- HV-DW TIC: temporary intercropping between durum wheat (cv. Claudio) and hairy vetch (*Vicia villosa* Roth cv. Capello);
- FP-DW TIC: temporary intercropping between durum wheat (cv. Claudio) and field pea (*Pisum sativum* L. cv. Corallo);
- PB-DW TIC: temporary intercropping between durum wheat (cv. Claudio) and pigeon bean (*Vicia faba* var. minor Beck cv. Torre Lama Scuro);
- N0: durum wheat pure crop unfertilized;
- N40: durum wheat pure crop fertilized with 40 N units ha$^{-1}$ applied as ammonium nitrate (26-0-0) at the end of the tillering stage of wheat (BBCH 29) (Stauss et al., 1994);
- N80: durum wheat pure crop fertilized with 80 N units ha$^{-1}$ applied as ammonium nitrate (26-0-0) in two different applications: 50% at the end of the tillering stage of wheat (BBCH 29), 50% in full stem elongation stage of wheat (BBCH 33) (Stauss et al., 1994);
- N120: durum wheat pure crop fertilized with 120 N units ha$^{-1}$ applied as ammonium nitrate (26-0-0) in two different applications: 50% at the end of the tillering stage of wheat (BBCH 29), 50% in full stem elongation stage of wheat (BBCH 33) (Stauss et al., 1994);
- N160: durum wheat pure crop fertilized with 160 N units ha$^{-1}$ applied as ammonium nitrate (26-0-0) in two different applications: 50% at the end of the tillering stage of wheat (BBCH 29), 50% in full stem elongation stage of wheat (BBCH 33) (Stauss et al., 1994);
- HV: hairy vetch pure crop unfertilized;
- FP: field pea pure crop unfertilized;
- PB: pigeon bean pure crop unfertilized.

In permanent intercropping treatments, the two companion crops, namely wheat and legume, were left grown together until harvest, which occurred simultaneously for both crops, whilst in temporary intercropping treatments the pulse crop was terminated and incorporated into the soil by rotary hoe at the beginning of the stem elongation stage of the wheat (BBCH 30) (Stauss et al., 1994).

*Figure 7 - Overview of the two field experiments after crop emergence in 2009/10 (on the left, 26/11/2009) and 2010/11 (on the right, 23/03/2011)*

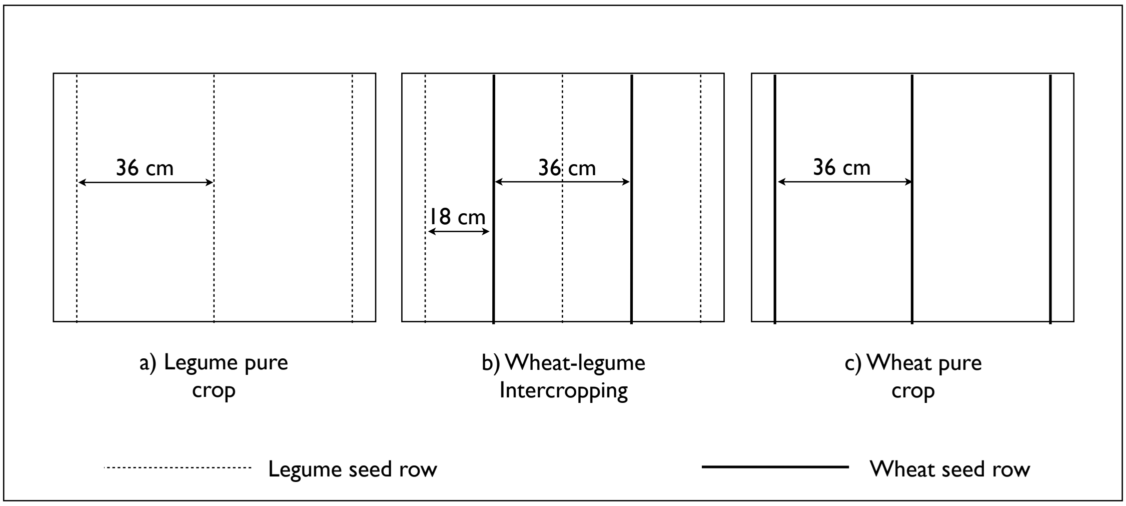
In both the strategies of intercropping, wheat and pulses were grown on alternated seed rows (i.e. one row of wheat alternated with one row of legume, see Fig. 8). The seeding of the two companion crops took place at the same date, by mean of 2 consecutive passes of a plot driller. For the first pass, the seed tank was filled only with wheat kernels, whilst for the second one, performed by driving the machine along the seed furrow of the previous pass, only the seeds of the legumes were drilled.

The seeding rate of each species grown in intercropping was determined according to an additive design, by using the same seed rate chosen for the pure stands. This latter was decided according to the normal technique followed by farmers in the area, and, exceptionally, depending on the seeding date. In 2009/10, wheat was seeded at a unique seeding rate of 400 seeds $m^{-2}$ regardless of the treatments (i.e. pure crop or intercropping). In 2010/11, the seeding rate of wheat was increased to 600 seeds $m^{-2}$ for all the treatments, due to the very late seeding date (Table 2). In both years, the seeding rate of the legumes was 80, 100 and 200 seeds $m^{-2}$, respectively, for pigeon bean, field pea and hairy vetch, grown in pure stands as well as intercropped with wheat.

Pure crop treatments of wheat and each of the three legumes were included in the experimental design as control treatments and consequently used as reference crops for the calculations of competition indexes.

In order to make the performances of pure crops and intercropping comparable, crops were established with the same inter-row distance, which was 36 cm in intercropping as well as in pure crops. The inter-row distance between wheat and legumes in intercropping was, consequently, 18 cm (Fig. 8).

*Figure 8 - Spatial disposition of seed rows of legumes and wheat in pure crops and intercropping*



Crops did not receive any fertilizer, except for the ammonium nitrate applied at proper rates on wheat pure crop plots. Moreover, no crop protection products were applied, and no weed control was performed.

In Table 2, the most relevant field operations performed in the two years are shown.

*Table 2 - Field operations scheme in 2009/10 and 2010/11*

| Field Operation | 2009/10 | 2010/11 | Notes |
|---|---|---|---|
| Ploughing | 05-10-09 | 14-09-10 | 30 cm depth |
| Harrowing | 29-10-09 | 24-01-11 | 1 pass of disk harrow + 2 passes of rotary harrow |
| Sowing | 30-10-09 | 07-02-11 | All the treatments |
| First application of ammonium nitrate | 29-01-10 | 13-04-11 | Only N40, N80, N120 and N160 plots |
| Termination of legumes | 31-03-10 | 06-05-11 | Only temporary intercropping treatments (HV-DW TIC, FP-DW TIC, PB-DW TIC). Rotary hoe (2 passes) |
| Second application of ammonium nitrate | 07-04-10 | 29-04-11 | Only N80, N120 and N160 plots |
| Harvest | 01-07-10 | 30-06-11 | All treatments |

In the second year, on the same field where the experiment was carried out in 2009/10, a durum wheat (cv. Claudio) crop was directly seeded in order to study the residual effect of fertility of treatments applied beforehand. Unfortunately, due to the adverse weather conditions, the establishment of the crop completely failed. The field was then kept free from weeds through the application of herbicides in the winter (Glyphosate 2 kg ha$^{-1}$ sprayed just once) and a minimum tillage performed in early spring with a disk harrow combined with a chisel. Afterwards, the seedbed was prepared with a rotary harrow and a grain maize crop (*Zea mays* L. hybrid PR36Y03, FAO Class 300) was seeded and harvested at maturity. More details are reported in Table 3.

*Table 3 - Field operations scheme for maize grown in 2010/11 on the experimental field of 2009/10*

| Field Operation | Date | Notes |
|---|---|---|
| Weed cutting | 23-09-10 | Plant crusher (1 pass) |
| Herbicide spray | 09-02-11 | Glyphosate 2 kg ha$^{-1}$ |
| Wheat direct sowing | 10-02-11 | Durum wheat cv. Claudio (250 kg ha$^{-1}$) |
| Main tillage | 19-04-11 | Disk harrow coupled with chisel (1 pass) |
| Seedbed preparation | 22-04-11 | Rotary harrow (2 passes) |
| Maize sowing | 27-05-11 | PR36Y03 hybrid FAO class 300 - 7 plants m$^{-2}$ |
| Maize harvesting | 3-10-11 | Combine harvester |

### *2.1.2.Sampling protocol and analytical methods*

The effect of intercropping was evaluated not only at the harvest time of wheat but also during its cycle, in order to study whether the treatments did affect or not the dynamics of growth, development and mineral nutrition of the crops.

Sampling schemes and procedures for all the two years are detailed in Table 4.

Table 4 - Sampling protocol of the main experiment in 2009/10 and 2010/11

| BBCH Stage[1] | Date | | Sampling area (m) | Nr of subsamples | Plant density[2] | Biomass yield[2] | NP accumulation[2] | Wheat grain quality | Plant height[2] | Soil Visual Coverage[2] | Weed biomass | $^{15}N$ natural abundance assessment | N equivalents | Competition indexes |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | Year 1 | Year 2 | | | | | | | | | | | | |
| 10 | 26-11-09 | 23-03-11 | 0.50 x 0.72 | 2 | x | | | | | | | | | |
| 14 | 28-01-10 | 12-04-11 | 0.50 x 0.72 | 1 | | | | | x | x | | | | |
| 30 | 16-03-10 | 28-04-11 | 0.50 x 0.72 | 1 | | x | x | | x | x | x | x | | x |
| 59 | 04-05-10 | 29-5-11 | 0.50 x 0.72 | 1 | | x | x | | x | x | x | | | |
| 69 | 12-05-10 | 25-05-11 | 0.50 x 0.72 | 1 | | x | x | | x | x | x | | | |
| 75 | 07-06-10 | 10-06-11 | 0.50 x 0.72 | 1 | | x | x | | x | x | x | | | |
| 89 | 01-07-10 | 30-06-10 | 0.50 x 0.72 | 3 | | x | x | x | x | x | x | x | x | x |

1 (Stauss et al., 1994)
2 Wheat, legume, total

Destructive assessments on wheat were performed on quite limited sampling area (0.36 $m^2$) in order to keep available enough material for the following samplings, and also to preserve until harvest a significative portion of the plot for the study of the residual effect of fertility of each treatment. The number of subsamples collected per plot was increased to three at final harvest date, in order to produce more reliable data, being harvest the growth stage where focus was mostly on. Anyway, sampling areas were carefully identified each time in order to consistently represent the real performance of the plots.

Biomass samples were collected in the field, separated in the different plant portions (i.e. straw, chaff and kernels for wheat; straw, pods and grain for legumes; total aboveground biomass for weeds) in the lab and then weighted after oven-dried at 60°C until constant weight. Dry samples of each material were then finely ground with a grinder and homogenized for chemical analyses, performed at the chemical lab of the Research Centre “Enrico Avanzi” (CIRAA) of the University of Pisa.

Total nitrogen content in the different portions of the biomass was evaluated according to the Kjeldahl method (Bremner and Mulvaney, 1982), which is based on the mineralization of organic N in ammonia.

Extractable phosphorus was evaluated according to the Olsen method (Olsen and Sommers, 1982).

Plant height of wheat and legumes was evaluated at canopy level (including awns for wheat) measuring 6 plants randomly selected on a 1 $m^2$ area per plot. The mean minimum height and the mean maximum height were then averaged to obtain the mean plant height of the plot.

Soil visual cover was assessed by visually estimating the percentage of the area covered by, respectively, wheat, legume and weeds on a 1 $m^2$ area randomly selected within each plot.

Wheat grain quality was evaluated at harvest through thousand kernel weight (dry mass of 1000 kernels weighted after oven-dried at 60°C until constant weight), and test weight (Shopper chondrometer with 250 ml cylinder), both measured on a subsample of the bulk of grain coming from the threshing of the whole plot.

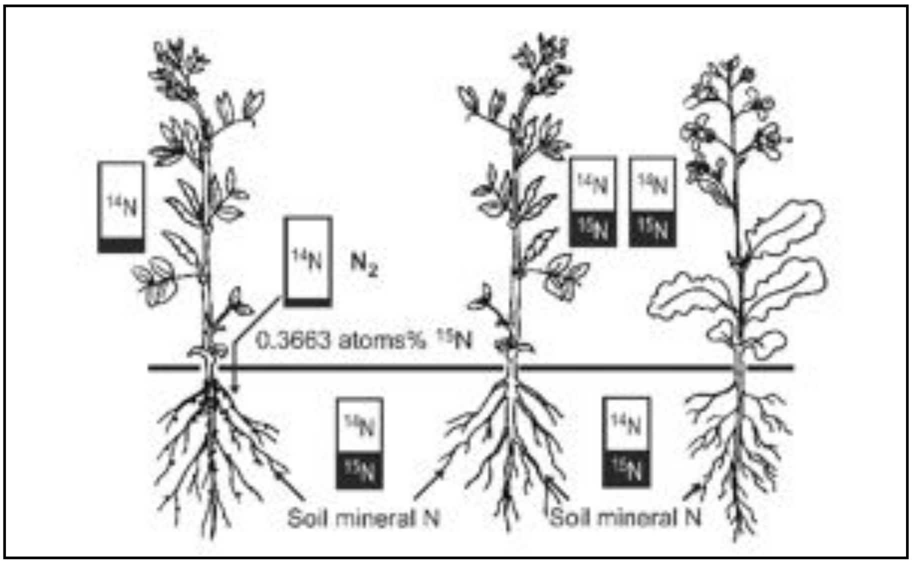
In cooperation with the laboratory of the Research Unit for Cereal Quality of the Agricultural Research Council (CRA-QCE), also grain protein content, gluten content (only in 2010/11), sodium dodecyl sulfate (SDS) sedimentation test were performed on subsamples of wheat grain. Protein content (g $kg^{-1}$) was measured by Near Infrared

Transmittance (NIT) technology employing the InfratecTM 1241 Grain Analyzer FOSS, (wavelength range: 570-1100 nm). Data Integration was carried out by FOSS DataLogger and DataLink software. SDS was determined on kernels of wheat, ground with a Cyclotec-PBI grind equipment (1 mm mesh) and added with 3% SDS solution (ICC method 151) (Preston et al., 1982). Samples from the three different replicates of the treatments were then merged in a unique sample, homogenized and milled with a Buhler MLU 202 (Uzwil, Switzerland) experimental mill. Dry gluten content (g $kg^{-1}$) was then determined following the EN ISO 21415 method. Wheat flour dough was washed with a buffer solution and weighted after oven-dried. The weight of dry gluten is then referred to dry matter of grain.

The estimation of biological $N_2$-fixation of legumes was performed with the $^{15}N$ natural abundance technique (Peoples et al., 2009b) in collaboration with the chemical laboratory of the Biosystems division of the Risø National Lab for Sustainable Energy, Danish Technical University (DTU), Roskilde (DK).

The rationale of the technique is that the two natural isotopes of nitrogen ($^{14}N$ and $^{15}N$) have a different abundance in the air and in the soil. In the atmosphere, $N_2$ gas has a fixed concentration of $^{15}N$ (0.3663% of total N atoms), whilst in the soil the relative abundance of this isotope is sensibly much higher than that of $^{14}N$, due to the effect of the transformation of the organic N caused by soil microorganisms. As a result, legumes, which mostly takes N from the atmosphere through symbiotic $N_2$-fixation, have a $^{15}N$ relative abundance very similar to the air. On the contrary, non-legume species have a pretty much higher $^{15}N$:$^{14}N$ ratio, as they mainly uptake N from the soil with roots (Fig. 9). More details about the technique are reported in Appendix 2, attached to the end of this manuscript.

*Figure 9 - Diagrammatic representation of the $^{15}N$ composition (indicated by the size of the black bar at the bottom of the histogram) of N accumulated by a nodulated legume using both soil mineral N and atmospheric $N_2$ for growth. Also the $^{15}N$ compositions of two non $N_2$–fixing plants, a non-nodulating legume and a non-legume, using only soil mineral N for growth are depicted (Peoples et al., 2009b)*



In both 2009/10 and 2010/11, at stem elongation stage and at harvest maturity, 2 0.5 $m^2$ subsamples of each plant material (straw and grain of wheat and legumes, total aboveground biomass of weeds) were: collected in all the plots, except for N40 and N120, oven-dried at 60°C for 48 hours, merged together in a unique sample per plot, homogenized and ground with a 1 mm mesh sieve. About 5 mg of each sample was put into tine capsule for micro-titration and, finally, shipped to the Risø Lab for analysis, which consisted in the assessment of N isotope abundance performed by coupling a carbon-hydrogen-nitrogen elemental analyzer (EA) with a gas mass-spectrometer (MS). The MS gives back the $^{15}N$ abundance of the sample expressed as δ value with reference to the standard which has the same isotope composition of the air (δ=0):

$$\delta^{15}N_{sample} = 1000 \times [(\text{atom\% }^{15}N_{sample} - 0.3663)/0.3663]$$

The natural abundance technique is considered extremely sensitive to environmental variability and also to mistakes occurring during sampling and sample handling. For instance, even a little contamination of samples of legume plants with grasses may dramatically change the δ value of the legume. This implies that a great attention has to be paid when defining sampling procedures. In this case, among all the different precautions listed and explained in the report appended to this dissertation (Appendix 2), particular emphasis was put on sampling tools (e.g. by using different sickles for collection of wheat biomass and legume biomass), oven-drying (e.g. by using different ovens to dry wheat and legumes) and grinding, e.g. by cleaning very carefully the grinding equipment before proceeding with a new sample, throwing away the first 2-3 spoons of the material, and also processing samples in this order:

*intercropped legume (all the replicates)→monocropped legume (all the replicates) →intercropped reference crop (all the replicates)→monocropped reference crop (all the replicates).*

The effect of treatments on weeds was evaluated assessing dry matter production and NP concentration and accumulation of weeds collected in the same frame where also crop samples were collected. Furthermore, as indicator of competition for space between crops and weeds, also soil visual cover of each crop, weeds and bare soil was estimated, and visual soil cover ratio (SCR) was then calculated.

### *2.1.3. Calculations*

N and P accumulations (g $m^{-2}$) in plant materials (straw, grain, ears, pods, weeds) were calculated by multiplying N and P concentration (g $kg^{-1}$) in the specific material by the respective dry biomass (kg $m^{-2}$).

Nitrogen equivalents of wheat grown in intercropping with legumes were calculated on the base of a regression curve (*y-axis*: wheat performance parameter; *x-axis*: level of N fertilization [kg N $ha^{-1}$]) built by fitting measured values of performance indicators of wheat grown as sole crop at the 5 different levels of N fertilization. Wheat performance indicators tested were: grain dry weight, total aboveground dry weight, N accumulation in grain, N accumulation in total aboveground biomass.

In both years, N recovery (Nrec) and N Use Efficiency (NUE) of wheat were computed at harvest maturity for wheat according to the methodology described by Wagger (1989) and Varvel (1990), respectively.
N recovery was computed for all intercropping and fertilized wheat treatments as:

$$N_{\mathrm{Re}c} = N_f - N_c$$

where $N_f$ is N accumulation in total aboveground dry matter of wheat fertilized or under intercropping, and $N_c$ is N accumulation in total aboveground dry matter of wheat unfertilized.

NUE was computed for temporary intercropping and fertilized wheat treatment with the difference method:

$$NUE(\%) = \frac{(N_f - N_c)}{R} * 100$$

where Nf is N accumulation in total aboveground dry matter of wheat fertilized or under intercropping, Nc is N accumulation in total aboveground dry matter of wheat unfertilized, and R is the fertilization rate or N accumulation in total aboveground dry matter of legume intercropped with wheat at the time of incorporation into the soil.

Competition between species grown together in intercropping treatments was investigated at stem elongation and at harvest maturity in both years. Three different indexes of competition were computed, namely the *Land Equivalent Ratio* (LER) (Willey, 1979), the *Relative Neighbour Effect* (RNE) (Markham and Chanway, 1996; Bartelheimer et al., 2006) and the *Aggressivity Index* (A) (McGilchrist, 1965).

The three competition indexes were computed for total aboveground dry weight and also for N accumulation in total aboveground dry biomass both for temporary (only at stem elongation stage, i.e. before the termination of the legume) and permanent (at stem elongation and harvest) intercropping. As wheat pure crop, the wheat fertilized with all the five level of N fertilization (from 0 to 160 kg N ha$^{-1}$) was considered, in order to test how the three competition indexes would vary with respect to different references.

LER is defined as "*the relative land area under sole crops that is required to produce the yields achieved in intercropping*" (Willey, 1979). For a legume-wheat intercropping LER is computed as:

$$LER = LER_L + LER_W;$$

$$LER_L = \frac{P_{LW}}{P_L};$$

$$LER_W = \frac{P_{WL}}{P_W}$$

where: $LER_L$ is the partial LER of legume; $LER_W$ is the partial LER of wheat; $P_{LW}$ and $P_L$ are the performances of the legume as intercrop and pure crop, respectively; $P_{WL}$ and $P_W$ are the performances of the wheat as intercrop and pure crop, respectively.

A LER>1 indicates a better use of environmental resources under intercropping than sole crops (in other words, a LER of 1.1, for instance, means that it would be necessary to crop 10% more land with pure crops to achieve the same yield produced by one unit of land managed with intercropping), whilst, on the contrary, a LER<1 or a LER=1 indicate, respectively, a less efficient or a likewise efficient use of resources under intercropping than pure crops (Willey, 1979). All these things provided that intercropping and sole crops are managed with the same crop technique (i.e. same fertilization level, same crop protection strategy, etc.).

RNE provides information on the occurrence of *facilitation* or *competition* between the component crops of intercropping. RNE was computed as:

$$RNE = \frac{(P_{control} - P_{mix})}{x}$$

with

$$x = P_{control} \quad \text{when} \quad P_{control} > P_{mix}$$

$$x = P_{mix} \quad \text{when} \quad P_{mix} > P_{control}$$

where $P$ is the wheat performance in the presence ($P_{mix}$) or absence ($P_{control}$) of legumes. RNE ranges from -1 to 1 with negative values indicating facilitation and positive values competition.

The A index is useful to highlight which of the two component species of an intercropping is the dominant one, and which other is the dominated. A was computed for legumes ($A_L$) and for wheat ($A_W$) as:

$$A_W = \frac{P_{WL}}{P_W * S_{WL}} - \frac{P_{LW}}{P_L * S_{LW}}; \qquad A_L = \frac{P_{LW}}{P_L * S_{LW}} - \frac{P_{WL}}{P_W * S_{WL}}$$

where $P_W$ and $P_{WL}$, and $P_L$ and $P_{LW}$, are the crop performance indicators in pure crops ($P_W$ and $P_L$) and intercropping ($P_{WL}$ and $P_{LW}$), respectively, of wheat and legumes. $S_{WL}$

and $S_{LW}$ are the proportions at sowing of wheat and legume in mixture, respectively. $S_{LW}$ was 17%, 20% and 33%, respectively, for bean, pea and vetch in both permanent and temporary intercropping. $S_{WL}$ was the difference to 100% of each seed ratio.

If $A_W = A_L = 0$, both species are competitive; if $A_W > 0$ (i.e. $A_L < 0$), then wheat is the dominant species; if $A_L > 0$ (i.e. $A_W < 0$), then wheat is the dominated species.

$N_2$-biological fixation of legumes (under intercropping and also as pure crops) and transfer of fixed N from legumes to the companion wheat in intercropping treatments were estimated on the basis of the δ values obtained by mass spectrometer analysis of plant material collected in the two years at stem elongation stage and at harvest maturity.

δ values were produced for each different part of the plants. At stem elongation stage, δ was computed for total aboveground biomass of wheat and of legumes; at harvest, δ was separately computed for grain and straw of wheat, as well as for legumes. Chaff and pods were considered of little importance for this research, and thus not analyzed. Unique δ values for the whole aboveground biomass of legumes and wheat were calculated as:

$$\delta\,^{15}N_{total\ biomass} = \left[\frac{\left(\delta\,^{15}N_{straw} * N\ accumulation_{straw}\right) + \left(\delta\,^{15}N_{grain} * N\ accumulation_{grain}\right)}{N\ accumulation_{total\ aboveground\ biomass}}\right]$$

The percentage of N derived from the atmosphere (Ndfa) of legumes was calculated by comparing $^{15}N$ enrichment of legumes with that of a reference plant, identified with the durum wheat sole crop unfertilized (N0):

$$\%\ Ndfa_{legume} = \left[\frac{\left(\delta\,^{15}N_{reference} - \delta\,^{15}N_{legume}\right)}{\left(\delta\,^{15}N_{reference} - B\right)}\right] * 100$$

where B is the $\delta^{15}N$ of legume grown in a N free medium (N is the only limiting factor) (Peoples et al., 2009b). B was -0.70 for hairy vetch, -0.66 for field pea and -0.50 for pigeon bean (Unkovich et al., 2008). As Ndfa values obtained from these calculations were considered as acceptable, no corrections for N content in crop seeds and roots were applied.

Ndfa was also calculated in mass units (g $m^{-2}$) by multiplying Ndfa% by N accumulation in total aboveground biomass of legumes (g $m^{-2}$).

The transfer of N from legumes to wheat through the release of root exudates, and the mineralization of legume biomass fallen down to the soil accidentally (temporary and permanent intercropping treatments) or as results of hoeing (temporary intercropping treatments only), was estimated on the basis of the following formula:

$$Ntransfer = \left[1 - \left(\frac{\delta\, {}^{15}N_{WL}}{\delta\, {}^{15}N_{W}}\right)\right] * 100$$

where $\delta^{15}N_{WL}$ is the $\delta^{15}N$ value of wheat under intercropping, and $\delta^{15}N_{W}$ is the $\delta^{15}N$ value of wheat pure crop unfertilized (N0).

Visual soil cover ratio (SCR) was computed as follows:

$$SoilCoverRatio = \frac{\left(SC_{WIC} + SC_{LIC}\right)}{x}$$

where:

$SC_{WIC}$ is the value of soil cover assessed for wheat under intercropping;

$SC_{LIC}$ is the value of soil cover assessed for the respective legume under intercropping;

$x$ is the maximum value of soil cover assessed for all the pure crops of wheat (all the N fertilization rates) and the specific legume included in intercropping. For instance, for PB-DW PIC:

$SC_{WIC}$ was the value of soil cover assessed for wheat under PB-DW PIC;

$SC_{LIC}$ was the value of soil cover assessed for pigeon bean under PB-DW PIC;

$x$ was the maximum value of soil cover among: N0, N40, N80, N120, N160 and PB.

SCR values bigger than 1 mean higher soil cover under intercropping than in every sole crop treatment.

### *2.1.4.Data analysis*

Results of the two years were analyzed separately, due to the high differences of weather conditions.

1-way analysis of variance (ANOVA) for a Randomized Complete Block design was performed using the CoStat Software (CoHort-Software, 2007). Differences between treatment means were compared using a Fisher's protected LSD test at $P<0.05$ (Gomez and Gomez, 1984).

Performance indicators of legumes grown as sole crops or under intercropping were compared species by species using a *t*-test pair comparison at $P < 0.05$ (Gomez and Gomez, 1984).

Before analysis, the Bartlett test was performed to test the homogeneity of error variances, and appropriated data transformation was applied when necessary. Soil visual cover data were transformed to:

$$\left\{ a\sin\left[\sqrt{\left(\frac{x}{100}\right)}\right]\right\} * \left(\frac{180}{\pi}\right)$$

where $x$ is soil cover (%) of wheat, legume or weeds.

*Nrec* data were transformed in:

$$\log_{10}\left(N_{rec} + x\right)$$

where $x$ was 30 in 2009/10, and 20 in 2010/11.

*NUE* data were transformed in:

$$\log_{10}\left(NUE + x\right)$$

where $x$ was 1 in 2009/10, and 5 in 2010/11.

N equivalents of the different types of intercropping were calculated through regression analysis. A second order polynomial regression was used for all the indicators listed in the previous paragraph, except for N accumulation in grain and total aboveground biomass of wheat in 2009/10, in whose case a linear regression fitted best. The coefficient of determination ($R^2$) for each regression analysis was computed with the least squares method (Gomez and Gomez, 1984).

The maize crop following wheat was sampled at harvest maturity, by collecting 1 sample of 2 $m^2$ per plot. Dry matter production and N accumulation of stubbles, cobs and grain were determined with the same methods described for wheat.

## 2.2 Lysimeter experiment

### 2.2.1. Site characteristics, experimental design, crop management

In parallel with the field experiment, a lysimeter experiment was carried out for two years (2009-2011) in order to study the effect of intercropping on N budget and mineral nutrition of wheat.

For this experiment, 18 open-top drainage lysimeters built in 2006 at the experimental station and uncropped since 2008 were used. Lysimeters were plastic-made tanks of about 1 $m^3$ volume (0.95 m length, 1.15 m width, 1.00 m height), placed into galvanized steel frames, leaning on bricks displaced on two parallel rows at 0.50 m from the ground (Fig. 10). The tanks have been surrounded by insulating panels, in order to decrease heat exchange between the soil and the air.

*Figure 10 - Open-top lysimeters used for the experiment. Pictures taken at the Rottaia Experimental Station of the Dipartimento di Scienze Agrarie, Alimentari e Agro-ambientali of the University of Pisa (26/11/2009)*

The tanks were originally drilled on the bottom, then the hole was covered by a 5 cm thick layer of gravel in order to facilitate drainage. On the top of the gravel layer a fine-maze gauze sheet was laid down to filter out solids from drainage water and to avoid clogging. At the end, the lysimeters were filled with soil collected from a near field owned by the Experimental Station.

The main characteristics of the soil are reported in Table 5.

As shown in the table, the soil type was a sandy-loam type, with a low level of organic matter. This coarse texture soil was mainly chosen with respect to the needs to have a good drainage of water, necessary to detect even minimum amount of N mineralized and leached, and a high rate of mineralization of organic material added to the soil with treatments.

*Table 5 - Main soil characteristics measured in the 0-30 cm soil layer before the beginning of the experiment in 2008*

| Parameter | Value | Measure Unit |
|---|---|---|
| Clay (<2 µm)[1] | 78.00 | g $kg^{-1}$ |
| Silt (2-50 µm)[1] | 63.00 | g $kg^{-1}$ |
| Sand (50-2000 µm)[1] | 859.00 | g $kg^{-1}$ |
| Soil Organic Matter[2] | 8.30 | g $kg^{-1}$ |
| pH[3] | 7.70 | - |
| Total N[4] | 0.40 | g $kg^{-1}$ |
| Extractable P[5] | 6.70 | mg $kg^{-1}$ |
| Electrical Conductivity[6] | 40.90 | µS $cm^{-1}$ |
| Cation Exchange Capacity[7] | 5.20 | meq 100 $g^{-1}$ |

[1] *USDA Method (Gee and Bauder, 1986)*
[2] *Walkley–Black method (Nelson and Sommers, 1982)*
[3] *pH meter using an extract of a 1 to 2.5 dilution of soil with water (McLean, 1982)*
[4] *Kjeldahl method (Bremner and Mulvaney, 1982)*
[5] *Olsen method (Olsen and Sommers, 1982)*
[6] *Potentiometric method (Violante and Adamo, 2000)*
[7] *Barium chloride-compulsive exchange method (Rhoades, 1982)*

The hole at the bottom of each lysimeter was connected by a multilayer water pipe to a 30 liter water tank, in order to allow sampling of drainage water. At the beginning of the experiment, 80 liters of irrigation water were applied on the soil surface of each lysimeter in order to saturate all the soil volume and remove residuals of N.

In 2009/10, 6 different treatments were applied to the lysimeters according to a completely randomized (CR) design, threefold replicated (Gomez and Gomez, 1984):

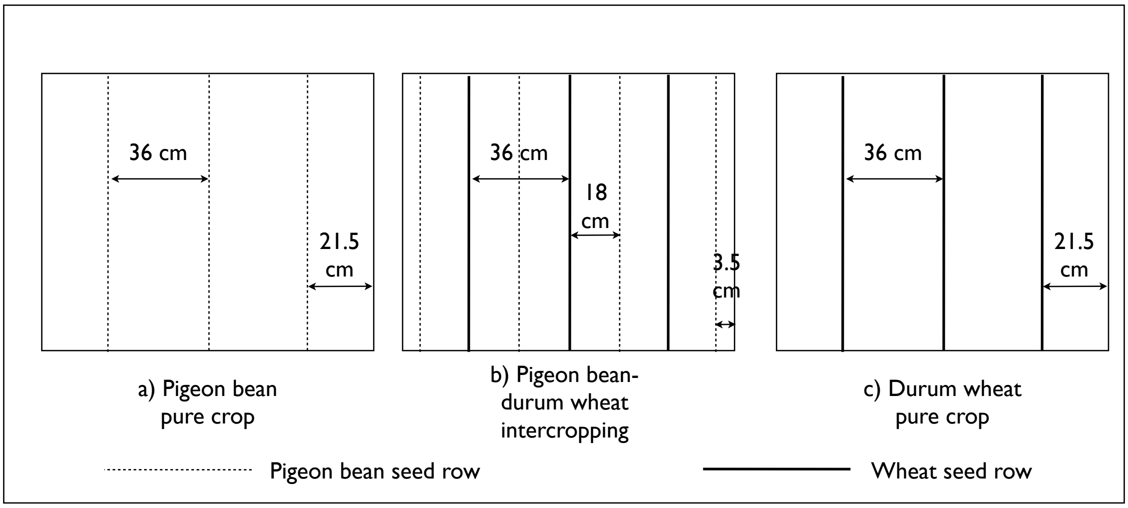
- PB-DW TIC: temporary intercropping between durum wheat (cv. Claudio) and pigeon bean (*Vicia faba* var. minor Beck cv. Torre Lama Scuro);
- N0: durum wheat pure crop unfertilized;
- N80 MIN: durum wheat pure crop fertilized with 80 N units $ha^{-1}$ applied as ammonium nitrate (26-0-0) in two different applications: 50% at the end of the tillering stage of wheat (BBCH 29), 50% in full stem elongation stage of wheat (BBCH 33) (Stauss et al., 1994);

- N80 ORG: durum wheat pure crop fertilized with 80 N units ha$^{-1}$ applied as dry blood (14-0-0) (Orgazot®, AGM S.R.L.) at sowing;
- N40+40 ORG: durum wheat pure crop fertilized with 80 N units ha$^{-1}$ applied as dry blood (14-0-0) (Orgazot®, AGM S.R.L.) in two different applications: 50% at sowing, 50% in full stem elongation stage of wheat (BBCH 33) (Stauss et al., 1994);
- PB: pigeon bean pure crop unfertilized.

Durum wheat-pigeon bean temporary intercropping was chosen among all the other typologies of intercropping studied in the field experiment because of its wide adoption by organic growers in the area. Intercropping was compared to 4 other fertilization strategies, including also mineral fertilization, in order to test whether the availability of N under intercropping with legume might be or not comparable to that achieved by the other potential alternatives for growers.

The seeding rate and inter-row distances used for this experiment were exactly the same as those performed in the field experiment (see paragraph 2.1.1). Seeding was performed manually according to the scheme reported in Figure 11.

*Figure 11 - Spatial disposition of seed rows of pigeon bean and wheat in pure crops and intercropping in the lysimeter trial*



Details on crop management are reported in Table 6.

*Table 6 - Crop management of pigeon bean and durum wheat in the lysimeter trial in 2009/10*

| **Crop Management Operation** | **Date** | **Notes** |
|---|---|---|
| Main tillage | 30-10-2009 | Manual Digging 20 cm depth |
| Seedbed preparation | 03-11-2009 | Manual Hoeing + Raking |
| Organic fertilization | 03-11-2009 | N40+40 ORG, N80 ORG |
| Sowing | 03-11-2009 | All the treatments |
| Hand weeding | 10-12-2009 | All the treatments |
| First application of ammonium nitrate | 29-01-2010 | N80 MIN |
| Termination of pigeon bean | 02-04-2010 | Manual cutting + hoeing |
| Second application of ammonium nitrate | 07-04-2010 | N80 MIN |
| Organic fertilization | 07-04-2010 | N40+40 ORG |
| Harvest | 30-06-2010 | All treatments |

Likewise the field experiment, crops did not receive any fertilizers nor crop protection products other than N fertilizers reported in the protocol. Lysimeters were kept weed-free by periodical hand weeding. In proximity to harvest, lysimeters were covered by fine-mesh exclosures in order to prevent seed predation by birds (Fig. 12).

*Figure 12 - Exclosures placed over lysimeters in 2009/10*

After harvest, aboveground plant residues were removed from each plot, then the soil was maintained weed free with hand weeding until the end of September 2010, when soil tillage (manual digging + hoeing) was performed in order to stimulate mineralization of residual N in roots and soil. In 2010/11, the soil was kept bare and only drainage water was collected.

### *2.2.2.Sampling protocol and analytical methods*

In the lysimeter trial, both crop biomass and drainage water were sampled over the two years of the experiment. As well as for field trial, samplings of crop biomass were performed not only at harvest maturity, but also at several intermediate growing stages, following the same schedule applied for the field trial. The protocol of plant biomass samplings is reported in Table 7.

Three to nine plants per species were randomly selected along the central rows of each lysimeter and cut at soil level (Table 7). Each plant sample was then kept separate from the other of the same plot and subdivided in the different portions (stems, leaves, pods or ears and grain), which were weighted after oven-dried at 60°C until constant weight. Chemical analyses (N and P accumulation in dry matter of each plant portion) were performed at the chemical laboratory of the Research Centre “Enrico Avanzi” of the University of Pisa, applying the same methodology above described for the field experiment. Due to the low amount of each material, N and P accumulation were determined on the bulk samples originating from pooling the three subsamples collected in each plot.

Plant height of wheat and pigeon bean was measured at canopy level selecting 6 plants per species (three among the tallest, three among the shortest) on the whole plot. Minimum mean plant height and maximum mean plant height were then averaged together in order to obtain one average value for each plot.

*Table 7 - Sampling protocol of the lysimeter experiment in 2009/10*

| BBCH Stage[1] | Date | Nr. of plants sampled | Plant dry matter[2] | | | | NP accumulation[2] | | | | Wheat grain quality | Plant height[2] |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | | Stems | Leaves | Ears/ Pods | Grain | Stems | Leaves | Ears/ Pods | Grain | | |
| 14 | 22-01-2010 | 3 of each species | x | x | | | x | x | | | | x |
| 30 | 30-03-2010 | 3 of each species | x | x | | | x | x | | | | x |
| 59 | 26-04-2010 | 3 of each species | x | x | x | | x | x | x | | | x |
| 69 | 18-05-2010 | 3 of each species | x | x | x | | x | x | x | | | x |
| 75 | 04-06-2010 | 3 of each species | x | x | x | | x | x | x | | | x |
| 89 | 30-06-2010 | 9 of each species | x | x | x | x | x | x | x | x | x | x |

[1] Stauss et al. (1994)
[2] Wheat and pigeon bean

I n

addition to plant measurements, in both 2009/10 and 2010/11 also the weight of water drainage from lysimeters and its content in nitrates were determined.

At the beginning of the experiment in 2009, all the empty water tanks were weighted and a mean tare of 835 g was computed and attributed to all the tanks, as their single weights differed very little from average. In the occasion of rainfall exceeding field capacity, all the drainage water collected in the tank of each lysimeter was collected in the day after. Tanks were cleaned by mud and dust, weighted with a digital field scale (the net weight of water was computed by subtracting the tare of 835 g from the gross weight), then shaken, in order to homogenize the distribution of solids in the solution, and emptied by pouring out water while collecting it in a 10 mL plastic tube at different intervals (namely, at the beginning. in the middle and at the end of the flow). Tubes were immediately closed with caps, numbered with the plot number, and stored into the fridge at 4°C until chemical analysis, performed within 48-72 hours. In the case that more than one tank had been necessary for collecting all the drainage water, additional samples of water (as many as the number of tanks) were collected with the same procedure above described.

The dates when water was sampled in the two years were:

- 2009: 08/10, 13/10, 15/10, 30/10, 06/11, 10/11, 18/11, 02/12, 10/12, 16/12, 22/12, 23/12, 28/12, 29/12, 31/12;
- 2010: 04/01, 05/01, 07/01, 09/01, 12/01, 13/01, 19/01, 08/02, 18/02, 22/02, 27/02, 05/03, 16/03, 29/04, 11/05, 17/05, 25/05, 30/06, 01/09, 26/10, 02/11, 09/11, 11/11, 15/11, 19/11, 24/11, 29/11, 03/12, 13/12, 22/12, 28/12;
- 2011: 14/01, 25/01, 18/02, 24/02, 01/03, 08/03, 15/03, 21/03, 30/03, 20/06, 09/08.

The concentration of nitrates ($NO_3^-$) leached from each lysimeter each time was determined by ion chromatography (Eaton et al., 1995) and expressed in µg $NO_3^-$ $g^{-1}$ $H_2O$.

### 2.2.3. Calculations

Plant dry matter production and NP accumulations were computed in the same way as for field experiment (see paragraph 2.1.3), but on a per plant base, rather than per unit area.

Nitrates content in the drainage water at a given date (g $NO_3^-$ $m^{-2}$) were computed by multiplying nitrate concentration by the weight of drainage water and referring the result to the unit area (by dividing it by 1.0925 $m^{-2}$). In order to simplify the interpretation of the results, the analysis of N content in the drainage water was performed by aggregating sampling dates for a given period with respect to the phenology of the wheat:

- Period 1 (P1): from the beginning of the experiment to the 4-leaves stage (BBCH 14) of wheat (8/10/2009 - 19/01/2010);
- Period 2 (P2): from 4-leaves to end of tillering stage (BBCH 29) of wheat (08/02/2010 - 13/03/2010);
- Period 3 (P3): from end of tillering to end of flowering (BBCH 69) of wheat (29/04/2010 - 17/05/2010);
- Period 4 (P4): from end of flowering to harvest maturity (BBCH 89) of wheat (25/05/2010 - 30/06/2010):
- Period 5 (P5): fallow (01/09/2010 - 09/08/2011).

For each period, total nitric nitrogen ($N\text{-}NO_3^-$) (mg) content in the water was calculated by multiplying the total weight of water drainage in all the given dates (g) by the mean $N\text{-}NO_3^-$ concentration in the water (µg $g^{-1}$) for the same period. N leached (mg $m^{-2}$) was estimated by dividing total nitric nitrogen content in the water by the area of the lysimeter (i.e. 1.0925 $m^2$).

Apparent budget of nitrogen was estimated for each treatment:

$$N_{Budget} = N_{input} - N_{output}$$

with the following entries:

- <u>N input</u>:

a.N from fertilizers ($N_{fert}$): N40+40 ORG, N80 ORG, N80 MIN;

b.N from biological $N_2$-fixation ($N_{fix}$): PB-DW TIC, PB;

c.N from rain ($N_{rain}$): all treatments;

d.N from soil organic matter mineralization ($N_{min}$): all treatments;

- <u>N output</u>

a.N leached ($N_{leach}$): all treatments;

b.N accumulation in aboveground dry matter ($N_{upt}$): all treatments.

$N_{fert}$ was 8 g N $m^{-2}$ for N40+40 ORG, N80 ORG, N80 MIN.

$N_{fix}$ was calculated for PB-DW TIC by multiplying $N_{upt}$ of pigeon bean by the percentage of N derived from the atmosphere (*Ndfa%*) determined in the 2009/10 field experiment for pigeon bean intercropped with wheat (79.80% at BBCH 30 of wheat, i.e. before termination of pigeon bean).

$N_{rain}$ was estimated on the base of the mean $N\text{-}NO_3^-$ concentration in the rain measured in the area (2 µg N $g^{-1}$ rain) (Masoni and Pampana, 2005).

$N_{leach}$ was computed by summing all $N_{leach}$ values computed for each sampling period.

$N_{upt}$ was determined by multiplying total N accumulated in 1 plant of wheat or pigeon bean by the crop density on a 1 $m^2$ base (400 plants $m^{-2}$ for wheat, 80 for pigeon bean).

N budget was determined in comparison with the control (N0), by reducing N inputs of the other treatments by $N_{rain}$, and N outputs by the N accumulation in the aboveground dry matter of wheat under N0 ($N_{upt(N0)}$), in order to estimate N coming from the mineralization of soil organic matter:

$$N_{NetBudget} = \left(N_{fert} + N_{fix} - N_{rain}\right) - \left(N_{upt} + N_{leach} - N_{upt(N0)}\right)$$

### 2.2.4. Data analysis

Analysis of variance (ANOVA) for a 1-way Completely Randomized design was performed using the CoStat Software (CoHort-Software, 2007). Differences between treatment means were compared using a Fisher's protected LSD test at $P < 0.05$ (Gomez and Gomez, 1984). Before analysis, the Bartlett test was performed to test the homogeneity of error variances, and appropriated data transformation was applied when necessary.

Data originated from plots number 1 (N0), 8 and 10 (N40+40 ORG) were excluded from the analysis, due to technical problems occurred to the lysimeters (periodical difficulties in water drainage).

For the purpose of this work, data on pigeon bean sole crop (PB) will be not presented, as the main focus was on N dynamics related to mineral nutrition of wheat.

# 3. Results and Discussion

## 3.1. Field Experiment

The results from the field experiment will be discussed in the following paragraphs in two different sections. In the first one, the main results on the performance (grain yield and quality at maturity, N use efficiency, dry matter production and N accumulation over the growing cycle) of wheat crop as affected by intercropping will be discussed. In the second section, an analysis on agroecological issues of intercropping (total dry matter and N accumulation of intercrops over the growing cycle, competition between component crops, level of $N_2$-fixation by legumes, weed abundance) will be performed.

### *Section 1 - Wheat performance under intercropping and as sole crop*

### *3.1.1. Wheat yield at harvest*

Wheat dry matter production at maturity (BBCH 89) in the two years of the field experiment is reported in Tables 8 and 9.

On average, wheat yielded appreciably lower than usual for the area (400 g d.m. of grain $m^{-2}$). This was possibly due, first, to adverse weather conditions (see chapter 2.1), above all to the huge amount of rain fallen down in fall-winter, which negatively affected seedbed preparation and crop establishment. And indeed, wheat seedling density assessed after emergence was, irrespective of the treatments, 291 and 302 plants $m^{-2}$, respectively, for 2009/10 and 2010/11, values pretty much inferior to the adopted seeding density (i.e. 400 and 600 plants $m^{-2}$, respectively).

Second, the high level of precipitations in winter in both years might have reduced a lot the amount of available N in the soil. According to the literature (Alzueta et al., 2012), this might have consequently depressed the production of secondary tillers, and this was specially true in 2009/10, when sowing occurred before winter, as confirmed also by the low ear density at harvest (304 ears $m^{-2}$, on average). In the second year, an analogue depression of tillering (334 ears $m^{-2}$ at harvest, on average) was indirectly caused by the late sowing, which excluded the crop from exposure to low temperatures, such as when overwintering, and thus did not stimulate the production of new tillers.

Finally, the wide row spacing adopted for the experiment might have generated an excessive intra-species competition among adult wheat plants displaced on the same row, due to the asymmetric plant displacement (wide row distance with a very high plant density within the row). This mechanism was also partially demonstrated by Hiltbrunner et al. (2005) and Tosti and Guiducci (2010).

*Table 8 - Effect of treatments on number of ears, dry matter of grain, chaff, straw, residues and total biomass, and harvest index of wheat grown in 2009/10 in the field experiment. Data expressed as means ± SD (n = 3). Within each column, data followed by different letters are significantly different.*

| **Treatment** | **Number of ears ($m^{-2}$)** | **Dry Weight (g $m^{-2}$)** | | | | | **Harvest Index (%)** |
|---|---|---|---|---|---|---|---|
| | | **Grain** | **Chaff** | **Straw** | **Residues** | **Total above-ground biomass** | |
| N0 | 261 ± 39 b | 114.00 ± 43.04 de | 45.51 ± 16.56 c | 201.97 ± 77.67 d | 247.48 ± 93.71 d | 361.48 ± 135.90 f | 31.58 c |
| N40 | 315 ± 53 ab | 199.15 ± 78.00 bc | 77.42 ± 28.24 bc | 349.11 ± 128.06 bc | 426.53 ± 156.20 bc | 625.68 ± 234.04 bc | 31.79 c |
| N80 | 325 ± 52 ab | 252.21 ± 25.54 ab | 113.01 ± 21.17 a | 397.58 ± 51.34 ab | 510.59 ± 72.47 ab | 762.80 ± 97.57 ab | 33.03 bc |
| N120 | 368 ± 43 a | 281.75 ± 45.33 a | 95.17 ± 8.16 ab | 444.93 ± 68.08 a | 540.10 ± 75.28 a | 821.85 ± 120.61 a | 34.31 bc |
| N160 | 360 ± 48 a | 313.50 ± 50.62 a | 117.86 ± 17.32 a | 464.11 ± 25.19 a | 581.97 ± 41.71 a | 895.47 ± 92.32 a | 35.04 b |
| PB-DW TIC | 347 ± 80 a | 205.75 ± 24.84 bc | 74.28 ± 7.65 bc | 258.92 ± 12.46 cd | 333.20 ± 19.95 cd | 538.95 ± 44.17 cde | 38.22 ab |
| FP-DW TIC | 365 ± 43 a | 169.50 ± 17.00 cd | 70.32 ± 39.22 bc | 242.62 ± 26.67 d | 312.94 ± 65.85 d | 482.44 ± 82.41 cdef | 35.20 b |
| HV-DW TIC | 370 ± 13 a | 213.77 ± 3.61 bc | 66.25 ± 23.21 bc | 272.13 ± 14.03 cd | 338.38 ± 34.62 cd | 552.15 ± 37.95 cd | 38.77 a |
| PB-DW PIC | 102 ± 38 c | 4.21 ± 1.28 f | 1.99 ± 0.56 d | 84.56 ± 7.47 e | 86.55 ± 8.03 e | 90.76 ± 9.29 g | 4.44 e |
| FP-DW PIC | 261 ± 23 b | 125.40 ± 22.66 de | 51.05 ± 8.79 c | 224.75 ± 33.02 d | 275.80 ± 41.57 d | 401.20 ± 82.41 def | 31.17 c |
| HV-DW PIC | 266 ± 32 b | 100.84 ± 38.79 e | 46.21 ± 12.12 c | 231.22 ± 62.08 d | 277.43 ± 70.71 d | 378.27 ± 109.25 ef | 26.72 d |
| Significance[1] | ** | ** | ** | ** | ** | ** | ** |
| *LSD* | *75* | *61.40* | *32.60* | *91.51* | *111.43* | *173.62* | *3.22* |
| *CV (%)[2]* | *14.5* | *20.0* | *27.7* | *18.6* | *18.5* | *19.0* | *6.1* |

*[1] **, *, ns are, respectively, significant for p≤.01, significant for p≤.05 and not significant (Fisher's Protected LSD test)*

*[2] Coefficient of variation*

As expected, in both years of the experiment, wheat reached the highest values of grain yield under sole crop with application of medium-high rates of N fertilizer (higher than 40 kg N $ha^{-1}$). In the first season, there were no significant differences among

N80, N120 and N160 (Table 8). In the second year, N160 was superior to all the other treatments (Table 9).

*Table 9 - Effect of treatments on number of ears, dry matter of grain, chaff, straw, residues and total biomass, and harvest index of wheat grown in 2010/11 in the field experiment. Data expressed as means ± SD (n = 3). Within each column, data followed by different letters are significantly different.*

| Treatment | Number of ears ($m^{-2}$) | Dry Weight ($g\ m^{-2}$) | | | | | Harvest Index (%) |
|---|---|---|---|---|---|---|---|
| | | Grain | Chaff | Straw | Residues | Total above-ground biomass | |
| N0 | 335 ± 37 b | 177.09 ± 14.69 def | 71.49 ± 10.73 de | 128.54 ± 24.48 cd | 200.03 ± 34.63 cd | 377.12 ± 49.30 def | 46.95 |
| N40 | 317 ± 45 bc | 256.19 ± 35.84 bc | 97.09 ± 16.55 bc | 180.49 ± 17.76 b | 277.58 ± 31.62 b | 533.77 ± 67.39 bc | 47.94 |
| N80 | 332 ± 11 b | 265.11 ± 2.21 bc | 103.99 ± 6.43 ab | 189.37 ± 18.14 ab | 293.36 ± 23.09 ab | 558.47 ± 25.28 b | 47.49 |
| N120 | 317 ± 24 bc | 283.50 ± 17.67 b | 100.65 ± 11.15 abc | 186.77 ± 15.38 ab | 287.42 ± 25.86 ab | 570.92 ± 43.50 b | 49.65 |
| N160 | 340 ± 20 b | 333.17 ± 15.39 a | 116.92 ± 9.53 a | 215.18 ± 14.74 a | 332.10 ± 24.27 a | 665.27 ± 39.60 a | 50.08 |
| PB-DW TIC | 424 ± 48 a | 187.08 ± 46.40 def | 73.36 ± 14.69 de | 117.66 ± 19.62 cd | 191.02 ± 34.00 cd | 378.10 ± 80.22 def | 49.47 |
| FP-DW TIC | 331 ± 48 b | 204.94 ± 54.88 de | 73.96 ± 9.74 de | 139.13 ± 24.32 cd | 213.09 ± 33.82 cd | 418.03 ± 88.69 de | 49.04 |
| HV-DW TIC | 324 ± 26 b | 223.95 ± 30.25 cd | 84.49 ± 7.47 cd | 141.88 ± 10.75 c | 226.37 ± 14.57 c | 450.32 ± 43.06 cd | 49.78 |
| PB-DW PIC | 257 ± 37 c | 105.29 ± 8.23 g | 43.29 ± 2.30 f | 86.46 ± 5.93 e | 129.75 ± 8.20 e | 235.04 ± 15.55 g | 44.68 |
| FP-DW PIC | 338 ± 23 b | 164.15 ± 27.21 ef | 62.55 ± 5.36 e | 121.45 ± 10.47 cd | 184.00 ± 15.65 cd | 348.15 ± 42.85 ef | 46.99 |
| HV-DW PIC | 358 ± 68 b | 141.93 ± 22.03 fg | 58.78 ± 9.36 ef | 110.00 ± 24.46 de | 168.78 ± 32.27 de | 310.71 ± 51.75 fg | 45.66 |
| Significance[1] | * | ** | ** | ** | ** | ** | *ns* |
| *LSD* | *63* | *48.39* | *17.44* | *31.06* | *46.00* | *90.49* | *3.38* |
| *CV (%)*[2] | *11.2* | *13.3* | *12.7* | *12.4* | *11.9* | *12.1* | *4.1* |

[1] **, *, ns are, respectively, significant for p≤.01, significant for p≤.05 and not significant (Fisher's Protected LSD test)

[2] Coefficient of variation

In 2009/10, wheat intercropped temporarily with hairy vetch (213.77 g $m^{-2}$) and pigeon bean (205.75 g $m^{-2}$) yielded significantly higher than the control (114.00 g $m^{-2}$), and even equal to wheat pure crop fertilized with 40 and 80 kg of N $ha^{-1}$ (199.15 and 252.21

g $m^{-2}$, respectively). FP-DW TIC showed a lower grain yield (169.50 g $m^{-2}$), although comparable to that produced by N40, as well as to wheat-field pea permanent intercropping (125.40 g $m^{-2}$). All the three permanent intercropping treatments performed equal or even lower (i.e. PB-DW PIC) than the control N0. In particular, permanent intercropping with pigeon bean determined a significant decrease in harvested ears of wheat (only 102 ears $m^{-2}$), revealing the occurrence of overgrowth and shading. Actually, only few wheat plants survived until harvest, and only some of them produced ears and grain. Only in the case of field pea, permanent intercropping did not significantly depress wheat production compared to temporary intercropping.

A similar trend was observed also for the other plant components, namely chaff and straw (Table 8). For this latter, the gap between wheat pure stand and intercropping was bigger than for grain, as wheat pure crop fertilized with ammonium nitrate produced much more straw than wheat grown under temporary and permanent intercropping. As results, total aboveground dry matter production of wheat under intercropping was statistically comparable only to N0 and N40, for temporary intercropping, and only to N0 for permanent intercropping treatments (Table 8).

The results of the second year experiment were in line with those of 2009/10, although with appreciable differences in terms of productivity (Table 9). On average, grain yield was actually 18% higher, whereas total aboveground dry matter production 18% lower than in the first year. This difference was mainly because of the shorter duration of vegetative growth stages caused by the late sowing in 2010/11, conditions which forced the crop to close the life cycle very quickly, by investing all the energy in the production of ears and, then, grain, rather than of straw. This evidence was also supported by the higher mean value of the harvest index in 2010/11 than in 2009/10 (48% vs 31%, respectively).

In terms of grain yield, all the intercropping treatments did not differ statistically from the control, or performed even worse, as in the case of PB-DW PIC, which was confirmed as the worst treatment together with HV-DW PIC. Anyway, in terms of absolute values, the grain yield of wheat was increased under temporary intercropping (187.08, 204.94, 223.95 g $m^{-2}$, respectively, for PB-, FP- and HV-DW TIC) compared to the control (177.09 g $m^{-2}$). Furthermore, in the case of HV-DW TIC the grain yield of wheat was also statistically equal to that of N40 and N80. Grain yield of wheat under FP-DW PIC was again not statistically different from FP-DW TIC.

Data on dry weight of straw confirmed the trend observed in the previous year, with significant differences between fertilized pure crops and all the other treatments.

Total aboveground dry biomass was higher in fertilized pure crops (582 g $m^{-2}$, on average) than temporarily intercropped wheat (415 g $m^{-2}$, on average) and permanently intercropped wheat (298 g $m^{-2}$, on average).

Globally, the results from the two years of the experiment confirmed the hypothesis that intercropping with pulses can increase the dry matter production of wheat in absence of N fertilization, but with differences depending on the strategy and the component legume crops. These results are consistent with those of other previous studies on legumes intercropped with wheat (Li Destri Nicosia et al., 2005; Fan et al., 2006; Szumigalski and Van Acker, 2006; Carpi et al., 2009; Di Miceli et al., 2009; Tosti and Guiducci, 2010; Amossé et al., 2013b) or other cereals (Kurdali et al., 1996; Hauggaard-Nielsen et al., 2001a; Chu et al., 2004), showing as well higher yields of these crops under intercropping than as sole crops. Nevertheless, in literature there is plenty of papers reporting lower yields of cereals under intercropping than as sole crops, whereas grain yield and total dry matter production of intercrops as a whole (i.e. cereals + legumes) over-yielded the single pure crops (Jensen, 1996; Haymes and Lee, 1999; Ghanbari-Bonjar et al., 2002; Trydeman Knudsen et al., 2004; Poggio, 2005; Hauggaard-Nielsen et al., 2006; Gooding et al., 2007; Gunes et al., 2007; Lauk et al., 2007; Lithourgidis et al., 2007; Lauk and Lauk, 2008; Pridham and Entz, 2008; Bedoussac and Justes, 2009; Mariotti et al., 2009; Naudin et al., 2010; Lithourgidis et al., 2011; Mariotti et al., 2012; Pelzer et al., 2012). In our case, the performance of intercrops as a whole will be discussed in the *Section 2* of this chapter.

Anyway, it is noteworthy that a comparison of our results to the cited references cannot be exhaustive as all these papers only dealt with permanent intercropping, i.e. of a kind of intercropping where the competition between the component species is at the highest level. Consistently, in our research wheat under permanent intercropping performed better than the control N0 only in the case of FP-DW PIC in the first year, albeit without statistical significance.

Furthermore, as also pointed out by Bedoussac and Justes (2010), many authors worked on wheat grown in spring in Central and Northern Europe. In these conditions, permanent intercropping could be more effective than temporary in terms of facilitation of wheat, as the crops grow together only for a short time and resources other than nutrients (e.g., water and radiation) may be not limited.

As regards the good performances of wheat under temporary intercropping, our results fully confirmed those obtained by Di Miceli et al. (2009) and by Tosti and Guiducci (2010), with durum wheat intercropped with pigeon bean. Anyway, in our conditions hairy vetch was the legume which positively affected the most the yield and the biomass production of wheat, as also shown by Li Destri Nicosia et al. (2005) and by our group in a former preliminary experiment (Carpi et al., 2009). This was likely due to a lower competition and a higher N supply of the vetch in comparison to, respectively, bean and pea, as also confirmed by the opposite results shown by wheat under permanent intercropping (i.e. with pea showing the highest facilitation and bean the highest competition). For pigeon bean, also Benincasa et al. (2012) demonstrated in similar conditions that a strong competition on the companion wheat crop might happen even from very early growth stages, mainly due to shading. Anyway, all these issues will be deeply examined in the *Section 2* of this chapter.

### *3.1.2. Grain quality of wheat at harvest*

Values of the main parameters of the quality of wheat kernels under the different treatments in the two years of the experiment are reported in Tables 10 and 11. All parameters showed higher values in the second year, revealing a better grain quality in addition to a higher grain yield compared to the first year. This might have been due to the shorter duration of growth season in 2010/11, caused by the late sowing, which has led the crop to allocate all the energy to sustain grain production. Moreover, the late sowing occurred in the second year might have avoided losses of N coming from legume $N_2$-fixation and mineral fertilizers during winter due to rainfalls.

Grain protein content was significantly affected by treatments in the years of the experiment. In year 1, averaged over all treatments, the mean value of protein content was 15% lower than in year 2 (112.85 vs 132.64 mg $g^{-1}$, respectively), and very low if compared to the standards imposed by the food industry (at least 12% of protein in the grain should be granted by growers to have their product accepted for pasta-making).

In year 1, the highest values were shown by all the intercropping treatments, together with N160 (Table 10). In particular, permanent intercropping with field pea significantly increased protein content of wheat compared to the control and N120. Temporary intercropping showed a mean value of protein content of 116.89 mg $g^{-1}$, which was 8% lower than FP-DW PIC, but also 10% and 8% higher than the control and all the fertilized sole crop treatments, respectively.

In year 2, all the temporary intercropping treatments and PB-DW PIC resulted in a significantly higher protein content than all the remaining treatments (Table 11). The mean value of protein for temporary intercropping, averaged over all the three treatments, was 143.33 mg $g^{-1}$, a really good value being 14% higher than the control, 6% and 13% higher than all permanently intercropped wheat and all the wheat sole crop fertilized with ammonium nitrate, respectively.

Albeit some authors did not report a significant positive effect of intercropping on grain protein (Pelzer et al., 2012; Amossé et al., 2013b), these results are in agreement to what found by many other previous studies (Li Destri Nicosia et al., 2005; Gooding et al., 2007; Lauk et al., 2007; Lauk and Lauk, 2008; Bedoussac and Justes, 2009; Carpi et al., 2009; Tosti and Guiducci, 2010; Zhang et al., 2011; Mariotti et al., 2012). In France, Bedoussac and Justes (2009) found a significant increase in grain protein content of durum wheat intercropped with winter pea, but only at low level of N fertilization. In that study, the authors reported that a better nutritional content of wheat grain under intercropping than sole crop was the result of a lower number of ears per unit area, at the same level of N accumulation in the grain. Thus, they concluded that, on one hand, interspecific competition for light and space between pea and wheat was responsible for decrease in the number of wheat ears. On the other hand, this competition increased the competitive ability of wheat to take up soil mineral N, and hence producing at the end a higher concentration of N in the grain compared to the sole crop. The same evidence was also reported by Mariotti et al. (2012), for a durum wheat-pigeon bean permanent intercropping for forage.

In a network of field experiments carried out over Europe, Gooding et al. (2007) observed a higher level of protein in wheat intercropped with faba bean and field pea, but with a parallel strong depletion of grain yield (25-30% of yield decrease for every 10 mg $g^{-1}$ of increase in crude protein). Our results are not consistent with these latter, as we observed a high protein content not only under permanent intercropping (i.e. with low grain yield), but also under temporary intercropping (i.e. with acceptable yields). Temporary intercropping with pulses are clearly reported to significantly increase protein content of wheat by other previous studies conducted in Italy. In a former field experiment carried out in our conditions, Carpi et al. (2009) observed 10% increase in grain protein in durum wheat grown under temporary intercropping with hairy vetch. In that experiment, the spatial arrangement of the crops was different from the present study (wheat sown in paired rows, alternated with single rows of vetch), but similar to that applied by Li Destri Nicosia et al. (2005), in a field experiment carried out in Foggia, Southern Italy, under dry conditions. Also this paper reported a 10% increase in grain protein content of wheat caused by temporary intercropping with hairy vetch. Even higher increments were observed by Tosti and Guiducci (2010), who reported a mean increase of 14% in protein content of durum wheat in temporary intercropping with pigeon bean. In this case, the spatial arrangement of the crops was the same as the ours.

Protein content is well known to be related to the status of nitrogen nutrition of wheat, and specifically to the level of N concentration in the kernels, as results of access to soil mineral N and translocation of photosynthates from leaves and stems to grain. This issue will be deeply analyzed in the next paragraph. What is relevant here is that intercropping significantly determined a better nutritional status of wheat kernels, compared to wheat sole crop.

*Table 10 - Effect of treatments on protein content (mg g$^{-1}$ grain dry matter), sodium-dodecyl sulphate sedimentation test (SDS), test weight, and thousand kernel weight (TKW) of wheat grain collected at maturity in 2009/10 in the field experiment. Data expressed as means ± SD (n = 3). Within each column, data followed by different letters are significantly different.*

| **Treatment** | **Protein content (mg g$^{-1}$)$^3$** | **SDS $^3$** | **Test weight (kg hl$^{-1}$) $^3$** | **TKW (g) $^3$** |
|---|---|---|---|---|
| N0 | 106.33 ± 4.60 bcd | 28.00 ± 2.65 | 76.47 ± 5.19 | 31.23 ± 2.04 |
| N40 | 101.33 ± 10.00 d | 28.33 ± 2.57 | 75.75 ± 3.59 | 33.04 ± 2.93 |
| N80 | 104.00 ± 9.00 cd | 29.67 ± 3.28 | 77.77 ± 2.15 | 34.26 ± 1.40 |
| N120 | 109.33 ± 0.60 bcd | 31.33 ± 1.80 | 79.10 ± 1.61 | 34.51 ± 2.12 |
| N160 | 118.00 ± 7.00 abc | 35.33 ± 3.01 | 78.80 ± 1.91 | 35.89 ± 2.16 |
| PB-DW TIC | 119.00 ± 11.40 ab | 32.33 ± 4.91 | 78.55 ± 2.97 | 35.53 ± 4.26 |
| FP-DW TIC | 113.67 ± 10.00 abcd | 30.00 ± 3.40 | 74.92 ± 6.36 | 33.37 ± 2.91 |
| HV-DW TIC | 118.00 ± 8.20 abc | 32.00 ± 2.47 | 77.90 ± 3.73 | 37.02 ± 1.56 |
| PB-DW PIC | n.a. | n.a. | n.a. | n.a. |
| FP-DW PIC | 126.00 ± 5.30 a | 32.33 ± 2.52 | 80.73 ± 0.35 | 37.11 ± 2.32 |
| HV-DW PIC | n.a. | n.a. | n.a. | n.a. |
| Significance[1] | * | ns | ns | ns |
| *LSD* | *14.18* | *4.97* | *5.01* | *3.85* |
| *CV (%)*[2] | *7.3* | *9.3* | *3.72* | *6.42* |

[1] ***, *, ns are, respectively, significant for p≤.01, significant for p≤.05 and not significant (LSD test)*

[2] *Coefficient of variation*

[3] *n.a. is not available for grain sample insufficient for analysis*

*Table 11 - Effect of treatments on protein content (mg $g^{-1}$ grain dry matter), gluten content (mg $g^{-1}$ grain dry matter) sodium-dodecyl sulphate sedimentation test (SDS), test weight, and thousand kernel weight (TKW) of wheat grain collected at maturity in 2010/11 in the field experiment. Data expressed as means ± SD (n = 3). Within each column, data followed by different letters are significantly different*

| **Treatment** | **Protein content (mg $g^{-1}$)** | **Gluten content (mg $g^{-1}$)** | **SDS** | **Test weight (kg $hl^{-1}$)** | **TKW (g)** |
|---|---|---|---|---|---|
| N0 | 124.00 ± 4.40 de | 81.33 ± 4.62 de | 31.00 ± 0.00 cd | 83.00 ± 0.26 b | 42.97 ± 1.47 bcd |
| N40 | 120.00 ± 0.00 e | 78.00 ± 1.00 e | 31.67 ± 0.58 bcd | 83.08 ± 0.43 b | 45.64 ± 0.14 a |
| N80 | 124.67 ± 6.70 cde | 82.67 ± 8.02 cde | 31.00 ± 1.00 cd | 83.15 ± 0.33 b | 45.66 ± 0.15 a |
| N120 | 128.00 ± 3.00 bcd | 86.33 ± 4.04 cd | 31.33 ± 1.15 cd | 83.45 ± 0.69 b | 46.73 ± 0.46 a |
| N160 | 129.00 ± 1.00 c | 87.67 ± 2.08 c | 32.33 ± 0.58 bc | 83.57 ± 0.73 b | 45.89 ± 0.22 a |
| PB-DW TIC | 145.00 ± 2.65 a | 104.00 ± 3.61 a | 35.33 ± 0.58 a | 84.42 ± 0.23 a | 43.29 ± 0.45 bcd |
| FP-DW TIC | 143.33 ± 5.51 a | 101.33 ± 5.03 a | 34.67 ± 1.53 a | 84.55 ± 0.26 a | 43.88 ± 1.25 bc |
| HV-DW TIC | 142.00 ± 2.00 a | 100.33 ± 1.15 a | 35.33 ± 1.15 a | 84.33 ± 0.35 a | 44.11 ± 0.44 b |
| PB-DW PIC | 143.67 ± 1.53 a | 102.67 ± 1.53 a | 33.00 ± 0.00 b | 84.35 ± 0.74 a | 42.75 ± 0.38 cd |
| FP-DW PIC | 123.67 ± 3.06 de | 81.67 ± 4.51 de | 30.67 ± 0.58 d | 83.22 ± 1.03 b | 42.29 ± 0.38 d |
| HV-DW PIC | 135.67 ± 3.06 b | 93.33 ± 2.08 b | 32.00 ± 1.00 bcd | 82.87 ± 0.24 b | 44.05 ± 0.24 b |
| Significance[1] | ** | ** | ** | ** | ** |
| *LSD* | *4.84* | *5.18* | *1.37* | *0.71* | *1.27* |
| *CV (%)*[2] | *2.1* | *3.3* | *2.5* | *0.5* | *1.7* |

*[1] **, *, ns are, respectively, significant for p≤.01, significant for p≤.05 and not significant (LSD test)*

*[2] Coefficient of variation*

Data on dry gluten content of grain at harvest in 2010/11 strongly correlated ($R^2$ = 0.99) with data on protein content in the same year (Table 11). The mean value of gluten content under temporary intercropping (101.89 mg $g^{-1}$) overyielded that of permanently intercropped wheat (+9%), the control (+20%) and also that of fertilized pure stands (+18%). Our results were in line with those of Li Destri Nicosia et al. (2005) and Zhang et al. (2011).

SDS test returned not very high values in both years (averaged over all treatments 31.04 and 32.58, respectively, in 2009/10 and 2010/11). The effect of treatments was significative only in year 2, when wheat under temporary intercropping was superior to all the remaining treatments (+13% than N0, +3% than PIC, +10% than fertilized wheat sole crop), with high values meaning a better quality of grain protein. These findings, confirmed by observations from others (Gooding et al., 2007; Zhang et al., 2011), suggest that temporary intercropping can actually improve grain protein quality, as well as increase their quantity.

Test weight (TW) in 2010/11 was increased by temporary intercropping, over all legume species, as well as by FP-DW PIC. Although significant, differences from the control and the other fertilized sole crops were very slight (+1-2%, on average), as also found by Li Destri Nicosia et al. (2005).

Thousand kernel weight (TKW) was significantly higher in fertilized sole wheat than other treatments only in 2010/11 (Table 11). There were very slight differences between wheat grown under temporary (43.76 g on average) or permanent (43.03 g on average) intercropping. Anyway, FP- and HV-DW TIC showed values significantly higher than FP-DW PIC. The weak effect of intercropping on TKW was also seen by Li Destri Nicosia et al. (2005) for temporary intercropping between durum wheat and hairy vetch, whereas Tosti and Guiducci (2010) observed a significant increase in TKW when durum what was temporarily intercropped with pigeon bean.

The joint analysis of TW and TKW suggests that under intercropping wheat produced smaller grain than under sole crop with fertilization, revealing that N supplied by legumes was something, but maybe not enough to fully sustain grain filling during ripening. Our hypothesis is that, under intercropping, most N fixed by legumes was made available for wheat during late stages (e.g. through direct N-transfer from legume to wheat, under permanent intercropping; or mediated by the mineralization of biomass of legumes incorporated into the soil with hoeing, under temporary intercropping). Therefore, wheat might have suffered from competition of legumes at early stages, with consequent depletion until heading of photosynthetic assimilation of C, and hence of biomass production compared to sole crop. The higher availability of N from legumes at late growing stages might have then determined a better N nutrition than under sole crop, but which was not enough good to achieve an optimal grain filling.

Data on N concentration and accumulation of wheat plant components at harvest can help to test this hypothesis.

### 3.1.3. N and P nutrition of wheat at harvest

For the aim of this dissertation, data on concentration and accumulation of phosphorus in the different components of wheat aboveground dry matter at harvest will be not exhibited. Still, for reader’s convenience data are reported in the Appendix 1 enclosed at the end of the manuscript.

In both years, N concentration in the different plant portions of wheat at harvest was significantly affected by treatments (Tables 12 and 13).

Compared to the control, intercropping increased N concentration whatever the plant components in both years. The highest values of N in wheat grain were observed under permanent intercropping with pigeon bean (22.33 mg N $g^{-1}$) and hairy vetch (24.13 mg N $g^{-1}$) in 2009/10, and under all the temporary intercropping treatments (24.30, 22.90 and 22.10 mg N $g^{-1}$, respectively, for PB-, FP- and HV-DW TIC), as well as permanent intercropping with pigeon bean (23.23 mg N $g^{-1}$), in 2010/11. Therefore, no clear distinction between the two strategies of intercropping was highlighted. Our results are in line with consistent literature (Jensen, 1996; Kurdali et al., 1996; Bulson et al., 1997; Hauggaard-Nielsen et al., 2001a; Trydeman Knudsen et al., 2004; Ghaley et al., 2005; Hauggaard-Nielsen et al., 2006; Szumigalski and Van Acker, 2006; Gooding et al., 2007; Gunes et al., 2007; Hauggaard-Nielsen et al., 2008; Di Miceli et al., 2009; Tosti et al., 2010).

The analysis of these data in comparison to those of dry matter production (Tables 8 and 9) supports the hypothesis that N concentration in the grain increased with yield depletion. In this sense, treatments producing low dry matter showed a high N concentration in their tissues. This mechanism is well-known for pure crops growing under adverse environmental conditions or suffering specific biotic or abiotic stresses. For intercropping this effect was also highlighted by other authors (Gooding et al., 2007; Lauk et al., 2007; Lauk and Lauk, 2008), who explained it with the higher competition for natural resources (e.g., light, space and water) suffered by the dominated crop under intercropping than as sole crop.

In our experiment, over all intercropping treatments the lowest values for N concentration in the grain were observed for wheat grown in permanent intercropping with field pea (Tables 12 and 13). Pea was actually the legume species in our study with the lowest competitive ability against wheat (see data on dry biomass production of legumes reported in *Section 2* of this chapter), and hence it did not significantly deplete the growth of the companion crop. Therefore, N concentration in wheat grown under FP-DW TIC and PIC was not increased compared to the control sole wheat.

N accumulation in all the components of the aboveground biomass of wheat was significantly affected by treatments in both years of experiments (Tables 12 and 13).

*Table 12 - Concentration and accumulation of N in the different plant portions (grain, chaff, straw, residues, total biomass) of wheat sampled at maturity in 2009/10 . Data expressed as means ± SD (n = 3). Within each column, data followed by different letters are significantly different*

| | N concentration (mg $g^{-1}$) | | | N accumulation (g $m^{-2}$) | | | | |
|---|---|---|---|---|---|---|---|---|
| **Treatment** | **Chaff** | **Straw** | **Grain** | **Chaff** | **Straw** | **Residues** | **Grain** | **Total** |
| N0 | 3.93 ± 0.53 c | 3.97 ± 0.61 d | 14.80 ± 1.46 f | 0.18 ± 0.07 de | 0.80 ± 0.42 e | 0.98 ± 0.49 e | 1.69 ± 0.77 f | 2.67 ± 1.26 ef |
| N40 | 4.07 ± 0.60 c | 4.17 ± 0.36 d | 14.83 1.87 f | 0.32 ± 0.15 bcd | 1.46 ± 0.62 cde | 1.78 ± 0.77 cde | 2.95 ± 1.47 cdef | 4.73 ± 2.22 cde |
| N80 | 4.20 ± 0.77 c | 5.60 ± 1.63 cd | 17.13 ± 1.60 def | 0.47 ± 0.09 b | 2.23 ± 0.70 bc | 2.70 ± 0.79 bc | 4.32 ± 0.74 bc | 7.02 ± 1.49 bc |
| N120 | 7.33 ± 2.12 b | 5.57 ± 1.56 cd | 18.93 ± 1.01 cde | 0.70 ± 0.24 a | 2.48 ± 0.87 ab | 3.18 ± 1.04 ab | 5.33 ± 0.71 ab | 8.51 ± 1.69 ab |
| N160 | 5.93 ± 0.36 bc | 7.10 ± 1.04 bc | 21.37 ± 1.23 bc | 0.70 ± 0.14 a | 3.30 ± 0.66 a | 4.00 ± 0.80 a | 6.70 ± 1.50 a | 10.70 ± 2.29 a |
| PB-DW TIC | 5.47 ± 0.23 bc | 5.13 ± 0.36 d | 19.50 ± 0.45 cd | 0.41 ± 0.06 bc | 1.33 ± 0.15 de | 1.74 ± 0.21 cde | 4.01 ± 0.58 bcd | 5.75 ± 0.79 cd |
| FP-DW TIC | 4.93 ± 1.81 bc | 4.40 ± 0.69 d | 16.67 ± 2.16 ef | 0.35 ± 0.07 bcd | 1.07 ± 0.29 de | 1.42 ± 0.35 de | 2.83 ± 0.59 def | 4.25 ± 0.95 de |
| HV-DW TIC | 6.87 ± 3.21 b | 5.40 ± 0.98 cd | 17.80 1.48 de | 0.46 ± 0.05 bc | 1.47 ± 0.29 cde | 1.93 ± 0.33 cde | 3.81 ± 0.36 cd | 5.74 ± 0.68 cd |
| PB-DW PIC | 10.10 ± 1.75 a | 11.43 ± 1.64 a | 22.33 ± 0.72 ab | 0.02 ± 0.00 e | 0.97 ± 0.16 de | 0.99 ± 0.17 e | 0.09 ± 0.03 g | 1.08 ± 0.19 f |
| FP-DW PIC | 5.27 ± 0.65 bc | 5.50 ± 0.90 cd | 18.10 ± 2.30 de | 0.27 ± 0.08 cd | 1.24 ± 0.31 de | 1.51 ± 0.38 de | 2.27 ± 0.68 f | 3.78 ± 1.05 de |
| HV-DW PIC | 6.93 ± 1.59 b | 7.63 ± 1.02 b | 24.13 ± 3.20 a | 0.32 ± 0.12 bcd | 1.76 ± 0.65 bcd | 2.08 ± 0.76 bcd | 2.43 ± 1.24 ef | 4.51 ± 1.96 cde |
| Significance[1] | ** | ** | ** | ** | ** | ** | ** | ** |
| *LSD* | *2.62* | *1.90* | *2.63* | *0.19* | *0.90* | *1.04* | *1.45* | *2.45* |
| *CV (%)[2]* | *26.1* | *18.7* | *8.3* | *29.7* | *32.0* | *30.4* | *25.4* | *26.8* |

*[1] **, *, ns are, respectively, significant for p≤.01, significant for p≤.05 and not significant (Fisher's Protected LSD test)*

*[2] Coefficient of variation*

*Table 13 - Concentration and accumulation of N in the different plant portions (grain, chaff, straw, residues, total biomass) of wheat sampled at maturity in 2010/11. Data expressed as means ± SD (n = 3). Within each column, data followed by different letters are significantly different*

| | **N concentration (mg g$^{-1}$)** | | | **N accumulation (g m$^{-2}$)** | | | | |
|---|---|---|---|---|---|---|---|---|
| **Treatment** | **Chaff** | **Straw** | **Grain** | **Chaff** | **Straw** | **Residues** | **Grain** | **Total** |
| N0 | 4.13 ± 0.19 de | 3.63 ± 0.09 cd | 18.70 ± 0.46 e | 0.30 ± 0.06 cd | 0.47 ± 0.09 c | 0.77 ± 0.14 cd | 3.31 ± 0.33 c | 4.08 ± 0.48 c |
| N40 | 4.03 ± 0.23 de | 3.37 ± 0.34 d | 18.97 ± 0.47 de | 0.39 ± 0.05 bc | 0.61 ± 0.12 bc | 1.00 ± 0.15 bc | 4.86 ± 0.78 b | 5.86 ± 0.93 b |
| N80 | 4.00 ± 0.56 e | 3.67 ± 0.28 cd | 19.67 ± 0.26 de | 0.42 ± 0.07 b | 0.69 ± 0.08 ab | 1.11 ± 0.12 b | 5.21 ± 0.11 b | 6.32 ± 0.21 b |
| N120 | 4.40 ± 0.48 cde | 3.93 ± 0.68 cd | 19.80 ± 0.35 de | 0.44 ± 0.03 b | 0.73 ± 0.10 ab | 1.17 ± 0.12 ab | 5.61 ± 0.36 ab | 6.78 ± 0.42 ab |
| N160 | 4.67 ± 0.43 bcde | 3.93 ± 0.29 cd | 20.07 ± 0.54 cde | 0.55 ± 0.07 a | 0.85 ± 0.11 a | 1.40 ± 0.17 a | 6.69 ± 0.46 a | 8.09 ± 0.62 a |
| PB-DW TIC | 5.03 ± 0.76 bc | 5.57 ± 0.48 a | 24.30 ± 3.21 a | 0.37 ± 0.11 bc | 0.66 ± 0.16 b | 1.03 ± 0.27 b | 4.55 ± 1.74 b | 5.58 ± 2.01 bc |
| FP-DW TIC | 5.17 ± 0.54 ab | 4.93 ± 0.60 b | 22.90 ± 1.39 ab | 0.38 ± 0.03 bc | 0.69 ± 0.19 ab | 1.07 ± 0.22 b | 4.69 ± 1.09 b | 5.76 ± 1.31 b |
| HV-DW TIC | 4.77 ± 0.39 bcd | 4.63 ± 0.17 b | 22.10 ± 2.15 abc | 0.40 ± 0.07 b | 0.66 ± 0.06 b | 1.06 ± 0.11 b | 4.95 ± 1.00 b | 6.01 ± 1.09 b |
| PB-DW PIC | 5.80 ± 0.27 a | 4.80 ± 0.40 b | 23.23 ± 0.42 ab | 0.25 ± 0.03 d | 0.42 ± 0.06 c | 0.67 ± 0.09 d | 2.45 ± 0.21 c | 3.12 ± 0.29 c |
| FP-DW PIC | 4.13 ± 0.41 de | 3.53 ± 0.31cd | 19.27 ± 0.48 de | 0.26 ± 0.01 d | 0.43 ± 0.05 c | 0.69 ± 0.06 d | 3.16 ± 0.44 c | 3.85 ± 0.48 c |
| HV-DW PIC | 4.97 ± 0.68 bc | 4.03 ± 0.09 c | 21.13 ± 0.95 bcd | 0.29 ± 0.07 cd | 0.44 ± 0.06 c | 0.73 ± 0.17 d | 3.00 ± 0.38 c | 3.73 ± 0.53 c |
| Significance[1] | ** | ** | ** | ** | ** | ** | ** | ** |
| *LSD* | *0.74* | *0.57* | *2.24* | *0.10* | *0.18* | *0.26* | *1.29* | *1.51* |
| *CV (%)*[2] | *9.3* | *8.0* | *6.3* | *16.2* | *17.3* | *15.4* | *17.2* | *16.4* |

*[1] **, *, ns are, respectively, significant for p≤.01, significant for p≤.05 and not significant (Fisher's Protected LSD test)*

*[2] Coefficient of variation*

In year 1, wheat grown under all the temporary intercropping except for FP-DW TIC accumulated more than 2-fold higher N in the grain than under permanent intercropping and as unfertilized sole crop (Table 12). Wheat under PB- and HV-DW

TIC showed, respectively, values statistically similar to those of N80 and N120. Concerning plant residues, like it was for dry matter production, also for N accumulation in residues no significant differences were observed between the control and intercropping, with the exception of HV-DW PIC which showed a statistically higher value (2.08 g N $m^{-2}$). Total N accumulation in aboveground biomass followed a similar trend as described for grain.

In year 2, the distinction between the two strategies of intercropping was more evident than in year 1. Over all temporary intercropping treatments, wheat showed values statistically equal to that of N120, accumulating 16% and 64% more N in the grain than the control and permanent intercropping, respectively. Also for residues, wheat under temporary intercropping overyielded N0, whereas permanent intercropping did not. In terms of accumulation of N in total aboveground biomass, a similar trend was observed, with temporary intercropping treatments (except for PB-DW TIC) being statistically superior to the control and equal to N120, whereas permanent treatments did not differ from N0.

Our results are in agreement with many other studies on this topic (Chu et al., 2004; Bedoussac and Justes, 2009; Di Miceli et al., 2009; Mariotti et al., 2009; Tosti et al., 2010; Tosti and Guiducci, 2010; Mariotti et al., 2012), but also in contrast to some others (Jensen, 1996; Ghaley et al., 2005; Naudin et al., 2010), which reported no significant increases in N accumulated in wheat biomass under intercropping. In our experiment the main determinant of these findings was clearly the entity of dry biomass production rather than N concentration in plant tissues. For instance, N accumulated in wheat grain at harvest significantly correlated positively with grain dry matter production in both years (with values of $R^2$ coefficient of 0.94 and 0.90, respectively, in 2009/10 and 2010/11), whilst it did not with N concentration in the grain. Therefore, the explanation of our results on N accumulation should match that of dry biomass production.

The high levels of N accumulated in wheat grown under temporary intercropping were probably due to a significant supply of N from the mineralization of the legumes incorporated into the soil at BBCH 30 of wheat, leading to a temporary N-sparing during the late stage of growth of cereals (Chu et al., 2004). Furthermore, the absence of an interspecific competition with legumes after heading stage, due to the interruption of co-growth, might have been another factor of the advantage gained by wheat under TIC than under PIC, as also demonstrated in similar conditions by Tosti and Guiducci (2010). In fact, legume are reported to take more advantage from intercropping from flowering stage onwards, when they become more competitive compared to wheat , whereas at early stages the cereal is dominant (Bedoussac and Justes, 2010).

A dependable hypothesis could arise that the higher was the N accumulated in the legume biomass incorporated into the soil, the higher should be the N accumulated in the wheat biomass at final maturity. Nevertheless this was only partially confirmed by the analysis of N accumulated in total aboveground biomass of legumes at BBCH 30 of wheat in the two years (Tables 26 and 29). Therefore, also the effect of early interspecific competition for resources between wheat and legumes should also be taken into account. Looking at the dry matter produced by wheat at BBCH 30 under the three different treatments of temporary intercropping (Tables 16 and 20), a clear deleterious effect on wheat biomass occurred in both years when grown together with pigeon bean, which was actually the legume with the highest N accumulated in the biomass then. The reasons of this strong early competition from pigeon bean, observed also by Benincasa et al. (2012), will be further discussed in the *Section 2* of this chapter. Here, what is noticeable to argue is that the facilitation offered by the legume component of temporary intercropping is not only the result of its specific ability to supply N to the cereal, but it depends also on its capacity to not reduce wheat growth right from early stages, by competing, for instance, for space, solar radiation and water (Bedoussac and Justes, 2010).

The calculations of N recovery and Nitrogen Use Efficiency (NUE) reported in Tables 14 and 15 support these considerations. N recovery was significantly affected by treatments in both years, whilst NUE was not in either. In year 1, wheat intercropped temporarily with legumes recovered significantly more N than PB-DW PIC, the only treatment showing a negative value (i.e. a N accumulation minor than the control N0). In year 2, the difference between the two strategies of intercropping was amplified, and hence all the temporary intercropping treatments resulted superior to the permanent ones, which resulted all in negative values. In spite of this, FP-DW PIC showed a N recovery inferior only to HV-DW TIC. Compared to sole wheat fertilized with mineral nitrogen, temporary intercropping did not lead to significant increases in N recovery in both years. Anyway, absolute values were generally higher than those of N40.

Concerning NUE, mean values averaged over all treatments were higher in the first than in the second year, possibly due to the shorter growth cycle in 2010/2011 if compared to 2009/10 (Tables 14 and 15). Noticeably, there were no clear differences among the different temporary intercropping in both years. Anyway, over all years wheat under intercropping with pea and vetch showed a N use efficiency very much higher than with bean. Furthermore, under FP- and HV-DW TIC, durum wheat had a NUE higher than 100%, revealing a N accumulation even higher the N accumulated in the legumes incorporated into the soil. This finding supports our above mentioned hypothesis that the facilitation provided to the wheat by these two legume species (i.e, pea and vetch) could have been also due to other services than N supply through mineralization of dead material.

*Table 14- N recovery ($N_{rec}$) and N use efficiency (NUE) of wheat grown under sole- and inter-cropping in 2009/10. $N_f$ and $N_c$ are N accumulation in total aboveground dry matter of wheat fertilized and unfertilized, respectively. R is the N fertilization rate applied in wheat sole crop plots or N incorporated into the soil with legumes at stem elongation stage of wheat under temporary intercropping (see Table _ for further details). Within each column, data followed by different letters are significantly different*

| **Treatment** | **$N_f$ (g m$^{-2}$)** | **$N_c$ (g m$^{-2}$)** | **R (g m$^{-2}$)** | **$N_{rec}$ (g m$^{-2}$)** | **NUE (%)** |
|---|---|---|---|---|---|
| N40 | 4.73 | 2.67 | 4.00 | 2.06 bc | 51.50 |
| N80 | 7.02 | 2.67 | 8.00 | 4.35 abc | 54.38 |
| N120 | 8.51 | 2.67 | 12.00 | 5.84 ab | 48.67 |
| N160 | 10.70 | 2.67 | 16.00 | 8.03 a | 50.19 |
| PB-DW TIC | 5.75 | 2.67 | 3.75 | 3.08 abc | 82.13 |
| FP-DW TIC | 4.25 | 2.67 | 1.14 | 1.58 bc | 138.60 |
| HV-DW TIC | 5.74 | 2.67 | 3.00 | 3.07 abc | 102.33 |
| PB-DW PIC | 1.08 | 2.67 | - | -1.59 d | - |
| FP-DW PIC | 3.78 | 2.67 | - | 1.11 c | - |
| HV-DW PIC | 4.51 | 2.67 | - | 1.84 bc | - |
| Significance[1] | - | - | - | ** | ns |
| *LSD* | - | - | - | *(0.32)* | *(0.35)* |
| *CV (%)*[2] | - | - | - | *10.9* | *84.5* |

[1] ***, *, ns are, respectively, significant for p≤.01, significant for p≤.05 and not significant (Fisher's Protected LSD test)*

[2] *Coefficient of variation*

[3] *Under brackets, values transformed as described in material and methods*

*Table 15- N recovery ($N_{rec}$) and N use efficiency (NUE) of wheat grown under sole- and inter-cropping in 2010/11. $N_f$ and $N_c$ are N accumulation in total aboveground dry matter of wheat fertilized and unfertilized, respectively. R is the N fertilization rate applied in wheat sole crop plots or N incorporated into the soil with legumes at stem elongation stage of wheat under temporary intercropping (see Table _ for further details). In each column, data followed by different letters are significantly different*

| **Treatment** | **$N_f$ (g m$^{-2}$)** | **$N_c$ (g m$^{-2}$)** | **R (g m$^{-2}$)** | **$N_{rec}$ (g m$^{-2}$)** | **NUE (%)** |
|---|---|---|---|---|---|
| N40 | 5.86 | 4.08 | 4.00 | 1.78 ab | 44.50 |
| N80 | 6.32 | 4.08 | 8.00 | 2.24 a | 28.00 |
| N120 | 6.78 | 4.08 | 12.00 | 2.70 a | 22.50 |
| N160 | 8.09 | 4.08 | 16.00 | 4.01 a | 25.06 |
| PB-DW TIC | 5.58 | 4.08 | 5.45 | 1.50 ab | 27.52 |
| FP-DW TIC | 5.76 | 4.08 | 1.65 | 1.68 ab | 101.82 |
| HV-DW TIC | 6.01 | 4.08 | 1.61 | 1.93 a | 119.88 |
| PB-DW PIC | 3.12 | 4.08 | - | -0.96 d | - |
| FP-DW PIC | 3.85 | 4.08 | - | -0.23 bc | - |
| HV-DW PIC | 3.73 | 4.08 | - | -0.35 cd | - |
| Significance[1] | - | - | - | ** | ns |
| *LSD* | - | - | - | *(0.32)* | *(9.40)* |
| *CV (%)*[2] | - | - | - | *12.9* | *9.76* |

*[1] **, *, ns are, respectively, significant for p≤.01, significant for p≤.05 and not significant (Fisher's Protected LSD test)*

*[2] Coefficient of variation*

*[3] Under brackets, values transformed as described in material and methods*

For instance, an increase in the mineralization of nitrogen content in the soil organic matter was also demonstrated, and hence related to the effect of inter-row hoeing for the incorporation of the legume into the soil under temporary intercropping (Di Miceli et al., 2009). The authors worked on temporary intercropping of durum wheat with pigeon bean under Mediterranean dry conditions. They observed that only a low amount of the N supplied by the biomass of the legume to the soil passed to the cereal. Additional estimations made on the basis of an analysis of the $^{15}N$ enrichment of wheat allowed them to conclude that wheat under TIC took up an extra of N from the soil than under sole crop due to the stimulation of soil respiration by inter-row hoeing.

Another possible explanation may be that, unlike what happened under TIC with pigeon bean, in the case of TIC with pea and vetch, wheat did not suffer from any overgrowth of the legumes since the very early stage of crop development. This complementarity between the companion crops might have preserved the growth of wheat until stem elongation, but also it might have enhanced it by increasing the competitive ability of the cereal for the uptake of soil mineral N, as also pointed out by Bedoussac and Justes (2009) and many others. Moreover, the increased competitive ability of wheat for soil N should also be put in relation to a complementary increased ability of the companion legume for biological $N_2$-fixation from the atmosphere. This issue will be deeply discussed in the *Section 2* of this chapter.

N equivalents of grain yield (Fig. 13 and 14), total aboveground dry matter production (Fig. 15 and 16), N accumulation in grain (Fig. 17 and 18) and N accumulation in total aboveground dry matter (Fig. 19 and 20) of wheat grown under intercropping were estimated both in 2009/10 and in 2010/11.

A clear difference between the N equivalents for grain yield of durum wheat grown under the two strategies of intercropping was observed in both years. Permanent intercropping revealed negative yields or at least close to null N fertilization. Temporary intercropping treatments, and especially with hairy vetch, showed grain yield of wheat comparable to wheat sole crop grown with the same spatial arrangement (i.e. 36 cm wide row distance, 400 seeds $m^{-2}$) and fertilized with up to 53 kg N $ha^{-1}$ (2009/10) and 32 kg N $ha^{-1}$ (2010/11).

These levels of fertilization can be considered very low if compared to the standard technique adopted in the region for durum wheat under conventional management, with 100-150 N units $ha^{-1}$ usually applied to the crop. On the other hand, they partially fit the requirements of N fertilizers usually considered under organic farming, which are about 60 N units $ha^{-1}$, applied in 1 or 2 applications as organic fertilizers. Due to the high cost of the N fertilizer unit for organic fertilizers (about 7-8 €/N unit, on average), temporary intercropping with hairy vetch can be a suitable way for organic farmers to save at least part of the money required for the purchase of commercial organic fertilizers.

*Figure 13 - N equivalents (kg N ha$^{-1}$) for grain yield of wheat grown under intercropping in 2009/10.*

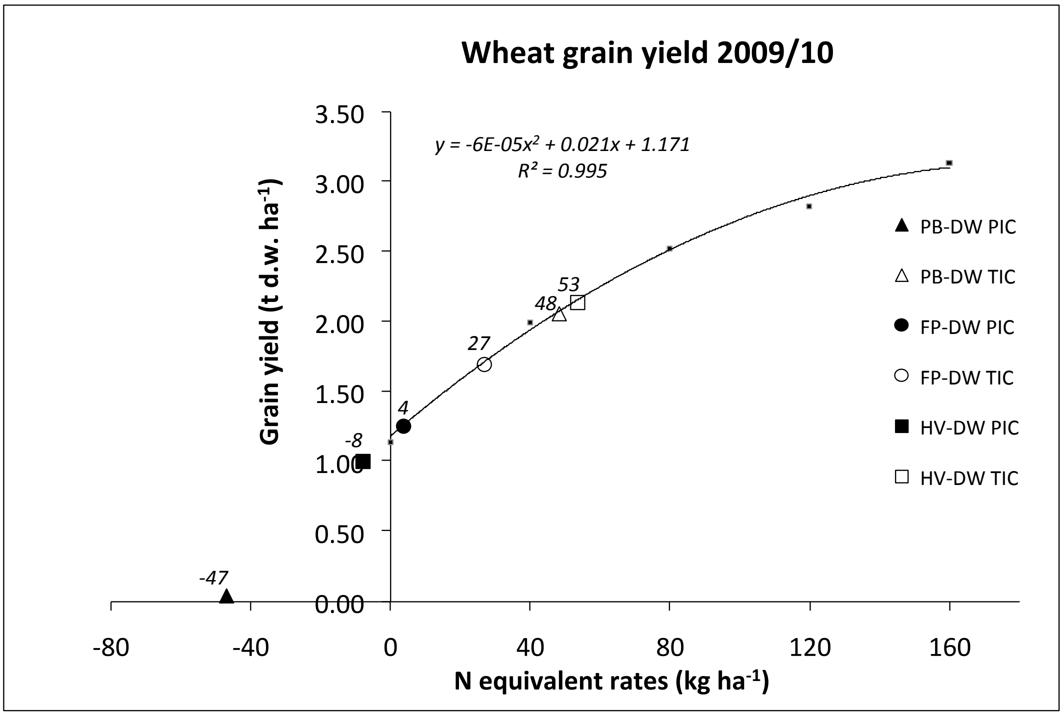
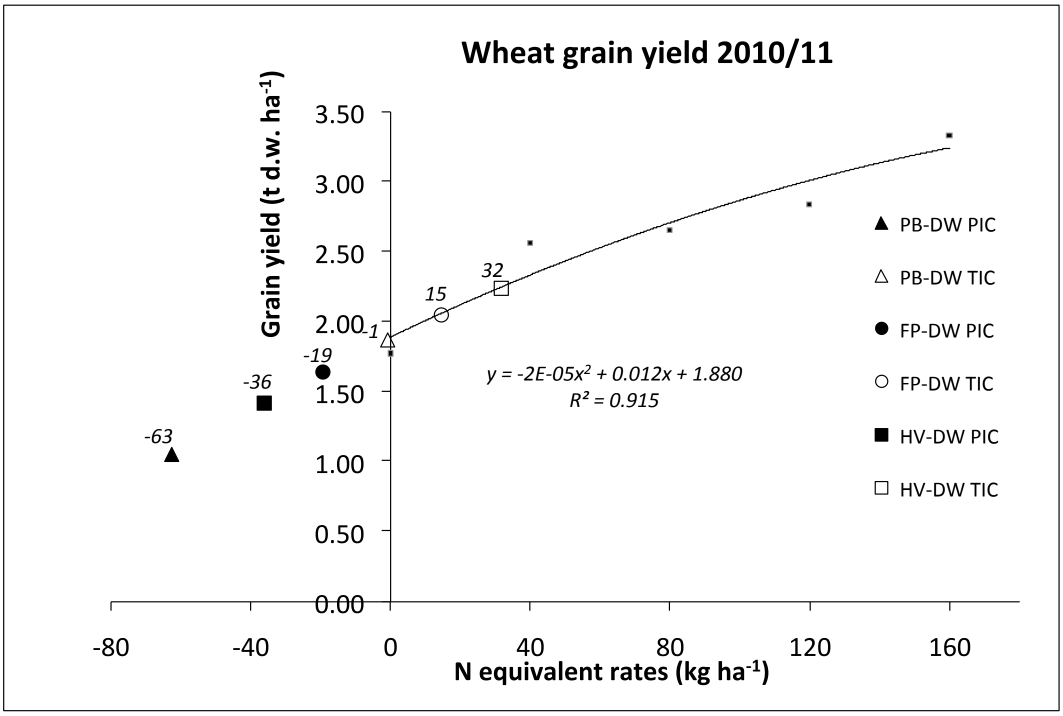


*Figure 14 - N equivalents (kg N ha$^{-1}$) for grain yield of wheat grown under intercropping in 2010/11.*



N equivalents estimated for total aboveground dry biomass (Figures 15 and 16) were lower in both years in comparison to those for grain yield. HV-DW TIC produced a total biomass equal to wheat sole crop fertilized with 30 and 21 units of N ha$^{-1}$, respectively, in year 1 and year 2. This evidence is in line with the observed differences in plant biomass partitioning between wheat under intercropping and as sole crop (Tables 13 and 14).

*Figure 15 - N equivalents (kg N ha$^{-1}$) for total aboveground dry biomass production of wheat grown under intercropping in 2009/10.*

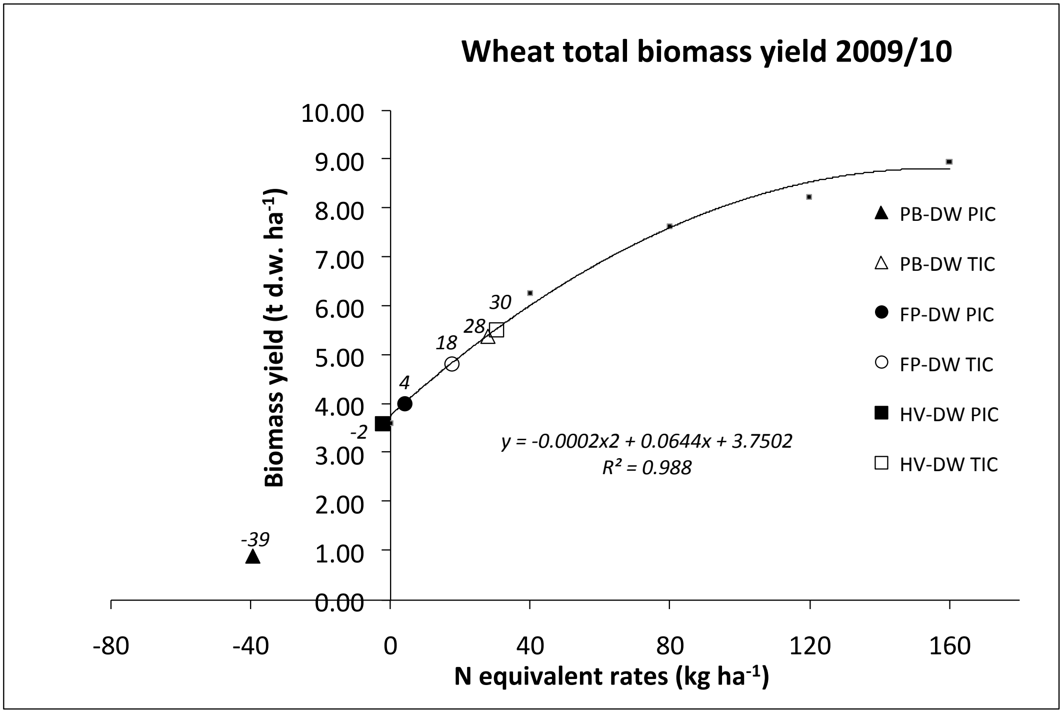
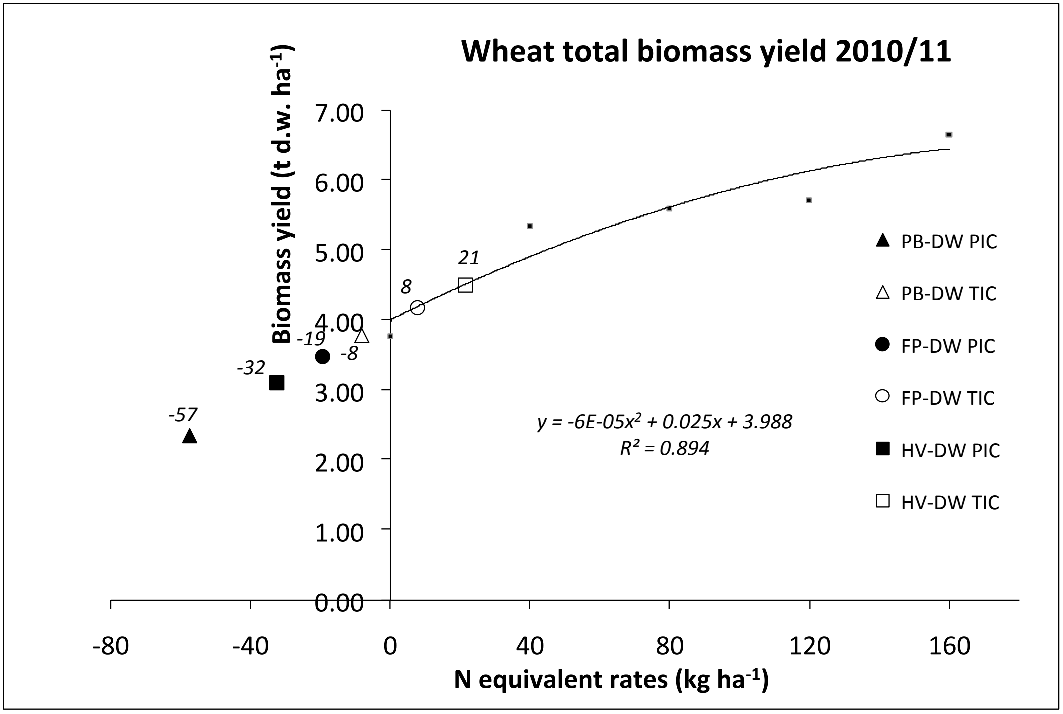


*Figure 16 - N equivalents (kg N ha$^{-1}$) for total aboveground dry biomass production of wheat grown under intercropping in 2010/11.*



From our results, it seems clear that under TIC wheat actually produces more output (i.e., the grain), than input (i.e. crop residues). Furthermore, as above mentioned, the mineralization of soil organic matter may be enhanced under TIC than PIC and sole crop, due to the effect of inter-row tillage, necessary to incorporate into the soil the legume at stem elongation stage of wheat (Di Miceli et al., 2009). Therefore, given these facts, it can be concluded that, additional provisions of organic C should be planned by farmers in order to avoid losses of soil organic matter in the long run. Nevertheless, also indirect savings of C, provided by TIC (such as the lower application of fertilizers and crop protection products, or the lower need for direct weed control), should be taken into account, leading to different conclusions.

The good results produced by temporary intercropping in terms of wheat dry matter production were even more positive if looking at N equivalents in terms of N accumulated in the grain (Fig. 17 and 18) and in total aboveground biomass (Fig. 19 and 20) at harvest.

Under TIC, wheat had a grain N yield comparable to that produced by fertilization with up to 73 (PB-DW TIC) and 65 kg N ha$^{-1}$ (HV-DW TIC), respectively, in year 1 and year 2. Maximum N equivalents of total N accumulation in aboveground biomass were 60 and 65 at harvest, respectively, for PB-DW TIC in 2009/10 and HV-DW TIC in 2010/11.

These results, fully in line with those on N accumulation in grain and total biomass of wheat previously described, reveal that the performance of intercropping in terms of N nutrition of wheat were even better than in terms of dry biomass production. This may suggest the occurrence of factors other than nitrogen, determining these strong limitations to the growth of the cereal. The peculiar spatial arrangement adopted for the crop in the experiments cannot be considered as the main reason behind that, as it was the same also for the sole crop treatments, and neither was phosphorus nutrition, as P accumulation in wheat biomass at harvest was in line with that of N (see Appendix 1).

Actually, the evidence of a lower dry matter production of straw than grain under TIC compared to wheat sole crop fertilized with ammonium nitrate (Tables 8 and 9) suggests that wheat might have suffered from competition by legumes also in the vegetative stages of its growth. Shading, but also removal of mineral N from the root space (possibly occurring in the early stages of the growth of legumes, when nodulation of roots was not completed yet) might have depressed the vegetative growth of wheat and then determined an advantage for legumes, conserved until the time of their termination, as also observed by Benincasa et al. (2012) and Amossé et al. (2013b).

*Figure 17 - N equivalents (kg N ha$^{-1}$) for N accumulated in grain of wheat grown under intercropping in 2009/10.*

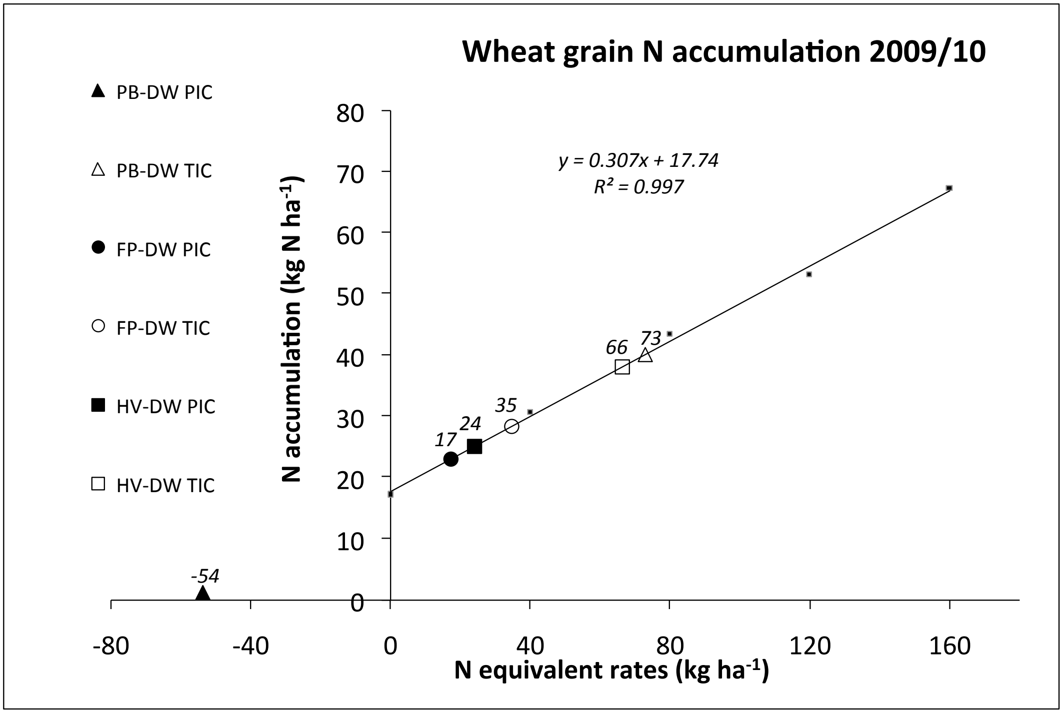


*Figure 18 - N equivalents (kg N ha$^{-1}$) for N accumulated in grain of wheat grown under intercropping in 2010/11.*

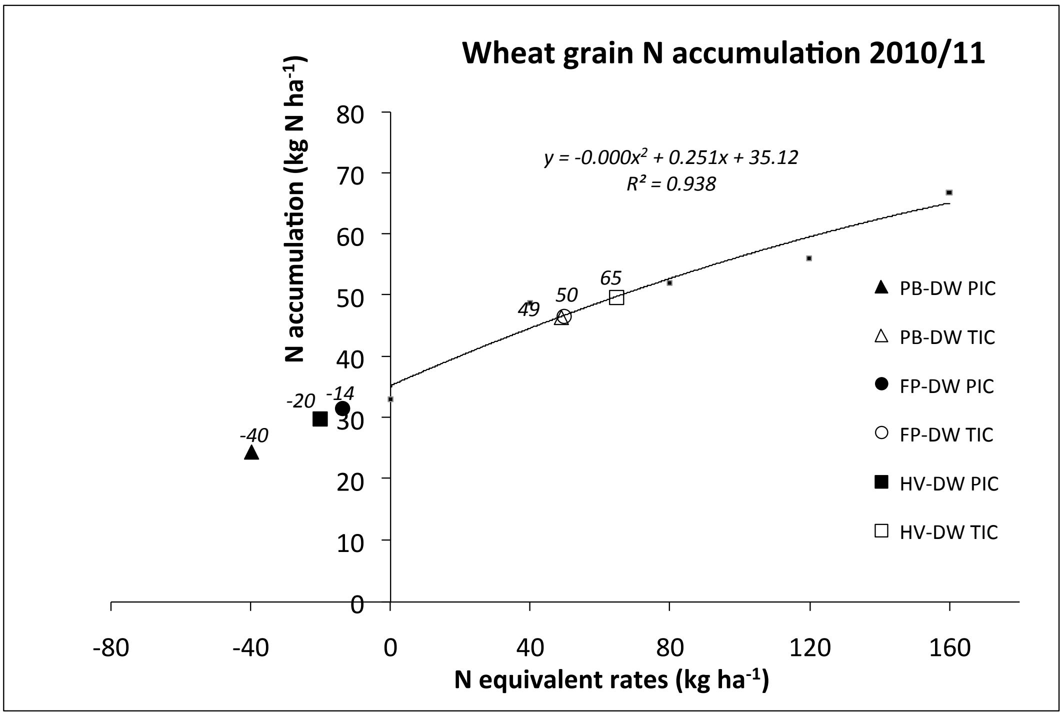


*Figure 19 - N equivalents (kg N ha$^{-1}$) for N accumulated in total aboveground dry matter of wheat grown under intercropping in 2009/10.*

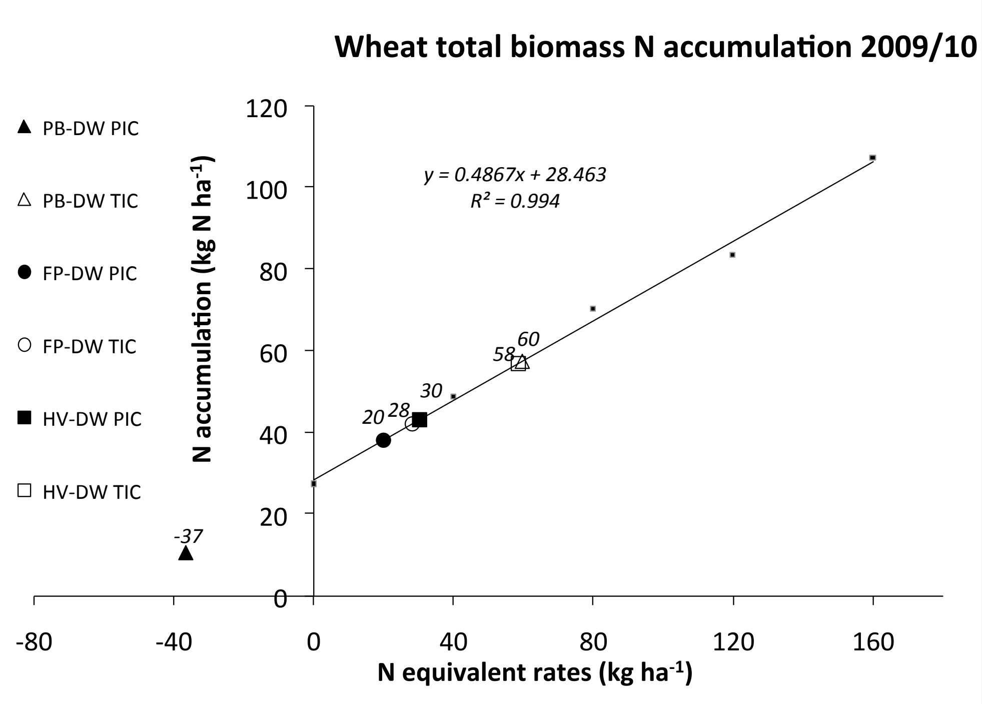
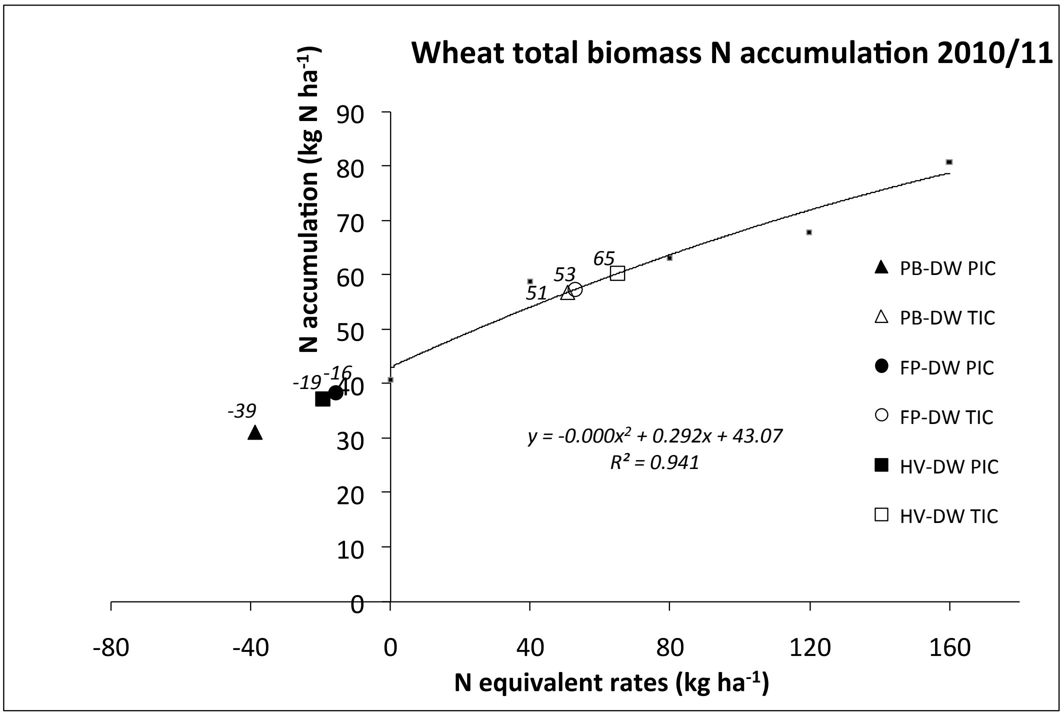


*Figure 20 - N equivalents (kg N ha$^{-1}$) for N accumulated in total aboveground dry matter of wheat grown under intercropping in 2010/11.*



To support or disprove these hypotheses, an analysis of the production of biomass and of N accumulation in aboveground dry matter over the growth period of wheat in the two years of experiment have been performed.

### *3.1.4. Wheat dry matter production, NP accumulations and plant height over time*

Data on dry matter production, N concentration and N accumulation in the different plant portions of wheat grown in 2009/10 at different growing stages are shown in Tables 16 to 19.

*Table 16 - Dry matter weight, N concentration and N accumulation of the different plant components of wheat sampled at stem elongation stage (**BBCH 30**) in 2009/10. Data expressed as means ± SD (n = 3). Within each column, data followed by different letters are significantly different*

| **BBCH 30** | | | | | | | | |
|---|---|---|---|---|---|---|---|---|
| **Treatment** | **Dry matter production (g m$^{-2}$)** | | | **N concentration (g kg$^{-1}$)** | | **N accumulation (g m$^{-2}$)** | | |
| | **Straw** | **Ears** | **Total Biomass** | **Straw** | **Ears** | **Straw** | **Ears** | **Total Biomass** |
| N0 | 94.17 ± 19.84 efg | - | 94.17 ± 19.84 efg | 10.86 ± 0.35 d | - | 1.02 ± 0.20 e | - | 1.02 ± 0.20 e |
| N40 | 176.02 ± 6.28 abcd | - | 176.02 ± 6.28 abcd | 13.05 ± 0.76 b | - | 2.30 ± 0.15 bc | - | 2.30 ± 0.15 bc |
| N80 | 192.91 ± 21.81 abc | - | 192.91 ± 21.81 abc | 12.06 ± 1.18 bcd | - | 2.33 ± 0.05 bc | - | 2.33 ± 0.05 bc |
| N120 | 200.54 ± 29.40 ab | - | 200.54 ± 29.40 ab | 12.79 ± 1.01 bc | - | 2.56 ± 0.16 b | - | 2.56 ± 0.16 b |
| N160 | 206.77 ± 55.92 a | - | 206.77 ± 55.92 a | 16.52 ± 2.43 a | - | 3.42 ± 0.47 a | - | 3.42 ± 0.47 a |
| PB-DW TIC | 87.00 ± 11.80 fg | - | 87.00 ± 11.80 fg | 12.23 ± 0.90 bcd | - | 1.06 ± 0.23 de | - | 1.06 ± 0.23 de |
| FP-DW TIC | 119.99 ± 36.24 defg | - | 119.99 ± 36.24 defg | 11.20 ± 1.11 cd | - | 1.34 ± 0.55 de | - | 1.34 ± 0.55 de |
| HV-DW TIC | 145.38 ± 79.93 bcde | - | 145.38 ± 79.93 bcde | 11.81 ± 0.76 bcd | - | 1.72 ± 0.88 cd | - | 1.72 ± 0.88 cd |
| PB-DW PIC | 77.66 ± 8.99 g | - | 77.66 ± 8.99 g | 12.74 ± 2.47 bc | - | 0.99 ± 0.08 e | - | 0.99 ± 0.08 e |
| FP-DW PIC | 124.02 ± 32.56 defg | - | 124.02 ± 32.56 defg | 10.66 ± 1.95 d | - | 1.32 ± 0.46 de | - | 1.32 ± 0.46 de |
| HV-DW PIC | 137.80 ± 2.87 cdef | - | 137.80 ± 2.87 cdef | 11.50 ± 1.38 bcd | - | 1.58 ± 0.22 de | - | 1.58 ± 0.22 de |
| Significance[1] | ** | - | ** | ** | - | ** | - | ** |
| *LSD* | *56.88* | - | *56.88* | *1.85* | - | *0.66* | - | *0.66* |
| *CV (%)*[2] | *23.5* | - | *23.5* | *8.8* | - | *22.0* | - | *22.0* |

*[1] **, *, ns are, respectively, significant for p≤.01, significant for p≤.05 and not significant (LSD test)*

*[2] Coefficient of variation*

At stem elongation (Table 16) dry matter production of wheat under intercropping, averaged over all treatments, was significantly lower than under sole crop with fertilization, whereas no differences were observed from the control. The two strategies of intercropping did not differed substantially at this stage, whereas some clear differences were shown among the legume species. Intercropping with pigeon bean determined lower dry matter production of wheat straw than with field pea, and then hairy vetch.

N concentration in wheat straw was significantly higher in N160, with no other differences among treatments.

N accumulated in wheat straw under intercropping was inferior to N40, except for HV-DW TIC which was also equal to N40 and N80.

Overall, intercropping did not facilitate at early stages the growth of durum wheat, neither its mineral nitrogen nutrition.

At heading stage (Table 17) the depletion in dry matter production of wheat under intercropping was more evident than at stem elongation in terms of absolute values. Only under PB-DW TIC wheat produced significantly more straw and ears than the control. There was an interesting interactions between the strategy of intercropping and the component legume crop, with pigeon bean determining, respectively, the higher increase and the higher depletion in wheat biomass under temporary and permanent intercropping. Data on the dry weight of ears and total biomass confirm this evidence.

A higher N concentration in wheat biomass was recorded for permanent intercropping with pigeon bean. N accumulation resulted in data similar to those of dry matter production. Averaged over all treatments, wheat intercropped with legumes differed very little from N0, revealing no intense facilitation compared to mineral fertilization. For intercropping with bean, a significantly higher N accumulation in wheat under temporary intercropping than permanent intercropping was shown. Pigeon bean confirmed to be the most competitive legume for wheat, whilst field pea resulted in the lowest disturbance.

*Table 17 - Dry matter weight, N concentration and N accumulation of the different plant components of wheat sampled at the end of heading stage (**BBCH 59**) in 2009/10. Data expressed as means ± SD (n = 3). Within each column, data followed by different letters are significantly different*

| **BBCH 59** | | | | | | | | |
|---|---|---|---|---|---|---|---|---|
| **Treatment** | **Dry matter production ($g\ m^{-2}$)** | | | **N concentration ($g\ kg^{-1}$)** | | **N accumulation ($g\ m^{-2}$)** | | |
| | **Straw** | **Ears** | **Total Biomass** | **Straw** | **Ears** | **Straw** | **Ears** | **Total Biomass** |
| N0 | 179.91 ± 74.31 e | 26.78 ± 8.77 ef | 206.69 ± 82.75 d | 6.22 ± 0.09 e | 13.43 ± 0.74 e | 1.12 ± 0.48 ef | 0.36 ± 0.10 ef | 1.48 ± 0.57 ef |
| N40 | 290.95 ± 76.01 bcd | 52.04 ± 29.84 bcde | 342.99 ± 104.31 bc | 6.59 ± 0.34 e | 13.38 ± 0.77 e | 1.92 ± 0.57 def | 0.70 ± 0.36 cde | 2.62 ± 0.93 de |
| N80 | 348.61 ± 129.99 bc | 64.52 ± 30.35 abc | 413.13 ± 160.10 b | 9.94 ± 1.40 bc | 16.67 ± 2.25 c | 3.47 ± 0.78 bc | 1.08 ± 0.51 abc | 4.55 ± 1.28 bc |
| N120 | 488.41 ± 19.51 a | 86.03 ± 16.32 a | 574.44 ± 34.30 a | 7.28 ± 0.58 de | 13.36 ± 0.06 e | 3.56 ± 0.34 b | 1.15 ± 0.22 ab | 4.71 ± 0.55 b |
| N160 | 373.33 ± 61.07 b | 74.68 ± 17.26 ab | 448.01 ± 77.12 b | 13.85 ± 4.20 a | 19.35 ± 0.95 b | 5.17 ± 2.06 a | 1.45 ± 0.31 a | 6.62 ± 2.27 a |
| PB-DW TIC | 278.63 ± 11.08 cd | 56.31 ± 11.38 bcd | 334.94 ± 21.81 bc | 8.11 ± 0.19 cde | 14.24 ± 0.64 de | 2.26 ± 0.09 cde | 0.80 ± 0.15 bcd | 3.06 ± 0.24 cd |
| FP-DW TIC | 229.64 ± 36.29 de | 46.04 ± 12.75 cde | 275.68 ± 48.76 cd | 7.72 ± 0.83 cde | 14.93 ± 0.38 cde | 1.77 ± 0.18 def | 0.69 ± 0.21 cde | 2.46 ± 0.39 def |
| HV-DW TIC | 233.24 ± 18.30 de | 47.37 ± 8.93 bcde | 280.61 ± 26.68 cd | 9.54 ± 0.15 bcd | 16.38 ± 1.22 cd | 2.23 ± 0.21 cde | 0.78 ± 0.15 bcd | 3.01 ± 0.35 cde |
| PB-DW PIC | 80.24 ± 7.64 f | 2.23 ± 2.46 f | 82.47 ± 9.98 e | 11.96 ± 0.30 ab | 22.33 ± 2.73 a | 0.96 ± 0.08 f | 0.05 ± 0.05 f | 1.01 ± 0.13 f |
| FP-DW PIC | 226.40 ± 68.50 de | 36.15 ± 17.94 de | 262.55 ± 86.27 cd | 7.14 ± 1.23 de | 13.74 ± 0.21 e | 1.62 ± 0.77 def | 0.50 ± 0.25 de | 2.12 ± 1.03 def |
| HV-DW PIC | 247.00 ± 24.66 de | 28.89 ± 24.27 def | 275.89 ± 31.17 cd | 10.78 ± 0.56 b | 16.08 ± 0.97 e | 2.66 ± 0.33 bcd | 0.46 ± 0.13 de | 3.12 ± 0.46 cd |
| Significance[1] | ** | ** | ** | ** | ** | ** | ** | ** |
| *LSD* | *91.96* | *28.02* | *117.01* | *2.50* | *2.24* | *1.27* | *0.41* | *1.56* |
| *CV (%)*[2] | *20.0* | *34.7* | *21.6* | *16.3* | *8.3* | *30.5* | *32.9* | *29.0* |

*[1] **, *, ns are, respectively, significant for $p \leq .01$, significant for $p \leq .05$ and not significant (LSD test)*

*[2] Coefficient of variation*

At end of flowering (Table 18), the deleterious effect of permanent intercropping on wheat growth was confirmed. Dry matter production of wheat straw under PIC was still increasing at BBCH 69, accounting for values slightly higher than under TIC. On the other hand, the biomass of ears produced by wheat under TIC was clearly higher than under PIC, showing a different trend in the crop phenology under the two strategies of intercropping.

*Table 18 - Dry matter weight, N concentration and N accumulation of the different plant components of wheat sampled at the end of flowering stage (**BBCH 69**) in 2009/10. Data expressed as means ± SD (n = 3). Within each column, data followed by different letters are significantly different*

| **BBCH 69** | | | | | | | | |
|---|---|---|---|---|---|---|---|---|
| **Treatment** | **Dry matter production (g m$^{-2}$)** | | | **N concentration (g kg$^{-1}$)** | | **N accumulation (g m$^{-2}$)** | | |
| | **Straw** | **Ears** | **Total Biomass** | **Straw** | **Ears** | **Straw** | **Ears** | **Total Biomass** |
| N0 | 224.33 ± 75.64 b | 47.78 ± 16.33 cd | 272.11 ± 91.75 c | 4.34 ± 0.09 ef | 11.73 ± 0.63 | 0.97 ± 0.33 d | 0.56 ± 0.16 ef | 1.53 ± 0.49 ef |
| N40 | 471.61 ± 195.67 a | 88.28 ± 42.41 bc | 559.89 ± 237.88 b | 4.22 ± 0.44 f | 11.48 ± 0.25 | 1.99 ± 0.98 bcd | 1.01 ± 0.50 cde | 3.00 ± 1.48 cde |
| N80 | 521.54 ± 114.00 a | 126.28 ± 26.78 ab | 647.82 ± 140.54 ab | 5.78 ± 0.69 def | 12.96 ± 0.63 | 3.01 ± 0.76 bc | 1.64 ± 0.27 bc | 4.65 ± 1.02 bc |
| N120 | 535.49 ± 37.21 a | 142.81 ± 83.47 a | 678.30 ± 120.28 ab | 6.19 ± 0.30 de | 14.16 ± 0.69 | 3.31 ± 0.19 b | 2.02 ± 1.29 ab | 5.33 ± 1.42 b |
| N160 | 564.98 ± 67.64 a | 165.24 ± 20.23 a | 730.22 ± 87.81 a | 10.01 ± 1.31 b | 15.29 ± 1.21 | 5.66 ± 1.30 a | 2.53 ± 0.42 a | 8.19 ± 1.60 a |
| PB-DW TIC | 274.81 ± 10.44 b | 89.02 ± 9.02 bc | 363.83 ± 19.07 c | 6.86 ± 0.19 cd | 14.99 ± 0.35 | 1.89 ± 0.12 cd | 1.33 ± 0.11 cd | 3.22 ± 0.21 cde |
| FP-DW TIC | 284.81 ± 59.67 b | 61.61 ± 16.84 c | 346.42 ± 75.33 c | 6.98 ± 3.20 cd | 11.90 ± 0.45 | 1.99 ± 1.43 bcd | 0.73 ± 0.19 def | 2.72 ± 1.60 de |
| HV-DW TIC | 285.80 ± 16.08 b | 94.02 ± 18.79 bc | 379.82 ± 34.64 c | 6.45 ± 0.04 d | 12.87 ± 0.08 | 1.84 ± 0.12 cd | 1.21 ± 0.25 cde | 3.05 ± 0.36 cde |
| PB-DW PIC | 81.21 ± 7.94 c | 2.72 ± 1.68 d | 83.93 ± 9.58 d | 12.16 ± 0.59 a | 19.70 ± 11.39 | 0.99 ± 0.15 d | 0.05 ± 0.04 f | 1.04 ± 0.19 f |
| FP-DW PIC | 289.04 ± 75.26 b | 52.11 ± 16.67 c | 341.15 ± 91.75 c | 6.14 ± 0.13 def | 12.98 ± 0.27 | 1.77 ± 0.48 cd | 0.68 ± 0.23 def | 2.45 ± 0.71 def |
| HV-DW PIC | 287.66 ± 79.95 b | 60.39 ± 14.72 c | 348.05 ± 92.82 c | 8.67 ± 0.64 bc | 14.70 ± 0.58 | 2.49 ± 0.86 bc | 0.89 ± 0.20 de | 3.38 ± 1.00 cd |
| Significance[1] | ** | ** | ** | ** | ns | ** | ** | ** |
| *LSD* | *130.77* | *48.65* | *164.68* | *1.96* | *5.96* | *1.32* | *0.70* | *1.72* |
| *CV (%)*[2] | *22.1* | *33.8* | *22.4* | *16.3* | *26.3* | *32.5* | *35.8* | *28.6* |

*[1] **, *, ns are, respectively, significant for $p \leq .01$, significant for $p \leq .05$ and not significant (LSD test)*

*[2] Coefficient of variation*

Total aboveground dry matter produced by wheat under intercropping at end of flowering stage was not different from the control, whilst for PB-DW PIC a value lower than N0 was registered.

N concentration in the straw of intercropped wheat compensated for lower dry biomass, with significantly higher values than the control, averaged over all treatments except for FP-DW PIC (Table 18).

N accumulated in wheat straw under intercropping was lower than under fertilized sole crop, but with very few significant differences. Fertilization with ammonium nitrate determined on the other hand higher N accumulation in ear biomass compared to intercropping, averaged over all treatments. As results of the combined effect of dry matter and N concentration, only wheat permanently intercropped with hairy vetch resulted in higher total N accumulation than the control.

*Table 19 - Dry matter weight, N concentration and N accumulation of the different plant components of wheat sampled at medium milky ripening stage (**BBCH 75**) in 2009/10. Data expressed as means ± SD (n = 3). Within each column, data followed by different letters are significantly different*

| BBCH 75 | | | | | | | | |
|---|---|---|---|---|---|---|---|---|
| **Treatment** | **Dry matter production (g m$^{-2}$)** | | | **N concentration (g kg$^{-1}$)** | | **N accumulation (g m$^{-2}$)** | | |
| | **Straw** | **Ears** | **Total Biomass** | **Straw** | **Ears** | **Straw** | **Ears** | **Total Biomass** |
| N0 | 207.65 ± 73.52 d | 134.61 ± 48.30 d | 342.26 ± 120.31 e | 3.79 ± 0.66 cd | 12.79 ± 2.03 de | 0.79 ± 0.36 d | 1.72 ± 0.87 e | 2.51 ± 1.20 ef |
| N40 | 357.19 ± 129.27 bc | 236.77 ± 99.04 c | 593.96 ± 227.68 bc | 3.47 ± 0.81 d | 11.37 ± 1.82 e | 1.24 ± 0.63 bcd | 2.69 ± 1.45 cde | 3.93 ± 2.08 cde |
| N80 | 404.41 ± 52.08 ab | 318.99 ± 26.07 ab | 723.40 ± 78.01 ab | 4.71 ± 0.60 cd | 13.81 ± 1.42 de | 1.90 ± 0.35 bc | 4.41 ± 0.57 bc | 6.31 ± 0.90 bc |
| N120 | 451.61 ± 66.43 a | 334.28 ± 48.93 a | 785.89 ± 110.54 a | 4.73 ± 1.18 cd | 15.64 ± 1.14 bcd | 2.14 ± 0.71 b | 5.23 ± 0.43 ab | 7.37 ± 1.03 b |
| N160 | 491.65 ± 32.10 a | 361.29 ± 49.90 a | 852.94 ± 80.50 a | 7.10 ± 2.00 b | 17.26 ± 3.71 bc | 3.49 ± 1.19 a | 6.24 ± 2.12 a | 9.73 ± 3.22 a |
| PB-DW TIC | 270.84 ± 9.93 cd | 244.38 ± 28.00 bc | 515.22 ± 37.93 cd | 4.44 ± 0.55 cd | 15.51 ± 1.13 bcd | 1.20 ± 0.19 cd | 3.79 ± 0.58 bcd | 4.99 ± 0.77 bcd |
| FP-DW TIC | 255.25 ± 35.83 d | 219.80 ± 48.03 c | 475.05 ± 83.80 cde | 3.87 ± 0.71 cd | 13.38 ± 2.42 de | 0.99 ± 0.33 d | 2.94 ± 1.19 cde | 3.93 ± 1.52 cde |
| HV-DW TIC | 277.88 ± 15.74 cd | 247.75 ± 24.45 bc | 525.63 ± 39.95 cde | 4.91 ± 1.55 cd | 15.10 ± 0.73 cd | 1.36 ± 0.51 bcd | 3.74 ± 0.40 bcd | 5.10 ± 0.89 bcd |
| PB-DW PIC | 82.05 ± 7.45 e | 3.37 ± 2.18 e | 85.42 ± 9.63 f | 11.91 ± 2.22 a | 22.65 ± 0.42 a | 0.98 ± 0.12 d | 0.08 ± 0.05 f | 1.06 ± 0.11 f |
| FP-DW PIC | 244.57 ± 37.52 d | 141.81 ± 19.09 d | 386.38 ± 56.24 de | 4.28 ± 0.72 cd | 14.07 ± 2.07 de | 1.05 ± 0.31 cd | 2.00 ± 0.52 e | 3.05 ± 0.82 def |
| HV-DW PIC | 244.29 ± 71.01 d | 119.67 ± 35.43 d | 363.96 ± 102.61 de | 5.82 ± 0.46 bc | 18.25 ± 1.51 b | 1.42 ± 0.35 bcd | 2.18 ± 0.84 de | 3.60 ± 1.11 de |
| Significance[1] | ** | ** | ** | ** | ** | ** | ** | ** |
| *LSD* | *92.75* | *75.14* | *162.98* | *2.07* | *3.01* | *0.89* | *1.64* | *2.41* |
| *CV (%)[2]* | *18.2* | *20.5* | *18.6* | *22.7* | *11.4* | *34.3* | *30.0* | *29.8* |

*[1] **, *, ns are, respectively, significant for p≤.01, significant for p≤.05 and not significant (LSD test)*

*[2] Coefficient of variation*

Data collected at medium milky ripening of wheat in 2009/10 are shown in Table 19.

For intercropping treatments a lower dry matter production than sole crop fertilized with ammonium nitrate was observed, whilst intercropped wheat was statistically similar to the performances of the control. Dry matter produced by wheat under temporary intercropping was higher at that stage compared to permanent intercropping for all plant components.

N concentration in wheat straw and ears was higher than the control only in PB- and HV-DW PIC, which compensated for the lower biomass production.

In terms of total N accumulation, only wheat intercropped temporarily with hairy vetch and bean showed values statistically higher than the control. No differences between the two strategies of intercropping were recorded. Wheat under PB-DW PIC was again the treatment with the lowest amount of N in wheat aboveground biomass.

In Figure 21 and 22 the patterns of total aboveground dry matter production and total N accumulation in aboveground dry matter production in the first year of the field experiment are depicted.

From Figure 21 a depletion in wheat biomass under intercropped wheat, as well as in the control, appears evident from early growth stage, in comparison to sole crop fertilized with the different rates of ammonium nitrate. Wheat under permanent intercropping grew at a steady rate until harvest, whereas under temporary intercropping a faster growth was recorded after flowering, possibly due to the effect of the availability of N from mineralization of legumes incorporated into the soil. Wheat under PB- and HV-DW TIC continued to grow also until harvest maturity, whereas under permanent intercropped wheat reached the maximum growth at BBCH 75. Intercropping with field pea determined the lowest growth curve among temporary treatments, and the highest among permanent ones. Permanent intercropping with pigeon bean determined a suppressed wheat growth right from early stages (BBCH 30).

The patterns of N accumulated in total aboveground biomass (Figure 22) were in line with dry biomass. Anyway, they allowed to differentiate the behavior of the different species. In terms of N uptake, all intercropping treatments except for PB-DW PIC showed clearly upper curves than N0. The pattern of N40 acted as the dividing line between the two strategies of intercropping, with temporary lying above and permanent below it. Under temporary intercropping, N accumulations grew at the highest rate between BBCH 69 and 75, and then they increased slowly until harvest. This behavior was particularly evident for FP-DW TIC, which suddenly stopped to grow and to accumulate N after BBCH 75. Concerning permanent intercropping, this strategy resulted in different kind of patterns, with an increasing rate higher than under temporary treatments in the last period of growth. This trend did not match that of dry matter production observed for the same treatments, suggesting the occurrence of a high N concentration in plant tissues, as confirmed by data previously discussed (Tables 12 and 19).

*Figure 21 - Time course of total aboveground dry matter production of durum wheat grown under different treatments in 2009/10. Error bars are standard deviations (n = 3).*

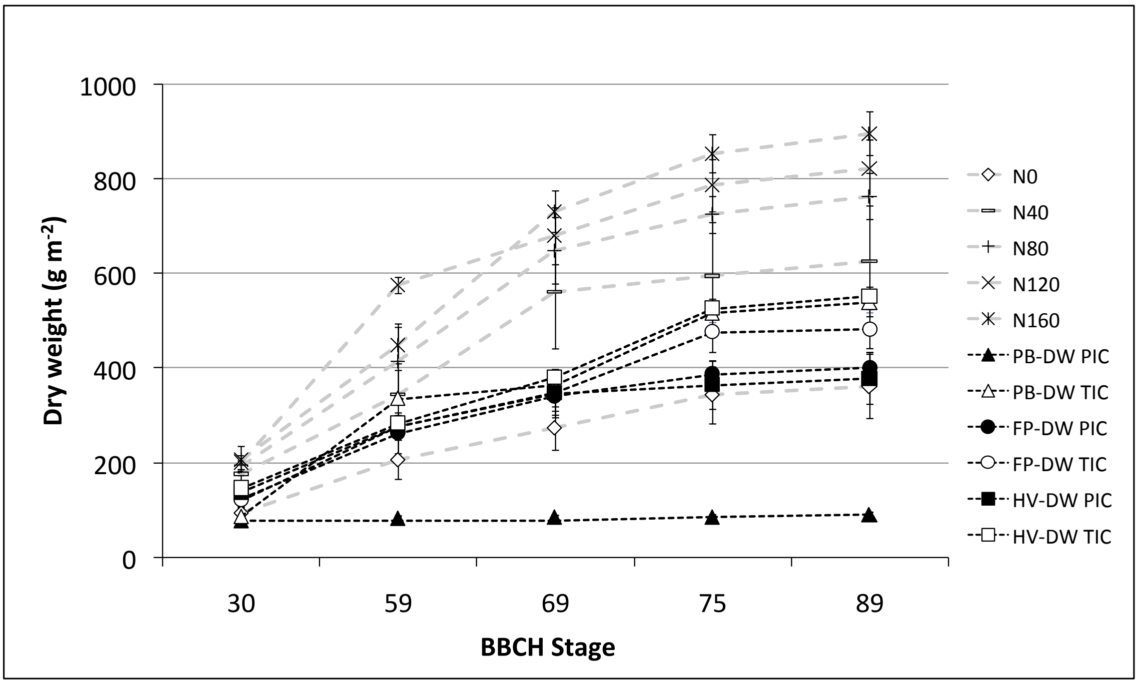
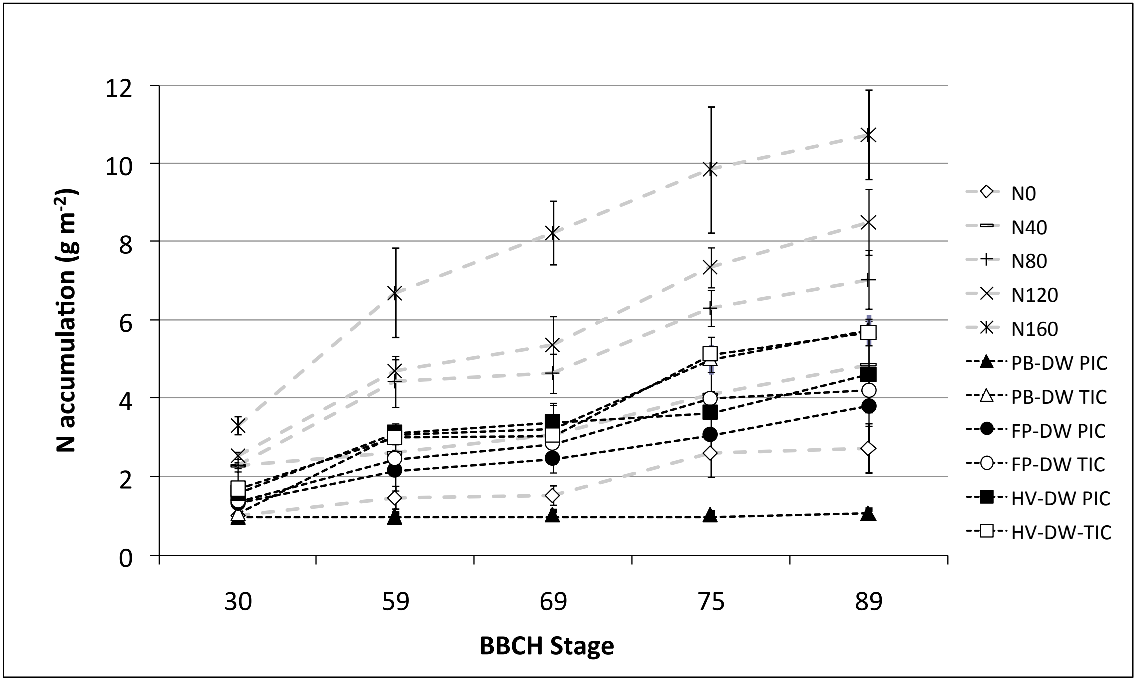


*Figure 22 - Time course of N accumulation in total aboveground dry matter of durum wheat grown under different treatments in 2009/10. Error bars are standard deviations (n = 3).*



The dry matter produced and the N accumulated by wheat grown under different treatments in 2010/11 at intermediate growth stages are shown in Tables 20 to 23 and in Figures 23 and 24.

*Table 20 - Dry matter weight, N concentration and N accumulation of the different plant components of wheat sampled at stem elongation stage (**BBCH 30**) in 2010/11. Data expressed as means ± SD (n = 3). Within each column, data followed by different letters are significantly different*

| BBCH 30 | | | | | | | | |
|---|---|---|---|---|---|---|---|---|
| **Treatment** | **Dry matter production ($g\ m^{-2}$)** | | | **N concentration ($g\ kg^{-1}$)** | | **N accumulation ($g\ m^{-2}$)** | | |
| | **Straw** | **Ears** | **Total Biomass** | **Straw** | **Ears** | **Straw** | **Ears** | **Total Biomass** |
| N0 | 47.67 ± 6.11 | - | 47.67 ± 6.11 | 18.73 ± 0.83 c | - | 0.89 ± 0.15 d | - | 0.89 ± 0.15 d |
| N40 | 59.00 ± 2.65 | - | 59.00 ± 2.65 | 25.13 ± 1.55 ab | - | 1.48 ± 0.06 ab | - | 1.48 ± 0.06 ab |
| N80 | 56.33 ± 12.10 | - | 56.33 ± 12.10 | 26.33 ± 2.27 ab | - | 1.48 ± 0.44 ab | - | 1.48 ± 0.44 ab |
| N120 | 55.00 ± 12.53 | - | 55.00 ± 12.53 | 26.17± 2.08 ab | - | 1.44 ± 0.44 abc | - | 1.44 ± 0.44 abc |
| N160 | 64.00 ± 12.53 | - | 64.00 ± 12.53 | 28.23 ± 3.27 a | - | 1.81 ± 0.49 a | - | 1.81 ± 0.49 a |
| PB-DW TIC | 38.00 ± 11.53 | - | 38.00 ± 11.53 | 23.73 ± 4.06 b | - | 0.90 ± 0.41 cd | - | 0.90 ± 0.41 cd |
| FP-DW TIC | 58.67 ± 8.08 | - | 58.67 ± 8.08 | 17.20 ± 0.53 c | - | 1.01 ± 0.12 bcd | - | 1.01 ± 0.12 bcd |
| HV-DW TIC | 42.67 ± 13.65 | - | 42.67 ± 13.65 | 19.43 ± 2.48 c | - | 0.83 ± 0.31 d | - | 0.83 ± 0.31 d |
| PB-DW PIC | 38.67 ± 7.51 | - | 38.67 ± 7.51 | 24.73 ± 2.45 ab | - | 0.95 ± 0.16 bcd | - | 0.95 ± 0.16 bcd |
| FP-DW PIC | 49.33 ± 13.05 | - | 49.33 ± 13.05 | 18.20 ± 0.70 c | - | 0.90 ± 0.26 d | - | 0.90 ± 0.26 d |
| HV-DW PIC | 48.33 ± 7.77 | - | 48.33 ± 7.77 | 19.23 ± 1.24 c | - | 0.93 ± 0.18 cd | - | 0.93 ± 0.18 cd |
| Significance[1] | ns | - | ns | ** | - | * | - | * |
| *LSD* | *18.30* | - | *18.30* | *3.95* | - | *0.55* | - | *0.55* |
| *CV (%)[2]* | *21.2* | - | *21.2* | *10.3* | - | *27.9* | - | *27.9* |

*[1] **, *, ns are, respectively, significant for p≤.01, significant for p≤.05 and not significant (LSD test)*

*[2] Coefficient of variation*

At BBCH 30 (Table 20) no significant differences were observed among treatments in terms of dry matter production. Anyway, among intercropping, only FP-DW TIC showed values higher than the control in terms of absolute values. Wheat sole cropping with mineral N fertilizer accounted for the highest N concentration level in wheat straw, with all intercropping, except those including pigeon bean, showing lower concentrations compared to the control.

Consequently, N accumulations in the straw of intercropped wheat, averaged over all treatments, were significantly inferior to fertilized sole crops, and not different from the control.

*Table 21 - Dry matter weight, N concentration and N accumulation of the different plant components of wheat sampled at the end of heading stage (**BBCH 59**) in 2010/11. Data expressed as means ± SD (n = 3). Within each column, data followed by different letters are significantly different*

| BBCH 59 | | | | | | | | |
|---|---|---|---|---|---|---|---|---|
| Treatment | Dry matter production (g $m^{-2}$) | | | N concentration (g $kg^{-1}$) | | N accumulation (g $m^{-2}$) | | |
| | Straw | Ears | Total Biomass | Straw | Ears | Straw | Ears | Total Biomass |
| N0 | 153.67 ± 83.15 bcd | 50.33 ± 24.58 bc | 204.00 ± 107.72 bc | 12.03 ± 1.25 cd | 14.30 ± 0.95 | 1.85 ± 0.76 cd | 0.72 ± 0.31 bc | 2.57 ± 1.06 bc |
| N40 | 202.67 ± 23.07 abc | 64.67 ± 9.61 ab | 267.34 ± 31.75 ab | 11.60 ± 1.35 cd | 13.93 ± 0.31 | 2.35 ± 0.03 abc | 0.90 ± 0.13 ab | 3.25 ± 0.13 ab |
| N80 | 230.00 ± 35.37 a | 78.00 ± 14.11 a | 308.00 ± 49.43 a | 11.87 ± 1.20 cd | 14.70 ± 1.01 | 2.73 ± 0.68 ab | 1.15 ± 0.28 a | 3.88 ± 0.97 a |
| N120 | 249.67 ± 21.50 a | 84.00 ± 7.21 a | 333.67 ± 26.01 a | 11.53 ± 1.31 cde | 14.57 ± 0.59 | 2.88 ± 0.56 a | 1.22 ± 0.14 a | 4.10 ± 0.64 a |
| N160 | 216.33 ± 38.70 ab | 78.00 ± 15.62 a | 294.33 ± 54.31 a | 12.97 ± 0.67 bc | 15.03 ± 0.84 | 2.81 ± 0.40 a | 1.17 ± 0.21 a | 3.98 ± 0.61 a |
| PB-DW TIC | 143.67 ± 23.25 cd | 44.00 ± 10.15 bc | 187.67 ± 33.31 bc | 9.67 ± 0.93 e | 14.53 ± 1.25 | 1.39 ± 0.37 d | 0.64 ± 0.19 bc | 2.03 ± 0.55 bc |
| FP-DW TIC | 153.00 ± 42.58 bcd | 50.67 ± 10.07 bc | 203.67 ± 52.52 bc | 12.17 ± 0.46 bcd | 14.57 ± 0.60 | 1.86 ± 0.59 bcd | 0.74 ± 0.14 bc | 2.60 ± 0.72 bc |
| HV-DW TIC | 143.33 ± 29.01 cd | 45.33 ± 10.69 bc | 188.66 ± 38.53 bc | 14.03 ± 1.27 ab | 14.70 ± 1.31 | 2.01 ± 0.58 abcd | 0.67 ± 0.19 bc | 2.68 ± 0.76 bc |
| PB-DW PIC | 108.33 ± 42.55 d | 31.33 ± 12.06 c | 139.66 ± 54.29 c | 15.03 ± 0.72 a | 16.00 ± 1.14 | 1.63 ± 0.70 cd | 0.50 ± 0.22 c | 2.13 ± 0.92 bc |
| FP-DW PIC | 117.33 ± 10.02 d | 37.00 ± 4.00 c | 154.33 ± 13.28 c | 11.03 ± 0.85 de | 13.77 ± 1.08 | 1.29 ± 0.04 d | 0.51 ± 0.09 c | 1.80 ± 0.08 c |
| HV-DW PIC | 111.00 ± 8.19 d | 37.00 ± 2.00 c | 148.00 ± 9.00 c | 13.97 ± 1.53 ab | 15.13 ± 0.25 | 1.55 ± 0.15 cd | 0.56 ± 0.03 c | 2.11 ± 0.18 bc |
| Significance[1] | ** | ** | ** | ** | ns | ** | ** | ** |
| *LSD* | *67.02* | *21.80* | *87.89* | *1.92* | *1.25* | *0.90* | *0.33* | *1.20* |
| *CV (%)*[2] | *23.7* | *23.5* | *23.4* | *9.1* | *5.0* | *25.9* | *24.6* | *24.9* |

*[1] **, *, ns are, respectively, significant for p≤.01, significant for p≤.05 and not significant (LSD test)*

*[2] Coefficient of variation*

After heading completion (Table 21), some differences among intercropping species started to arise. Pea was the legume facilitating the most wheat, whatever the strategy of intercropping. In terms of dry matter production of all plant components, there were no significant differences between temporary and permanent intercropping, even though these latter were only similar to the control, and the first also to N40. Averaged over all treatments, intercropping determined a strong biomass depletion of wheat compared to sole cropping (about 50% less). This deleterious effect was partially offset by an increase in N concentration in plant tissues, which was significantly affected by treatments for straw component. In some intercropping treatments (i.e. FP- and HV-DW TIC, as well as HV-DW PIC), N concentration in straw showed levels comparable to the sole crop fertilized with the highest rates of ammonium nitrates. PB-DW PIC was even superior to N160.

As results, N accumulations in aboveground wheat biomass was significantly higher at highest rates of ammonium nitrate in sole crop than other treatments. Intercropping overall produced results statistically comparable to N0 and N40 (except for FP-DW PIC, being only similar to N0), with not significant differences between the two strategies. Still, temporarily intercropped wheat produced N accumulations higher than permanently in terms of absolute values.

The difference between fertilized sole and intercropped wheat was the same also at end of flowering (Table 22). Total dry biomass produced by wheat under intercropping, averaged over all treatments, was approximately half that of the fertilized sole crop. Anyway no difference was observed compared to the control, even though only for FP-DW TIC higher absolute values were recorded. The species of legume intercropped with wheat did not affect significantly wheat growth, albeit a clear trend (FP > HV >PB) arose.

Permanent intercropping with pigeon bean resulted again in the highest N concentration in plant tissues, and particularly in ears, with only wheat sole crop fertilized with 160 N units being comparable to. An effect of compensation of the low dry matter production was then assessed.

N accumulated in total wheat biomass was not significantly affected by the strategy of intercropping which, averaged over all treatments, resulted still in lower values than sole crops with mineral N fertilization. Although appreciably lower in absolute terms, N accumulation in wheat temporarily intercropped with vetch and pea resulted in values statistically comparable to all the sole crop treatments. Permanent intercropping produced, on average, N accumulations only slightly lower than temporary.

*Table 22 - Dry matter weight, N concentration and N accumulation of the different plant components of wheat sampled at the end of flowering stage (**BBCH 69**) in 2010/11 Data expressed as means ± SD (n = 3). Within each column, data followed by different letters are significantly different. Within each column, data followed by different letters are significantly different*

| **BBCH 69** | | | | | | | | |
|---|---|---|---|---|---|---|---|---|
| **Treatment** | **Dry matter production ($g\ m^{-2}$)** | | | **N concentration ($g\ kg^{-1}$)** | | **N accumulation ($g\ m^{-2}$)** | | |
| | **Straw** | **Ears** | **Total Biomass** | **Straw** | **Ears** | **Straw** | **Ears** | **Total Biomass** |
| N0 | 170.00 ± 69.07 c | 64.33 ± 28.31 c | 234.33 ± 97.27 b | 9.50 ± 0.00 | 15.37 ± 1.00 bc | 1.62 ± 0.66 | 0.99 ± 0.51 bcd | 2.61 ± 1.17 bcd |
| N40 | 275.00 ± 37.00 a | 100.33 ± 13.58 a | 375.33 ± 50.56 a | 9.20 ± 0.56 | 13.80 ± 0.17 c | 2.53 ± 0.30 | 1.38 ± 0.18 abc | 3.91 ± 0.46 abc |
| N80 | 264.67 ± 25.93 a | 99.33 ± 16.77 ab | 364.00 ± 42.67 a | 9.57 ± 1.99 | 14.83 ± 1.46 bc | 2.53 ± 0.75 | 1.47 ± 0.36 ab | 4.00 ± 1.09 ab |
| N120 | 280.33 ± 31.21 a | 111.00 ± 14.80 a | 391.33 ± 45.71 a | 9.17 ± 0.84 | 14.37 ± 0.67 bc | 2.57 ± 0.45 | 1.60 ± 0.29 a | 4.17 ± 0.73 a |
| N160 | 248.67 ± 46.01 ab | 104.00 ± 22.65 a | 352.67 ± 68.52 a | 9.37 ± 1.33 | 16.07 ± 0.47 ab | 2.33 ± 0.75 | 1.67 ± 0.35 a | 4.00 ± 1.09 ab |
| PB-DW TIC | 153.33 ± 29.26 c | 54.67 ± 9.87 c | 208.00 ± 36.51 b | 10.23 ± 2.75 | 15.53 ± 0.51 bc | 1.57 ± 0.70 | 0.85 ± 0.18 d | 2.42 ± 0.88 cd |
| FP-DW TIC | 184.00 ± 33.45 bc | 66.67 ± 12.58 c | 250.67 ± 45.80 b | 9.60 ± 1.73 | 15.17 ± 1.76 bc | 1.77 ± 0.62 | 1.01 ± 0.07 bcd | 2.78 ± 0.70 abcd |
| HV-DW TIC | 165.67 ± 48.95 c | 70.67 ± 12.86 bc | 236.34 ± 59.94 b | 11.23 ± 2.34 | 15.40 ± 1.01 bc | 1.88 ± 0.93 | 1.09 ± 0.22 bcd | 2.95 ± 1.14 abcd |
| PB-DW PIC | 145.00 ± 49.73 c | 50.00 ± 14.73 c | 195.00 ± 64.37 b | 10.70 ± 0.36 | 17.63 ± 1.10 a | 1.55 ± 0.59 | 0.88 ± 0.32 cd | 2.43 ± 0.91 cd |
| FP-DW PIC | 152.00 ± 20.52 c | 51.33 ± 4.51 c | 203.33 ± 25.03 b | 8.33 ± 0.70 | 15.13 ± 1.27 bc | 1.27 ± 0.24 | 0.78 ± 0.13 d | 2.05 ± 0.34 d |
| HV-DW PIC | 153.00 ± 45.71 c | 48.67 ± 17.62 c | 201.67 ± 62.31 b | 9.37 ± 0.12 | 15.17 ± 0.70 bc | 1.43 ± 0.41 | 0.74 ± 0.23 d | 2.17 ± 0.64 d |
| Significance[1] | ** | ** | ** | ns | * | ns | ** | * |
| *LSD* | *74.09* | *29.21* | *101.69* | *2.39* | *1.76* | *1.06* | *0.51* | *1.53* |
| *CV (%)[2]* | *21.8* | *23.0* | *21.8* | *14.6* | *6.8* | *32.3* | *246.4* | *29.4* |

*[1] **, *, ns are, respectively, significant for p≤.01, significant for p≤.05 and not significant (LSD test)*

*[2] Coefficient of variation*

At medium milky ripening (Table 23) the trend of wheat growth observed at the previous stage was substantially confirmed. In terms of total dry matter, intercropped wheat produced values statistically comparable only to N0 and, in the case of FP-DW TIC also to N40. Averaged over treatments, temporary intercropping resulted in higher dry matter production than permanent, with statistical significance assessed only for ears component.

*Table 23 - Dry matter weight, N concentration and N accumulation of the different plant components of wheat sampled at medium milky ripening stage (**BBCH 75**) in 2010/11 Data expressed as means ± SD (n = 3). Within each column, data followed by different letters are significantly different*

| | **BBCH 75** | | | | | | | |
|---|---|---|---|---|---|---|---|---|
| **Treatment** | **Dry matter production (g m$^{-2}$)** | | | **N concentration (g kg$^{-1}$)** | | **N accumulation (g m$^{-2}$)** | | |
| | **Straw** | **Ears** | **Total Biomass** | **Straw** | **Ears** | **Straw** | **Ears** | **Total Biomass** |
| N0 | 141.00 ± 40.04 c | 177.00 ± 66.43 cde | 318.00 ± 106.45 cd | 4.53 ± 0.06 c | 15.77 ± 0.59 bc | 0.64 ± 0.18 bc | 2.79 ± 1.12 cd | 3.43 ± 1.30 cde |
| N40 | 206.00 ± 21.66 ab | 284.67 ± 17.24 ab | 490.67 ± 38.89 ab | 4.43 ± 0.93 c | 14.37 ± 0.64 c | 0.91 ± 0.09 abc | 4.09 ± 0.10 abc | 5.00 ± 0.10 abc |
| N80 | 226.00 ± 32.92 a | 291.67 ± 29.02 ab | 517.67 ± 61.24 a | 4.27 ± 0.15 c | 14.57 ± 0.51 c | 0.97 ± 0.12 ab | 4.25 ± 0.57 ab | 5.22 ± 0.67 ab |
| N120 | 231.33 ± 42.36 a | 295.33 ± 40.27 ab | 526.66 ± 82.28 a | 4.93 ± 0.42 bc | 15.33 ± 0.31 bc | 1.14 ± 0.31 a | 4.53 ± 0.67 ab | 5.67 ± 0.97 ab |
| N160 | 233.33 ± 26.69 a | 345.67 ± 33.98 a | 579.00 ± 60.56 a | 5.10 ± 0.40 bc | 15.33 ± 0.91 bc | 1.19 ± 0.20 a | 5.30± 0.74 a | 6.49 ± 0.87 a |
| PB-DW TIC | 130.00 ± 18.19 c | 208.33 ± 35.50 cd | 338.33 ± 50.50 cd | 6.50 ± 2.02 ab | 17.17 ± 1.70 ab | 0.85 ± 0.35 abc | 3.58 ± 0.85 bc | 4.43 ± 1.17 bcd |
| FP-DW TIC | 156.00 ± 36.10 bc | 226.33 ± 72.23 bc | 382.33 ± 108.09 bc | 5.37 ± 1.10 abc | 17.00 ± 0.92 ab | 0.84 ± 0.13 abc | 3.85 ± 1.07 bc | 4.69 ± 1.13 bc |
| HV-DW TIC | 155.67 ± 33.17 bc | 194.33 ± 30.35 cde | 350.00 ± 63.51 cd | 6.80 ± 1.48 a | 17.13 ± 2.75 ab | 1.06 ± 0.48 a | 3.33 ± 1.08 bcd | 4.39 ± 1.56 bcd |
| PB-DW PIC | 107.00 ± 13.00 c | 124.67 ± 6.51 e | 231.67 ± 19.09 d | 5.77 ± 0.64 abc | 17.70 ± 0.61 a | 0.62 ± 0.02 bc | 2.21 ± 0.18 d | 2.83 ± 0.20 de |
| FP-DW PIC | 115.33 ± 18.50 c | 148.67 ± 25.42 de | 264.00 ± 43.71 cd | 4.30 ± 0.56 c | 14.57 ± 0.31 c | 0.50 ± 0.14 c | 2.17 ± 0.40 d | 2.67 ± 0.52 e |
| HV-DW PIC | 134.00 ± 39.61 c | 128.33 ± 23.50 e | 262.33 ± 62.85 cd | 5.73 ± 0.42 abc | 16.70 ± 1.06 ab | 0.77 ± 0.25 abc | 2.14 ± 0.53 d | 2.91 ± 0.75 de |
| Significance$^1$ | ** | ** | ** | * | ** | * | ** | ** |
| *LSD* | *54.39* | *69.69* | *120.79* | *1.66* | *1.91* | *0.43* | *1.30* | *1.65* |
| *CV (%)$^2$* | *19.1* | *18.6* | *18.3* | *18.6* | *7.0* | *29.0* | *21.9* | *22.4* |

*[1] **, *, ns are, respectively, significant for $p \leq .01$, significant for $p \leq .05$ and not significant (LSD test)*

*[2] Coefficient of variation*

N concentration was higher than the control in wheat temporarily intercropped with, vetch and pea, and with pigeon bean, respectively, for straw and ears.

The analysis of N accumulated in wheat straw at BBCH 75 revealed a weak effect from treatments. Apparently, also wheat sole crop was forced to invest most energy to sustain the growth of reproductive organs, i.e. ears, than vegetative one, i.e. straw, due to the short duration of growing season. For ears, a more clear distinction between temporary and permanent intercropping treatments can be made, with the first accounting for the highest values, although without statistical significance. Total N accumulations of intercropping did not differentiate from the control, but for temporary intercropping with vetch and pea a slight advantage was demonstrated.

Figures 23 and 24 depict the pattern of, respectively, total dry matter production and N accumulation of wheat in 2010/11 over the crop cycle.

*Figure 23 - Time course of total aboveground dry matter production of durum wheat grown under different treatments in 2010/11. Error bars are standard deviations (n = 3).*

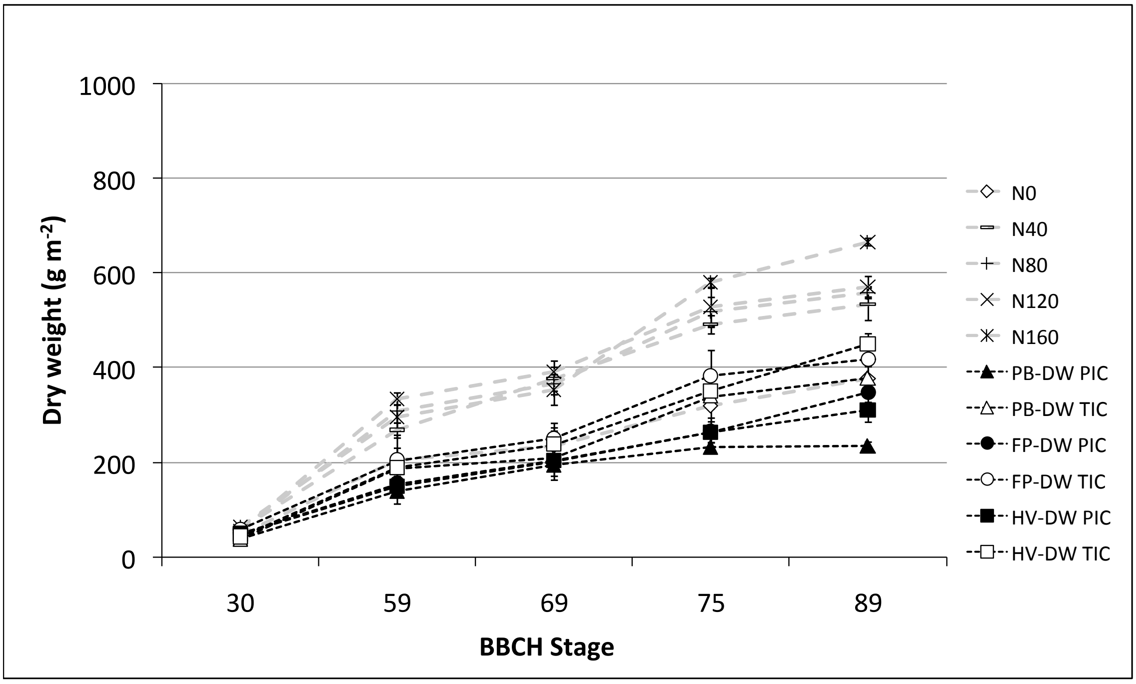
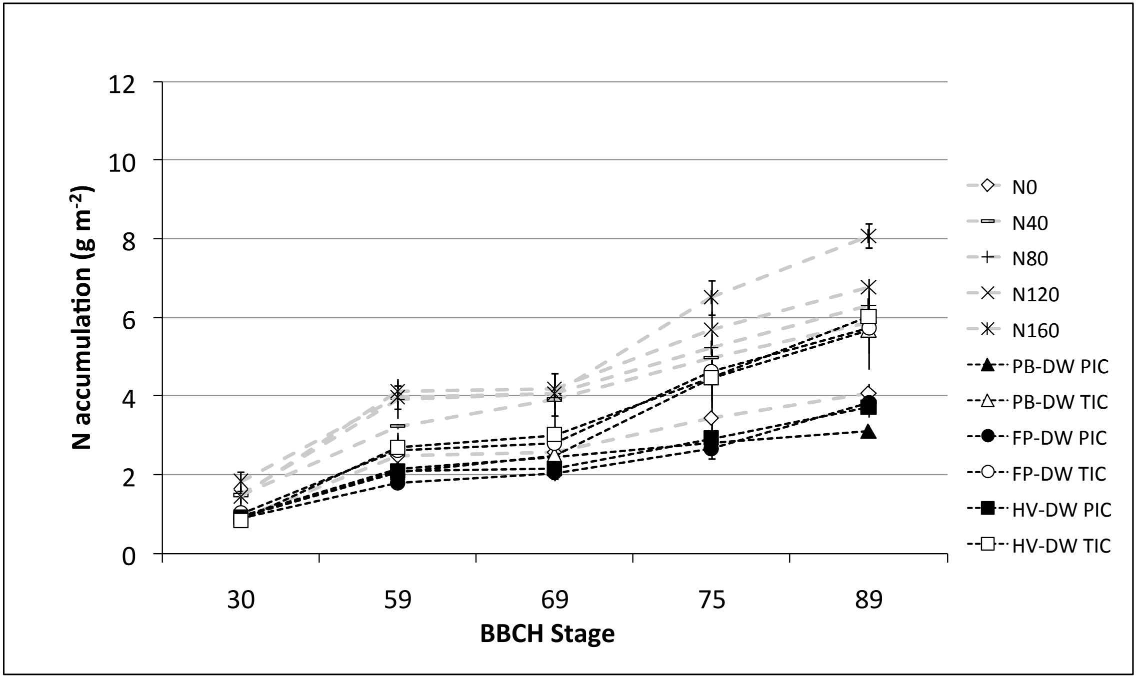


*Figure 24 - Time course of N accumulation in total aboveground dry matter of durum wheat grown under different treatments in 2010/11. Error bars are standard deviations (n = 3).*



Although the higher grain yield assessed at harvest (Table 9), the shorter duration of growing season in comparison to year 1 significantly reduced the total dry biomass production and the total N accumulations of wheat in 2010/11.

In terms of dry matter, N fertilization produced only little increase in wheat production if compared to year 1 (Figure 23). This evidence confirms our impression that N fertilization determined evident advantages for wheat sole crop compared to intercropped mostly by stimulating vegetative growth at early stage. When this was not possible, like in the year 2, due to the late sowing, the gap between the two groups of treatment clearly decreased.

The two strategies of intercropping clearly distinguished from each other right from flowering, and the difference became amply higher between BBCH 69 and 75, when temporarily intercropped wheat grew at a higher rate than permanently.

It is noteworthy that permanent intercropping with pigeon bean did not suppress wheat growth with the same strength than in year 1, when wheat dry biomass did not increased at all over sampling dates (Figure 21). Differently, in the second year durum wheat under PB-DW PIC steadily grew at the same rate than the other PIC treatments until BBCH 69, then it started to slowed down from 69 to 75, and finally it stopped to grow until harvest. The reason behind it might have been the increased competition imposed by pigeon bean on wheat after flowering stage of the legume, probably due to shading and reduction of photosynthesis, as well documented also for field pea by Bedoussac and Justes (2010). In comparison to the first year, the duration of co-growth was shorter in year 2, leading to a lower competition since very early crop development stages and to a final better performance of wheat.

The patterns of N accumulations in wheat total biomass (Figure 24) put even more emphasis on the effect of temporary intercropping, which significantly increased N accumulations in comparison to the control and permanent intercropping treatments. A clear distinction between the two strategies of intercropping was evidenced at early stages yet (BBCH 59), but it was between BBCH 69 and 75 that a clear line of separation between them was drawn. After flowering, wheat under temporary intercropping accumulated N in plant tissues at a rate almost twice that of wheat under PIC, and even comparable to that of N160.

Compared to year 1, the effect of the species of legume grown together with wheat was weaker than intercropping strategy, leading to a more fuzzy segregation of intercropping treatments.

The analysis of wheat plant height at each sampling time highlighted significant differences among treatments in both year (Tables 24 and 25), supporting what shown for dry matter production.

In both 2009/10 (Table 24) and 2010/11 (Table 25), the height of wheat was affected by treatments only in late stages (from heading onwards).

In year 1, the long growth season stressed more the difference among wheat sole crops, with the crop being tallest under N160 and then becoming progressively shorter at decreasing rate of mineral N fertilizer (Table 24). In year 2, the range of variation was smaller, with all wheat sole crop fertilized with ammonium nitrate, whatever the rate, showing the highest values for all the cycle (Table 25).

*Table 24 - Plant height (cm) at canopy level of wheat grown in 2009/10. Within each column, data followed by different letters are significantly different*

| **Treatment** | **BBCH 14** | **BBCH 30** | **BBCH 59** | **BBCH 69** | **BBCH 75** | **BBCH 89** |
|---|---|---|---|---|---|---|
| N0 | 16.92 | 33.33 | 47.33 d | 61.59 d | 65.00 de | 65.00 de |
| N40 | 19.17 | 38.17 | 56.33 bc | 67.58 bcd | 68.67 bcd | 68.67 bcd |
| N80 | 17.17 | 41.83 | 60.17 ab | 72.54 abc | 75.67 ab | 75.67 ab |
| N120 | 19.33 | 42.17 | 61.33 ab | 73.95 ab | 74.17 bc | 74.17 bc |
| N160 | 18.00 | 45.00 | 67.33 a | 81.04 a | 83.67 a | 83.67 a |
| PB-DW TIC | 20.50 | 39.83 | 59.83 ab | 61.06 d | 64.40 de | 64.40 de |
| FP-DW TIC | 18.83 | 36.33 | 52.17 cd | 59.22 d | 59.50 e | 59.50 e |
| HV-DW TIC | 19.83 | 38.83 | 58.17 bc | 64.90 bcd | 66.33 cde | 66.33 cde |
| PB-DW PIC | 20.50 | 42.00 | 62.00 ab | 64.50 cd | 71.35 bcd | 71.35 bcd |
| FP-DW PIC | 18.75 | 33.67 | 56.50 bc | 64.95 bcd | 69.50 bcd | 69.50 bcd |
| HV-DW PIC | 19.67 | 41.33 | 56.17 bc | 65.00 bcd | 72.83 bcd | 72.83 bcd |
| *Significance[1]* | *ns* | *ns* | ** | ** | ** | ** |
| *LSD* | *3.75* | *7.16* | *7.65* | *9.10* | *8.77* | *8.75* |
| *CV (%)[2]* | *11.6* | *10.7* | *7.75* | *8.0* | *7.3* | *7.4* |

[1] ***, *, ns are, respectively, significant for p≤.01, significant for p≤.05 and not significant (LSD test)*

[2] *Coefficient of variation*

*Table 25 - Plant height (cm) at canopy level of wheat grown in 2010/11. Within each column, data followed by different letters are significantly different*

| Treatment | BBCH 14 | BBCH 30 | BBCH 59 | BBCH 69 | BBCH 75 | BBCH 89 |
|---|---|---|---|---|---|---|
| N0 | 21.67 | 27.92 | 55.67 c | 56.93 b | 57.67 b | 57.67 b |
| N40 | 21.00 | 33.92 | 64.67 a | 64.80 a | 67.83 a | 67.83 a |
| N80 | 21.33 | 35.17 | 65.00 a | 67.17 a | 67.50 a | 67.50 a |
| N120 | 19.33 | 33.58 | 66.00 a | 66.07 a | 66.50 a | 66.50 a |
| N160 | 19.17 | 34.50 | 66.67 a | 68.17 a | 68.33 a | 68.33 a |
| PB-DW TIC | 20.00 | 32.83 | 51.67 cd | 53.67 b | 55.33 b | 55.33 b |
| FP-DW TIC | 18.67 | 29.00 | 51.00 cd | 55.07 b | 55.17 b | 55.17 b |
| HV-DW TIC | 18.67 | 29.75 | 52.67 cd | 55.10 b | 56.00 b | 56.00 b |
| PB-DW PIC | 21.67 | 35.00 | 64.00 ab | 66.83 a | 66.93 a | 66.93 a |
| FP-DW PIC | 19.00 | 24.33 | 48.33 d | 54.63 b | 54.83 b | 54.83 b |
| HV-DW PIC | 19.83 | 28.33 | 57.33 bc | 57.60 b | 58.00 b | 58.00 b |
| *Significance[1]* | *ns* | *ns* | ** | ** | ** | ** |
| *LSD* | *3.40* | *7.08* | *6.78* | *6.09* | *5.68* | *5.70* |
| *CV (%)[2]* | *10.0* | *13.3* | *6.8* | *5.9* | *5.4* | *5.0* |

*[1] **, *, ns are, respectively, significant for p≤.01, significant for p≤.05 and not significant (LSD test)*

*[2] Coefficient of variation*

Intercropping determined different effects depending on the species of legume and the strategy of intercropping. In both years, temporary intercropping with legumes produced wheat plants as much high as the control (N0) or, at most as the lowest rate of mineral fertilization (N40). On the other hand, permanent intercropping stimulated more the upright growth of wheat, resulting in final values (at BBCH 89) statistically similar to sole wheat fertilized. For field pea in 2009/10 and for pigeon bean in 2010/11, this increase under permanent intercropping compared to temporary was significant.

The pattern of plant height of wheat in the two years (Figures 25 and 26) showed clear differences in the dynamics of growth. In year 1 (Figure 25), N160 produced the tallest plants right from BBCH30, whereas the other sole wheat fertilized treatments started to differentiate from intercropping only between BBCH 59 and 69. In year 2 (Figure 26), there were less differences among treatments, which showed similar trend of growth.

Wheat permanently intercropped with pigeon bean had similar plant height to fertilized sole wheat at all sampling dates.

*Figure 25 - Time course of plant height of wheat over the growing cycle in 2009/10. Bars are standard deviation (n =3).*

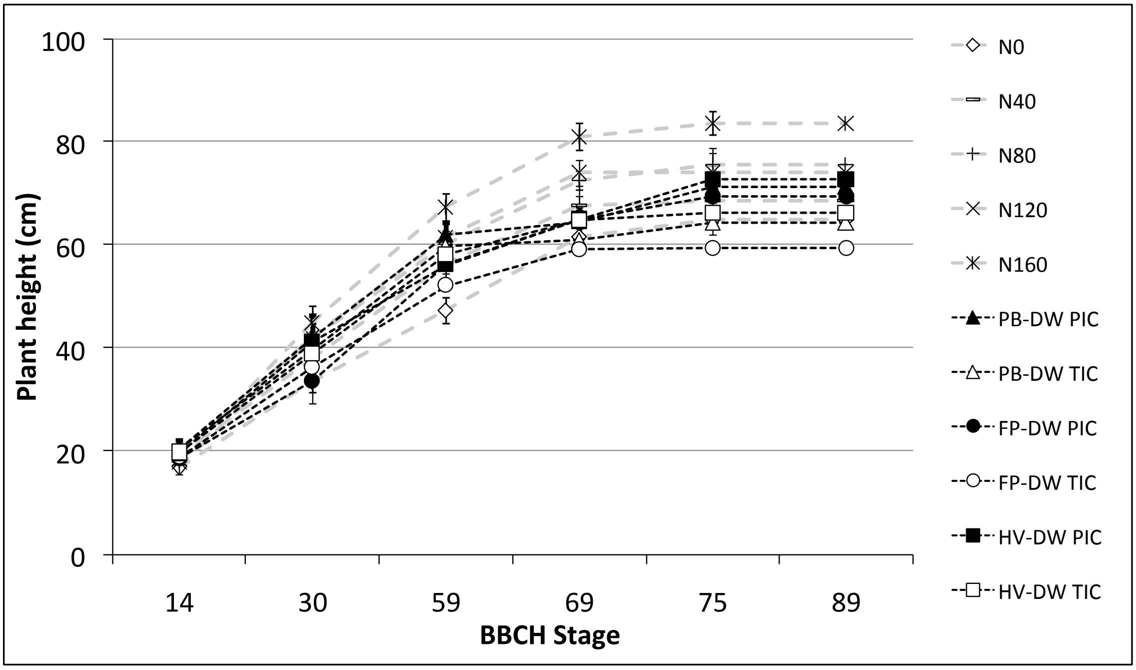
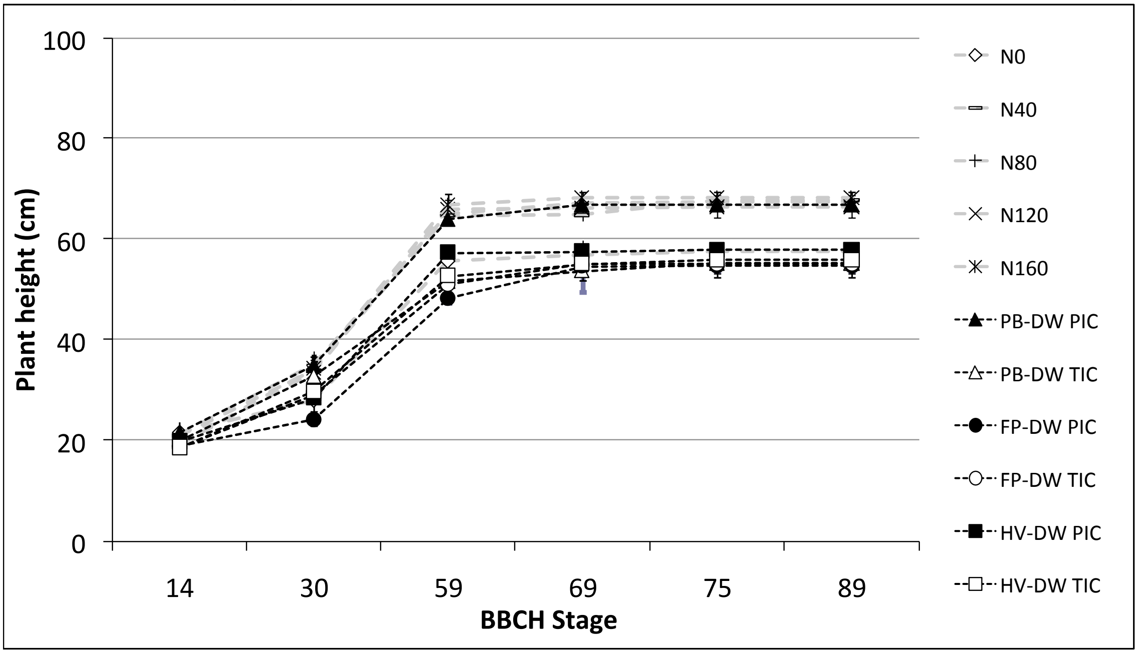


*Figure 26 - Time course of plant height of wheat over the growing cycle in 2009/10. Bars are standard deviation (n =3).*



The interpretation of these results integrate that of dry matter production of wheat. The difference in terms of plant height between wheat under intercropping and as fertilized sole crop was lower than observed for dry matter and N accumulation. In this sense, our findings are in agreement with those of Corre-Hellou et al. (2006). This may lead to the conclusion that what made mostly the difference between the two groups of treatments were the size and the weight of the tissues (above all stems and leaves during vegetative stages, and ears from flowering onwards), rather than vertical growth.

Conversely, the higher wheat height observed under permanent than temporary intercropping suggest the occurrence of a strong competition for light between wheat and the companion legume, as well among wheat plants, under permanent intercropping. And this was especially true for PB-DW PIC, where pigeon bean clearly overgrow wheat, as also reported by Haymes and Lee (1999), Tosti and Guiducci (2010), Mariotti et al. (2012).

Tosti and Guiducci (2010) confirmed our findings and also highlighted the peculiarity of wheat growth under temporary intercropping with pigeon bean, compared to permanent intercropping. Indeed, they observed that pigeon bean already dominated wheat in terms of plant height at time of its incorporation into the soil (i.e. end of tillering of wheat), and that durum wheat, whatever the variety, was taller under intercropping than sole cropping. Anyway, this predominance of pigeon bean did not affect to any further extent the growth of wheat, as the co-growth of the two species was stopped when the legume was terminated by hoeing.

From our results, a higher importance of the trait of wheat height for intercropping practiced in long season arose too. Haymes and Lee (1999) tested the same wheat-field bean intercropping sown either in autumn and in spring. For autumn drilled wheat, plant height was the real determinant for its adaptation to intercropping with bean, whereas for the spring-sown wheat the competitive ability against weeds was more crucial. For this latter, a quick establishment of wheat, a large foliar area ensuring a good soil cover might be only some of the most important traits of wheat genotypes able to fit such conditions of intercropping.

Over all the two years, the analysis of dry matter production, N concentration and accumulation, and plant height of aboveground biomass of wheat over time revealed peculiar dynamics of growth and N nutrition of wheat under the different treatments, and hence it confirmed to be of extreme importance in studies on intercropping, as well also pointed out by Andersen et al. (2007).

The interpretation of growth curves, which implied the inclusion of both intra-specific and inter-specific factors, allowed to discriminate temporary from permanent intercropping treatments, with the latter resulting in higher competition for wheat at early (year 1) or late (year 2) growing stages. Paradoxically, the strong difference in the weather between the two years of the experiment was helpful, as it allowed to clearly identify factors playing a role in the competition suffered by wheat under certain treatments of intercropping. Accordingly to Mariotti et al. (2012), early competition -from emergence to end of tillering- for natural resources (space, solar radiation and nutrients) was identified as the pivotal factor negatively affecting wheat growth under intercropping and determining the gap with fertilized sole crop. As early N fertilization is reported to be helpful to substantially increase the vegetative growth of wheat (Naudin et al., 2010), it could be considered as a valuable option also under intercropping with legumes, in order to enhance the competitive ability of wheat at early stages and to preserve it from growth depletion due to shading.

In the next section of this chapter, the results of wheat performance discussed so far will be related to the ones achieved on legumes grown together with wheat under intercropping. A comprehensive analysis of competition for, and efficiency use of natural resources, as well as of the effect of intercropping on $N_2$ biological fixation and weed abundance, will follow then. At the light of all these things, other suitable options to improve the efficiency of intercropping will be proposed.

## Section 2 - Agroecological evaluation of intercropping

### 3.1.5. Legume dry matter production, NP accumulation, $N_2$-fixation and plant height

Data on dry matter production, N concentration and N accumulation in aboveground biomass of legumes over their growth cycle in the two years are reported in Tables 26 to 31. As for wheat, also for legumes data on P concentration and accumulation in aboveground dry matter are excluded from the discussion, but reported in the Appendix 1.

The dry biomass produced by legumes at harvest in year 1 (Table 26) was much higher than in year 2 (Table 27), due to the longer vegetative period. In 2010/11, pigeon bean, both under permanent and temporary intercropping, produced one third of the aboveground biomass measured in 2009/10. Field pea sole crop produced 60% less total biomass than in the first year, whereas under intercropping it accounted almost for the same amount of dry matter than in 2009/10. For hairy vetch, biomass depletion was -33% for sole crop, -22% for intercropped.

In year 1 (Table 26), intercropping negatively affected the growth of the legumes only for pigeon bean, for which a significant depletion in dry matter production was observed right from early stage (-41% at BBCH 30 of the wheat) until harvest (-18% at BBCH 89). At harvest, only the grain component of the biomass of bean was unaffected by treatment.

For the other species, the negative effect of intercropping determined a decrease in biomass production between -62% (at BBCH 59) and -70% (at BBCH 30), for field pea, and between -35% (at BBCH 89) and -52% (at BBCH 59), for hairy vetch.

Differently from year 1, in year 2 (Table 27) intercropping decreased dry matter production of pigeon bean only at harvest, whilst for all the previous stages the two treatments were absolutely comparable. Anyway, none of the difference between pigeon bean sole crop and intercropped was statistically significant, and neither was for pea and vetch at all sampling dates. For pea and vetch, a decrease in biomass production under intercropping was observed, but being only of modest entity for pea (-6% at harvest) and moderate for hairy vetch (-25% at harvest). Field pea grown in intercropping with wheat showed the highest biomass depletion compared to the sole crop at the beginning (-16% at BBCH 30), and the lowest at the end (-3% at BBCH 75) of its growing cycle.

Table 26 - Aboveground dry matter production (g m$^{-2}$) of legumes under sole- (PB, FP, HV) and inter-cropping (PB-DW PIC, FP-DW PIC, HV-DW PIC, PB-DW TIC, FP-DW TIC, HV-DW TIC) in 2009/10

| Treatment | BBCH 30† | | BBCH 59‡ | | | BBCH 69‡ | | | BBCH 75‡ | | | BBCH 89‡ | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | **Straw** | **Total** | **Straw** | **Pods** | **Total** | **Straw** | **Pods** | **Total** | **Straw** | **Pods** | **Total** | **Straw** | **Pods** | **Grain** | **Total** |
| PB | 228.00 ± 42.51 a | 228.00 ± 42.51 a | 455.67 ± 58.53 a | 20.33 ± 4.16 | 476.00 ± 58.62 a | 495.00 ± 74.51 | 144.00 ± 42.23 | 639.00 ± 106.66 a | 541.00 ± 84.54 a | 558.33 ± 132.79 a | 1099.33 ± 216.82 a | 556.15 ± 85.60 a | 174.11 ± 26.49 a | 441.44 ± 86.43 | 1171.70 ± 190.80 a |
| PB-DW PIC | 135.00 ± 18.33 b | 135.00 ± 18.33 b | 315.67 ± 73.04 b | 20.00 ± 25.24 | 335.67 ± 96.84 b | 385.33 ± 21.08 | 68.67 ± 43.66 | 454.00 ± 64.58 b | 414.00 ± 76.22 b | 370.00 ± 105.08 b | 784.00 ± 180.63 b | 422.67 ± 88.82 b | 132.56 ± 32.69 b | 406.84 ± 94.85 | 962.07 ± 213.53 b |
| PB-DW-TIC | 137.33 ± 20.43 b | 137.33 ± 20.43 b | - | - | - | - | - | - | - | - | - | - | - | - | - |
| *$P_{.05}$* | *0.03* | *0.03* | *0.01* | *0.99* | *0.04* | *0.07* | *0.06* | *0.02* | *0.02* | *0.01* | *0.01* | *0.00* | *0.02* | *0.18* | *0.01* |
| FP | 68.67 ± 58.14 | 68.67 ± 58.14 | 107.00 ± 78.25 | 102.00 ± 105.08 | 209.00 ± 176.60 | 124.33 ± 91.66 | 222.33 ± 221.68 | 346.66 ± 304.37 | 165.67 ± 126.30 | 291.67 ± 251.62 | 457.34 ± 376.34 | 177.51 ± 132.16 | 198.11 ± 176.54 | 145.49 ± 115.42 | 521.11 ± 417.66 |
| FP-DW PIC | 21.00 ± 9.17 | 21.00 ± 9.17 | 32.00 ± 11.53 | 47.33 ± 34.08 | 79.33 ± 45.61 | 36.00 ± 12.29 | 80.00 ± 47.76 | 116.00 ± 60.01 | 51.67 ± 9.71 | 95.00 ± 49.57 | 146.67 ± 59.08 | 63.35 ± 11.04 | 20.59 ± 9.98 | 105.02 ± 48.00 | 188.96 ± 68.86 |
| FP-DW-TIC | 39.67 ± 25.03 | 39.67 ± 25.03 | - | - | - | - | - | - | - | - | - | - | - | - | - |
| *$P_{.05}$* | *0.03* | *0.03* | *0.19* | *0.32* | *0.23* | *0.19* | *0.31* | *0.25* | *0.23* | *0.24* | *0.23* | *0.24* | *0.21* | *0.42* | *0.24* |
| HV | 156.33 ± 54.54 | 156.33 ± 54.54 | 258.33 ± 59.18 | 0.00 | 258.33 ± 59.18 | 291.00 ± 61.88 | 0.00 | 291.00 ± 61.88 | 308.33 ± 65.68 | 45.00 ± 6.00 | 353.33 ± 71.58 | 328.40 ± 71.76 | 23.28 ± 1.30 | 23.46 ± 2.11 | 375.14 ± 74.39 |
| HV-DW PIC | 86.67 ± 14.01 | 86.67 ± 14.01 | 123.00 ± 26.63 | 0.00 | 123.00 ± 26.63 | 149.00 ± 36.76 | 0.00 | 149.00 ± 36.76 | 171.67 ± 45.35 | 16.00 ± 187.67 | 187.67 ± 55.47 | 213.83 ± 35.01 | 13.70 ± 12.37 | 14.60 ± 12.64 | 242.13 ± 59.93 |
| HV-DW-TIC | 79.67 ± 7.37 | 79.67 ± 7.37 | - | - | - | - | - | - | - | - | - | - | - | - | - |
| *$P_{.05}$* | *0.12* | *0.12* | *0.11* | *-* | *0.11* | *0.13* | *-* | *0.13* | *0.16* | *0.09* | *0.15* | *0.20* | *0.36* | *0.28* | *0.22* |

*† Treatments analyzed under one-way ANOVA (Fisher's protected LSD test)*

*‡ Paired comparison t-test performed with treatments concerning the same legume species (i.e. PB vs PB-DW PIC; FP vs FP-DW PIC, HV vs HV-DW PIC)*

*Table 27 - Aboveground dry matter production (g m-²) of legumes under sole- (PB, FP, HV) and inter-cropping (PB-DW PIC, FP-DW PIC, HV-DW PIC, PB-DW TIC, FP-DW TIC, HV-DW TIC) in 2010/11*

| **Treatment** | **BBCH 30†** | | **BBCH 59‡** | | | **BBCH 69‡** | | | **BBCH 75‡** | | | **BBCH 89‡** | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | **Straw** | **Total** | **Straw** | **Pods** | **Total** | **Straw** | **Pods** | **Total** | **Straw** | **Pods** | **Total** | **Straw** | **Pods** | **Grain** | **Total** |
| PB | 190.00 ± 11.36 | 190.00 ± 11.36 | 278.33 ± 28.99 | 52.33 ± 25.77 | 330.66 ± 53.63 | 207.33 ± 51.00 | 128.67 ± 10.02 | 336.00 ± 61.00 | 200.33 ± 54.54 | 142.00 ± 12.12 | 342.33 ± 66.61 | 186.33 ± 41.62 | 57.67 ± 16.50 | 144.33 ± 55.22 | 388.33 ± 112.19 |
| PB-DW PIC | 178.67 ± 24.91 | 178.67 ± 24.91 | 272.33 ± 24.95 | 58.67 ± 24.42 | 331.00 ± 1.73 | 247.33 ± 17.21 | 93.67 ± 24.42 | 341.00 ± 7.21 | 217.67 ± 29.70 | 134.00 ± 48.00 | 351.67 ± 21.22 | 205.33 ± 27.47 | 56.67 ± 13.32 | 99.25 ± 35.70 | 361.67 ± 24.83 |
| PB-DW-TIC | 184.00 ± 61.88 | 184.00 ± 61.88 | - | - | - | - | - | - | - | - | - | - | - | - | - |
| *$P_{.05}$* | *0.94* | *0.94* | *0.82* | *0.82* | *0.99* | *0.30* | *0.17* | *0.90* | *0.51* | *0.83* | *0.85* | *0.33* | *0.96* | *0.46* | *0.74* |
| FP | 92.67 ± 35.12 | 92.67 ± 35.12 | 112.00 ± 46.36 | 55.67 ± 41.53 | 167.67 ± 87.87 | 100.33 ± 29.94 | 77.33 ± 51.60 | 177.66 ± 81.46 | 86.00 ± 24.76 | 107.67 ± 57.27 | 193.67 ± 82.03 | 75.33 ± 26.65 | 72.00 ± 16.70 | 62.67 ± 32.32 | 210.00 ± 75.61 |
| FP-DW PIC | 76.67 ± 66.27 | 76.67 ± 66.27 | 91.33 ± 58.80 | 59.33 ± 39.32 | 150.66 ± 98.08 | 88.33 ± 51.08 | 85.67 ± 41.40 | 174.00 ± 92.48 | 70.67 ± 40.08 | 118.67 ± 52.20 | 189.34 ± 92.23 | 61.67 ± 33.86 | 90.33 ± 25.97 | 45.67 ± 22.19 | 197.67 ± 81.35 |
| FP-DW-TIC | 69.33 ± 23.12 | 69.33 ± 23.12 | - | - | - | - | - | - | - | - | - | - | - | - | - |
| *$P_{.05}$* | *0.66* | *0.66* | *0.69* | *0.93* | *0.95* | *0.77* | *0.85* | *0.97* | *0.65* | *0.84* | *0.96* | *0.67* | *0.38* | *0.54* | *0.87* |
| HV | 61.00 ± 12.49 | 61.00 ± 12.49 | 162.00 ± 14.80 | 47.00 ± 27.22 | 209.00 ± 41.61 | 122.67 ± 13.05 | 115.33 ± 41.48 | 238.00 ± 54.37 | 109.67 ± 17.21 | 139.67 ± 39.50 | 249.34 ± 56.70 | 92.67 ± 11.93 | 54.33 ± 18.18 | 106.00 ± 26.46 | 253.00 ± 54.45 |
| HV-DW PIC | 53.33 ± 22.50 | 53.33 ± 22.50 | 124.33 ± 25.03 | 24.33 ± 7.37 | 148.66 ± 30.73 | 88.67 ± 15.95 | 78.00 ± 32.23 | 166.67 ± 44.46 | 72.33 ± 12.06 | 115.00 ± 51.26 | 187.33 ± 63.13 | 67.33 ± 14.01 | 39.67 ± 13.05 | 83.67 ± 35.12 | 190.67 ± 62.61 |
| HV-DW-TIC | 52.00 ± 30.32 | 52.00 ± 30.32 | - | - | - | - | - | - | - | - | - | - | - | - | - |
| *$P_{.05}$* | *0.88* | *0.88* | *0.23* | *0.28* | *0.28* | *0.18* | *0.45* | *0.33* | *0.16* | *0.68* | *0.46* | *0.23* | *0.50* | *0.59* | *0.45* |

*† Treatments analyzed under one-way ANOVA (Fisher's protected LSD test)*

*‡ Paired comparison t-test performed with treatments concerning the same legume species (i.e. PB vs PB-DW PIC; FP vs FP-DW PIC, HV vs HV-DW PIC)*

*Table 28 - N concentration (g kg$^{-1}$) in aboveground dry matter of legumes under sole- (PB, FP, HV) and inter-cropping (PB-DW PIC, FP-DW PIC, HV-DW PIC, PB-DW TIC, FP-DW TIC, HV-DW TIC) in 2009/10*

| **Treatment** | **BBCH 30†** | **BBCH 59‡** | | **BBCH 69‡** | | **BBCH 75‡** | | **BBCH 89‡** | | |
|---|---|---|---|---|---|---|---|---|---|---|
| | **Straw** | **Straw** | **Pods** | **Straw** | **Pods** | **Straw** | **Pods** | **Straw** | **Pods** | **Grain** |
| PB | 30.00 ± 0.79<br>a | 23.33 ± 0.47 | 50.07 ± 1.25 | 19.37 ± 0.35<br>a | 33.87 ± 1.46<br>b | 9.33 ± 1.00 | 33.83 ± 1.57 | 7.97 ± 0.73 | 12.06 ± 1.23 | 44.13 ± 1.54 |
| PB-DW PIC | 29.70 ± 0.69<br>a | 25.67 ± 1.46 | 48.83 ± 5.84 | 17.90 ± 0.36<br>b | 43.37 ± 1.58<br>a | 14.43 ± 9.28 | 35.27 ± 0.49 | 7.95 ± 0.90 | 12.89 ± 1.65 | 42.58 ± 2.47 |
| PB-DW-TIC | 27.33 ± 1.95<br>b | - | - | - | - | - | - | - | - | - |
| $P_{.05}$ | *0.04* | *0.14* | *0.69* | *0.05* | *0.01* | *0.43* | *0.18* | *1.00* | *0.12* | *0.10* |
| FP | 31.10 ± 5.68 | 20.30 ± 7.89 | 29.63 ± 4.31 | 14.40 ± 2.39 | 21.57 ± 2.11 | 13.50 ± 1.25 | 24.83 ± 4.00 | 13.14 ± 2.44 | 13.29 ± 2.77 | 32.86 ± 2.94 |
| FP-DW PIC | 28.83 ± 6.17 | 17.53 ± 2.90 | 33.07 ± 2.83 | 13.10 ± 1.05 | 23.17 ± 1.19 | 16.03 ± 2.27 | 30.37 ± 1.72 | 14.91 ± 1.73 | 18.11 ± 1.95 | 34.14 ± 1.20 |
| FP-DW-TIC | 28.80 ± 7.50 | - | - | - | - | - | - | - | - | - |
| $P_{.05}$ | *0.09* | *0.44* | *0.13* | *0.37* | *0.28* | *0.27* | *0.07* | *0.53* | *0.22* | *0.61* |
| HV | 33.53 ± 4.46 | 26.43 ± 1.25 | 0.00 | 25.90 ± 0.27<br>a | 0.00 | 20.00 ± 0.17 | 35.27 ± 1.01 | 23.72 ± 1.93 | 12.86 ± 0.98 | 44.03 ± 2.04 |
| HV-DW PIC | 34.30 7.32 | 27.87 ± 0.49 | 0.00 | 24.20 ± 0.70<br>b | 0.00 | 18.70 ± 2.39 | 34.33 ± 1.36 | 17.92 ± 0.61 | 15.66 ± 0.94 | 44.32 ± 2.18 |
| HV-DW-TIC | 37.63 ± 1.97 | - | - | - | - | - | - | - | - | - |
| $P_{.05}$ | *0.70* | *0.28* | - | *0.03* | - | *0.42* | *0.51* | *0.06* | *0.08* | *0.92* |

*† Treatments analyzed under one-way ANOVA (Fisher's protected LSD test)*

*‡ Paired comparison t-test performed with treatments concerning the same legume species (i.e. PB vs PB-DW PIC; FP vs FP-DW PIC, HV vs HV-DW PIC)*

*Table 29 - N concentration (g kg$^{-1}$) in aboveground dry matter of legumes under sole- (PB, FP, HV) and inter-cropping (PB-DW PIC, FP-DW PIC, HV-DW PIC, PB-DW TIC, FP-DW TIC, HV-DW TIC) in 2010/11*

| Treatment | BBCH 30[†] | BBCH 59[‡] | | BBCH 69[‡] | | BBCH 75[‡] | | BBCH 89[‡] | | |
|---|---|---|---|---|---|---|---|---|---|---|
| | Straw | Straw | Pods | Straw | Pods | Straw | Pods | Straw | Pods | Grain |
| PB | 32.10 ± 0.95 | 16.97 ± 0.67 | 32.87 ± 0.78 | 16.63 ± 2.78 | 30.43 ± 3.75 | 16.03 ± 0.96 a | 31.87 ± 0.85 a | 9.40 ± 0.66 | 16.37 ± 2.12 | 37.23 ± 2.81 |
| PB-DW PIC | 28.97 ± 0.50 | 14.80 ± 1.71 | 31.77 ± 0.45 | 14.37 ± 0.06 | 30.57 ± 0.40 | 12.10 ± 1.59 b | 28.90 ± 0.79 b | 9.43 ± 0.15 | 17.00 ± 1.13 | 38.87 ± 1.62 |
| PB-DW-TIC | 29.60 ± 2.09 | - | - | - | - | - | - | - | - | - |
| *P.05* | *0.15* | *0.22* | *0.26* | *0.71* | *0.95* | *0.03* | *0.01* | *0.94* | *0.68* | *0.47* |
| FP | 21.17 ± 2.11 | 8.87 ± 0.93 | 22.20 ± 1.91 | 8.13 ± 0.68 | 22.67 ± 2.16 | 7.53 ± 1.80 | 22.67 ± 0.81 a | 7.63 ± 0.83 | 9.50 ± 3.65 | 31.80 ± 0.00 a |
| FP-DW PIC | 22.93 ± 2.68 | 8.23 ± 1.27 | 21.30 ± 2.70 | 7.37 ± 0.95 | 21.30 ± 2.21 | 6.83 ± 1.62 | 17.30 ± 0.95 b | 8.00 ± 0.56 | 9.40 ± 0.70 | 25.50 ± 1.59 b |
| FP-DW-TIC | 23.83 ± 1.50 | - | - | - | - | - | - | - | - | - |
| *P.05* | *0.50* | *0.67* | *0.74* | *0.41* | *0.64* | *0.65* | *0.03* | *0.68* | *0.96* | *0.02* |
| HV | 30.50 ± 4.45 | 23.63 ± 0.12 | 35.77 ± 0.59 | 18.03 ± 0.12 | 29.50 ± 0.69 | 11.07 ± 3.42 | 31.97 ± 2.80 | 11.60 ± 1.57 | 8.07 ± 1.16 | 43.27 ± 0.59 |
| HV-DW PIC | 31.90 ± 1.38 | 22.50 ± 1.28 | 35.10 ± 0.85 | 17.70 ± 1.32 | 29.70 ± 0.17 | 10.43 ± 2.15 | 29.57 ± 2.40 | 12.43 ± 0.21 | 8.23 ± 2.25 | 43.40 ± 3.03 |
| HV-DW-TIC | 30.97 ± 0.42 | - | - | - | - | - | - | - | - | - |
| *P.05* | *0.81* | *0.23* | *0.37* | *0.29* | *0.63* | *0.48* | *0.49* | *0.43* | *0.93* | *0.95* |

[†] *Treatments analyzed under one-way ANOVA (Fisher's protected LSD test)*

[‡] *Paired comparison t-test performed with treatments concerning the same legume species (i.e. PB vs PB-DW PIC; FP vs FP-DW PIC, HV vs HV-DW PIC)*

*Table 30 - N accumulation (g m$^{-2}$) in aboveground dry matter of legumes under sole- (PB, FP, HV) and inter-cropping (PB-DW PIC, FP-DW PIC, HV-DW PIC, PB-DW TIC, FP-DW TIC, HV-DW TIC) in 2009/10*

| **Treatment** | **BBCH 30†** | | **BBCH 59‡** | | | **BBCH 69‡** | | | **BBCH 75‡** | | | **BBCH 89‡** | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | **Straw** | **Total** | **Straw** | **Pods** | **Total** | **Straw** | **Pods** | **Total** | **Straw** | **Pods** | **Total** | **Straw** | **Pods** | **Grain** | **Total** |
| PB | 6.84 ± 1.38 a | 6.84 ± 1.38 a | 10.63 ± 1.30 a | 1.02 ± 3.07 | 11.65 ± 1.28 | 9.59 ± 1.62 | 4.88 ± 1.56 | 14.47 ± 2.93 a | 5.05 ± 1.10 | 18.89 ± 4.73 a | 23.94 ± 5.54 | 4.43 ± 0.29 a | 2.10 ± 0.11 a | 19.48 ± 3.20 | 26.01 ± 3.50 a |
| PB-DW PIC | 4.01 ± 0.56 b | 4.01 ± 0.56 b | 8.10 ± 1.50 b | 0.98 ± 1.04 | 9.08 ± 2.44 | 6.90 ± 0.34 | 2.98 ± 1.90 | 9.88 ± 2.23 b | 5.97 ± 2.39 | 13.05 ± 3.85 b | 19.02 ± 2.15 | 3.36 ± 0.55 b | 1.71 ± 0.23 b | 17.32 ± 3.11 | 22.39 ± 3.71 b |
| PB-DW-TIC | 3.75 ± 0.43 b | 3.75 ± 0.43 b | - | - | - | - | - | - | - | - | - | - | - | - | - |
| *$P_{.05}$* | *0.01* | *0.01* | *0.00* | *0.87* | *0.07* | *0.07* | *0.10* | *0.02* | *0.81* | *0.01* | *0.13* | *0.02* | *0.03* | *0.07* | *0.05* |
| FP | 2.14 ± 2.20 | 2.14 ± 2.20 | 2.17 ± 2.00 | 3.02 ± 3.06 | 5.19 ± 4.74 | 1.79 ± 1.46 | 4.80 ± 5.40 | 6.59 ± 6.81 | 2.24 ± 1.84 | 7.24 ± 7.29 | 9.48 ± 9.08 | 2.33 ± 1.90 | 2.63 ± 2.87 | 4.78 ± 4.02 | 9.74 ± 8.50 |
| FP-DW PIC | 0.61 ± 0.40 | 0.61 ± 0.40 | 0.56 ± 0.25 | 1.57 ± 1.21 | 2.13 ± 1.45 | 0.47 ± 0.19 | 1.85 ± 1.17 | 2.32 ± 1.36 | 0.83 ± 0.19 | 2.89 ± 1.57 | 3.72 ± 1.73 | 0.94 ± 0.12 | 0.37 ± 0.16 | 3.59 ± 1.60 | 4.90 ± 1.85 |
| FP-DW-TIC | 1.14 ± 1.02 | 1.14 ± 1.02 | - | - | - | - | - | - | - | - | - | - | - | - | - |
| *$P_{.05}$* | *0.18* | *0.18* | *0.20* | *0.31* | *0.21* | *0.21* | *0.35* | *0.30* | *0.26* | *0.28* | *0.27* | *0.32* | *0.27* | *0.42* | *0.31* |
| HV | 5.24 ± 1.18 a | 5.24 ± 1.18 a | 6.83 ± 1.67 | 0.00 | 6.83 ± 1.67 | 7.54 ± 1.61 | 0.00 | 7.54 ± 1.61 | 6.17 ± 1.30 | 1.59 ± 0.24 | 7.76 ± 1.53 | 7.79 ± 1.79 | 0.30 ± 3.20 | 1.03 ± 0.09 | 9.12 ± 1.89 |
| HV-DW PIC | 2.97 ± 0.18 b | 2.97 ± 0.18 b | 3.43 ± 0.74 | 0.00 | 3.43 ± 0.74 | 3.61 ± 0.86 | 0.00 | 3.61 ± 0.86 | 3.21 ± 1.05 | 0.55 ± 0.35 | 3.76 ± 1.39 | 3.83 ± 0.74 | 0.21 ± 3.11 | 0.65 ± 0.52 | 4.69 ± 1.47 |
| HV-DW-TIC | 3.00 ± 0.38 b | 3.00 ± 0.38 b | - | - | - | - | - | - | - | - | - | - | - | - | - |
| *$P_{.05}$* | *0.05* | *0.05* | *0.13* | *-* | *0.13* | *0.11* | *-* | *0.11* | *0.16* | *0.09* | *0.14* | *0.11* | *0.63* | *0.36* | *0.15* |

*† Treatments analyzed under one-way ANOVA (Fisher's protected LSD test)*

*‡ Paired comparison t-test performed with treatments concerning the same legume species (i.e. PB vs PB-DW PIC; FP vs FP-DW PIC, HV vs HV-DW PIC)*

*Table 31 - N accumulation (g $m^{-2}$) in aboveground dry matter of legumes under sole- (PB, FP, HV) and inter-cropping (PB-DW PIC, FP-DW PIC, HV-DW PIC, PB-DW TIC, FP-DW TIC, HV-DW TIC) in 2010/11*

| Treatment | BBCH 30† | | BBCH 59‡ | | | BBCH 69‡ | | | BBCH 75‡ | | | BBCH 89‡ | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | **Straw** | **Total** | **Straw** | **Pods** | **Total** | **Straw** | **Pods** | **Total** | **Straw** | **Pods** | **Total** | **Straw** | **Pods** | **Grain** | **Total** |
| PB | 6.10 ± 0.34 | 6.10 ± 0.34 | 4.72 ± 0.31 a | 1.72 ± 0.80 | 6.44 ± 1.09 | 3.45 ± 0.80 | 3.92 ± 0.35 a | 7.37 ± 1.06 | 3.21 ± 1.06 | 4.53 ± 0.47 | 7.74 ± 1.52 | 1.75 ± 0.34 | 0.94 ± 0.39 | 5.37 ± 1.76 | 8.06 ± 2.48 |
| PB-DW PIC | 5.18 ± 0.64 | 5.18 ± 0.64 | 4.03 ± 0.20 b | 1.86 ± 0.77 | 5.89 ± 0.82 | 3.55 ± 0.24 | 2.86 ± 0.75 b | 6.41 ± 0.50 | 2.63 ± 0.73 | 3.87 ± 1.30 | 6.50 ± 0.62 | 1.94 ± 0.29 | 0.96 ± 0.28 | 3.87 ± 1.41 | 6.77 ± 1.44 |
| PB-DW-TIC | 5.45 ± 2.05 | 5.45 ± 2.05 | - | - | - | - | - | - | - | - | - | - | - | - | - |
| $P_{.05}$ | *0.69* | *0.69* | *0.01* | *0.85* | *0.55* | *0.82* | *0.05* | *0.19* | *0.28* | *0.56* | *0.36* | *0.10* | *0.98* | *0.45* | *0.59* |
| FP | 1.96 ± 0.93 | 1.96 ± 0.93 | 0.99 ± 0.40 | 1.24 ± 0.89 | 2.23 ± 1.28 | 0.82 ± 0.19 | 1.75 ± 1.17 | 2.57 ± 1.36 | 0.65 ± 0.09 | 2.44 ± 1.32 | 3.09 ± 1.37 | 0.57 ± 0.19 | 0.69 ± 0.08 | 1.99 ± 1.04 | 3.24 ± 1.14 |
| FP-DW PIC | 1.76 ± 1.34 | 1.76 ± 1.34 | 0.75 ± 0.41 | 1.26 ± 0.73 | 2.01 ± 1.14 | 0.65 ± 0.32 | 1.82 ± 0.74 | 2.47 ± 1.05 | 0.48 ± 0.19 | 2.05 ± 0.83 | 2.53 ± 1.03 | 0.49 ± 0.24 | 0.85 ± 0.23 | 1.16 ± 0.53 | 2.50 ± 1.00 |
| FP-DW-TIC | 1.65 ± 0.62 | 1.65 ± 0.62 | - | - | - | - | - | - | - | - | - | - | - | - | - |
| $P_{.05}$ | *0.77* | *0.77* | *0.45* | *1.00* | *0.81* | *0.51* | *0.96* | *0.90* | *0.38* | *0.71* | *0.63* | *0.67* | *0.20* | *0.52* | *0.08* |
| HV | 1.86 ± 0.14 | 1.86 ± 0.14 | 3.83 ± 0.34 | 1.68 ± 0.99 | 5.51 ± 1.33 | 2.21 ± 0.24 | 3.40 ± 1.16 | 5.61 ± 1.39 | 1.21 ± 0.54 | 4.47 ± 0.95 | 5.68 ± 1.43 | 1.07 ± 0.29 | 0.44 ± 0.11 | 4.59 ± 1.10 | 6.10 ± 1.45 |
| HV-DW PIC | 1.70 ± 0.74 | 1.70 ± 0.74 | 2.80 ± 0.66 | 0.85 ± 0.24 | 3.65 ± 0.82 | 1.57 ± 0.15 | 2.32 ± 0.95 | 3.89 ± 1.06 | 0.75 ± 0.04 | 3.40 ± 1.30 | 4.15 ± 1.27 | 0.84 ± 0.16 | 0.33 ± 0.03 | 3.63 ± 1.33 | 4.80 ± 1.53 |
| HV-DW-TIC | 1.61 ± 0.91 | 1.61 ± 0.91 | - | - | - | - | - | - | - | - | - | - | - | - | - |
| $P_{.05}$ | *0.90* | *0.90* | *0.22* | *0.34* | *0.27* | *0.10* | *0.45* | *0.34* | *0.23* | *0.27* | *0.40* | *0.44* | *0.29* | *0.53* | *0.50* |

*† Treatments analyzed under one-way ANOVA (Fisher's protected LSD test)*

*‡ Paired comparison t-test performed with treatments concerning the same legume species (i.e. PB vs PB-DW PIC; FP vs FP-DW PIC, HV vs HV-DW PIC)*

In both years N concentration in legume biomass was never significantly affected by intercropping (Tables 28 and 29).

Data on N accumulated in the different components of legume plants are shown in Tables 30 (2009/10) and 31 (2010/11).

In the first year, N accumulated in total biomass at final harvest showed a significant negative effect of intercropping only for pigeon bean, for which a 14% lower value under intercropping than sole crop was observed (Table 30). For pea (-50%) and vetch (-48%), the decrease in N accumulation was even higher than for bean, but with high variability. For pigeon bean, significant decrements in N accumulation were also observed at BBCH 30 and 69, whereas for vetch only at BBCH 30. Therefore, it was confirmed the trend described for dry matter production, with an early competition between component crops under permanent intercropping right from the beginning of their cycles.

Under temporary intercropping, legumes accumulated before incorporation into the soil (BBCH 30) 3.75, 1.14, and 3.00 g N $m^{-2}$, respectively in pigeon bean, field pea and hairy vetch.

In the second year, there were no significant differences in N accumulations averaged over all treatments and sampling dates (Table 31). Still, there was a clear decrease in total N accumulation in dry biomass of legumes, which at harvest accounted for -16%, -12% and -21%, respectively, for pigeon bean, field pea and hairy vetch. Compared to the first year, the decrease of N accumulation was clearly similar for pigeon bean, and appreciably much lower for hairy vetch and field pea.

N accumulations in legumes grown under temporary intercropping before termination (BBCH 30) were 5.45, 1.65, and 1.61, respectively for pigeon bean, field pea and hairy vetch.

It is clear that the completely different weather conditions and the consequently different crop technique characterizing the two experimental years produced data only partially consistent with each other. Anyway, a clear distinction between the most vigorous legume species, i.e. pigeon bean, and the other ones can be made.

Despite the strong deleterious effect on the biomass production of the companion wheat crop, pigeon bean grown under permanent intercropping suffered itself from the presence of the cereal for all the duration of the growing cycle. Significant decrements in biomass production were observed only in the first year, because of the longer duration of the co-growth period than in year 2. Decrease in N accumulated in aboveground biomass were less evident, but still strongly correlated with dry matter production, rather than N concentration in plant tissues. Therefore, the supposed compensation of N accumulation through the increased level of N concentration in plant tissues did not take place.

The main reason behind this difference between bean and the other legumes may be the different architecture of the plants, with bean growing more in vertical, and other species more prostrate. Furthermore, the choice of the specific cultivar of pigeon bean grown in the experiment, namely *cv. Torre Lama Scuro*, amplified even more this trait of the species. This variety actually is well known to produce luxuriant vegetation, with lot of ramifications, and hence it is able to produce huge biomass and supply large amount of N even in short time. Under intercropping, the limited space available for pigeon bean might have reduced from the beginning the space available for its growth. On the other hand, the quick vertical growth of this bean variety might have disturbed the interception of solar radiation in wheat plants from early stages. Obviously, this reciprocal competition was of higher importance in the first year, when the two crops grew together for a longer time, and pigeon bean was more luxuriant than in the second year.

The findings of Benincasa et al. (2012) fully support our interpretation of the results. Besides the competition for space and radiation, the same authors also hypothesized the occurrence of a strong root competition for mineral N in the soil at very early stages of plantlet growth. The two component crops have different competitive ability for soil N, because of their contrasting root architecture, with the taproot of pigeon bean more located in upper soil, and the fasciculate root system of wheat more distributed along soil profile. Anyway, with scarcity of N in the soil, like in our conditions, the specific ability of wheat of taking up N from the soil becomes even more important than distribution of root system in the soil (Bedoussac and Justes, 2009), giving a strong advantage to wheat compared to the legume. Thus, it can be supposed that also the competition for soil mineral N could have affected negatively the growth of pigeon bean under intercropping. Still, on the other hand our data revealed that N concentration in pigeon bean under intercropping was not significantly reduced compare to sole bean. Therefore, pigeon bean might have compensated the reduced availability of mineral N by increasing the rate of symbiotic $N_2$-fixation. This hypothesis will be tested in the following paragraph.

Data on measurements of $\delta^{15}N$ of wheat and legumes under sole and inter-cropping, as well as calculations of N derived from the atmosphere (Ndfa), N derived from the soil and N transferred from legume to companion wheat (N transfer) are reported in Tables 32 to 35. Tables 32 and 33 show data collected in the first year, respectively at stem elongation (BBCH 30) and at harvest (BBCH 89), whereas Tables 34 and 35 refer to data collected in the second year, respectively at stem elongation (BBCH 30) and at harvest (BBCH 89).

In both years at stem elongation (Tables 32 and 34), the analysis of $^{15}N$ natural abundance resulted in variable values of δ for wheat and also for legumes. Wheat showed higher δ values in year 1 than in year 2. In 2009/10, delta values of wheat under either temporary or permanent intercropping were much higher than the reference wheat sole crop (N0) (18.21, averaged over all intercropping, vs. 7.92 of N0). This evidence was not observed in the second year, when the delta ranged 2.42 (HV-DW PIC) and 8.74 (FP-DW PIC).

The estimated percentage of N derived from the atmosphere (Ndfa%) in legumes was not significantly affected by treatments in 2009/10, while, conversely, it was in 2010/11, when sole pea and pea under temporary intercropping with wheat resulted in values inferior to all the remaining treatments (Table 34).

On average, the estimated level of symbiotic $N_2$-fixation was 87.1% in the first year, and 84.4% in the second one, values absolutely plausible if related to the low availability of soil N hypothesized at the end of winter in our conditions. Pigeon bean resulted in the highest mean values in the two years (90.61% and 91.70, respectively in 2009/10 and 2010/11), pea had the lowest level of $N_2$-fixation (84.73% in year 1, 76.00% in year 2), vetch was in between (85.96% in year 1, 85.56% in year 2).

Noteworthy for each treatment the same trend was observed in the two years, although without statistical significance. Pigeon bean showed higher level of $N_2$-fixation under sole crop than intercropping,.hairy vetch exactly the opposite, and field pea resulted unaffected by treatment.

As a result of the combination of Ndfa% and dry matter production, the amount of N derived from atmosphere in legumes (Ndfa) resulted in significant differences in both years, with treatments including pigeon bean overyielding all the others (Tables 32 and 34).

N transfer from legumes to wheat under intercropping did not occur at stem elongation in any of the experiments.

*Table 32 - δ $^{15}$N, N derived from the atmosphere (Ndfa) and from the soil (Ndfs) in legumes, and transfer of N from legumes to the wheat companion crop under intercropping at* ***BBCH 30*** *of wheat in 2009/10. $N_{leg}$ and $N_{wheat}$ are, respectively, N accumulation in total aboveground dry matter of legumes and wheat.*

| Treatment | δ $^{15}$N values | | | Ndfa (%) | $N_{leg}$ (g N m$^{-2}$) | Ndfa (g N m$^{-2}$) | Ndfs (g N m$^{-2}$) | N transfer (%) | $N_{wheat}$ (g N m$^{-2}$) | N transfer (g N m$^{-2}$) |
|---|---|---|---|---|---|---|---|---|---|---|
| | Legume | Wheat | Reference | | | | | | | |
| N0 | - | 7.92 | 7.92 | - | - | - | - | - | 1.02 | - |
| PB-DW TIC | 0.44 | 21.33 | 7.92 | 88.84 | 3.75 | 3.33 bc | 0.42 | -169.32 | 1.06 | -1.79 |
| FP-DW TIC | 0.83 | 17.28 | 7.92 | 82.63 | 1.14 | 0.94 c | 0.20 | -118.18 | 1.34 | -1.58 |
| HV-DW TIC | 0.62 | 18.98 | 7.92 | 84.69 | 3.00 | 2.54 b | 0.46 | -139.65 | 1.72 | -2.40 |
| PB-DW PIC | 0.42 | 17.11 | 7.92 | 89.07 | 4.01 | 3.57 b | 0.44 | -116.04 | 0.99 | -1.15 |
| FP-DW PIC | 0.51 | 17.72 | 7.92 | 86.36 | 0.61 | 0.53 c | 0.08 | -123.74 | 1.32 | -1.63 |
| HV-DW PIC | 0.12 | 16.85 | 7.92 | 90.49 | 2.97 | 2.69 b | 0.28 | -112.75 | 1.58 | -1.78 |
| PB | 0.01 | - | 7.92 | 93.94 | 6.84 | 6.43 a | 0.41 | - | - | - |
| FP | 0.61 | - | 7.92 | 85.20 | 2.14 | 1.82 b | 0.32 | - | - | - |
| HV | 0.79 | - | 7.92 | 82.71 | 5.24 | 4.33 ab | 0.91 | - | - | - |
| *Significance[1]* | *ns* | *ns* | - | *ns* | - | * | *ns* | *ns* | - | *ns* |
| *LSD* | *1.06* | *8.53* | - | *31.80* | - | *2.47* | *1.47* | *217.90* | - | *1.20* |
| *CV (%)[2]* | *127.3* | *28.6* | - | *23.8* | - | *56.6* | *1.06* | *28.9* | - | *341.1* |

*[1] **, *, ns are, respectively, significant for $p \leq .01$, significant for $p \leq .05$ and not significant (LSD test)*

*[2] Coefficient of variation*

*[3] B-values: -0.5, -0.66 and -0.7 for pigeon bean, field pea and hairy vetch, respectively (Unkovich et al., 2008)*

*Table 33 - δ $^{15}$N, N derived from the atmosphere (Ndfa) and from the soil (Ndfs) in legumes, and transfer of N from legumes to the wheat companion crop under intercropping at **BBCH 89** of wheat in 2009/10. $N_{leg}$ and $N_{wheat}$ are, respectively, N accumulation in total aboveground dry matter of legumes and wheat.*

| **Treatment** | **δ $^{15}$N values** | | | **Ndfa (%)** | **$N_{leg}$ (g N m$^{-2}$)** | **Ndfa (g N m$^{-2}$)** | **Ndfs (g N m$^{-2}$)** | **N transfer (%)** | **$N_{wheat}$ (g N m$^{-2}$)** | **N transfer (g N m$^{-2}$)** |
|---|---|---|---|---|---|---|---|---|---|---|
| | **Legume** | **Wheat** | **Reference** | | | | | | | |
| N0 | - | 9.22 b | 9.22 | - | - | - | - | - | - | - |
| PB-DW TIC | - | 7.94 b | 9.22 | - | - | - | - | 13.83 a | 5.75 | 0.80 |
| FP-DW TIC | - | 9.87 b | 9.22 | - | - | - | - | -7.02 a | 4.25 | -0.30 |
| HV-DW TIC | - | 8.21 b | 9.22 | - | - | - | - | 10.93 a | 5.74 | 0.63 |
| PB-DW PIC | 0.50 abc | 16.78 a | 9.22 | 89.76 ab | 22.39 | 20.10 a | 2.29 ab | -81.98 c | 1.08 | -0.89 |
| FP-DW PIC | -0.07 c | 11.94 b | 9.22 | 94.07 a | 4.90 | 4.61 b | 0.31 c | -29.55 b | 3.78 | -1.12 |
| HV-DW PIC | -0.07 c | 8.27 b | 9.22 | 93.65 ab | 4.69 | 4.39 b | 0.30 c | 10.34 a | 4.51 | 0.47 |
| PB | 0.58 ab | - | 9.22 | 88.90 ab | 26.01 | 23.10 a | 2.91 a | - | - | - |
| FP | 0.14 bc | - | 9.22 | 91.90 ab | 9.74 | 8.95 b | 0.79 b | - | - | - |
| HV | 0.97 a | - | 9.22 | 83.13 b | 9.12 | 7.58 b | 1.54 abc | - | - | - |
| *Significance[1]* | * | * | - | * | - | ** | ** | ** | - | *ns* |
| *LSD* | *1.04* | *4.49* | - | *10.56* | - | *8.95* | *1.83* | *38.82* | - | *1.81* |
| *CV (%)[2]* | *81.2* | *24.5* | - | *3.5* | - | *30.0* | *41.7* | *127.7* | - | - |

[1] **, *, ns are, respectively, significant for $p \leq .01$, significant for $p \leq .05$ and not significant (LSD test)

[2] Coefficient of variation

[3] B-values: -0.5, -0.66 and -0.7 for pigeon bean, field pea and hairy vetch, respectively (Unkovich et al., 2008)

*Table 34 - δ $^{15}$N, N derived from the atmosphere (Ndfa) and from the soil (Ndfs) in legumes, and transfer of N from legumes to the wheat companion crop under intercropping at **BBCH 30** of wheat in 2010/11. $N_{leg}$ and $N_{wheat}$ are, respectively, N accumulation in total aboveground dry matter of legumes and wheat.*

| **Treatment** | **δ $^{15}$N values** | | | **Ndfa (%)** | **$N_{leg}$ (g N m$^{-2}$)** | **Ndfa (g N m$^{-2}$)** | **Ndfs (g N m$^{-2}$)** | **N transfer (%)** | **$N_{wheat}$ (g N m$^{-2}$)** | **N transfer (g N m$^{-2}$)** |
|---|---|---|---|---|---|---|---|---|---|---|
| | **Legume** | **Wheat** | **Reference** | | | | | | | |
| N0 | - | 6.41 | 6.41 | - | - | - | - | - | 0.89 | - |
| PB-DW TIC | 0.14 ab | 8.31 | 6.41 | 90.74 a | 5.45 | 4.95 a | 0.50 | -29.64 | 0.90 | -0.27 |
| FP-DW TIC | 1.26 a | 7.82 | 6.41 | 72.84 b | 1.65 | 1.20 b | 0.45 | -22.00 | 1.01 | -0.22 |
| HV-DW TIC | 0.28 ab | 8.30 | 6.41 | 86.22 a | 1.61 | 1.39 b | 0.22 | -29.49 | 0.83 | -0.25 |
| PB-DW PIC | 0.17 ab | 8.68 | 6.41 | 90.30 a | 5.18 | 4.68 a | 0.50 | -35.41 | 0.95 | -0.34 |
| FP-DW PIC | 0.83 ab | 8.74 | 6.41 | 78.93 a | 1.76 | 1.39 b | 0.37 | -36.35 | 0.90 | -0.33 |
| HV-DW PIC | 0.23 ab | 2.42 | 6.41 | 86.92 a | 1.70 | 1.48 b | 0.22 | 62.25 | 0.93 | 0.58 |
| PB | -0.09 b | - | 6.41 | 94.07 a | 6.10 | 5.74 a | 0.36 | - | - | - |
| FP | 1.02 ab | - | 6.41 | 76.24 b | 1.96 | 1.49 b | 0.47 | - | - | - |
| HV | 0.47 ab | - | 6.41 | 83.54 a | 1.86 | 1.55 b | 0.31 | - | - | - |
| *Significance*[1] | * | *ns* | - | ** | - | ** | *ns* | *ns* | - | *ns* |
| *LSD* | *1.22* | *7.14* | - | *17.72* | - | *2.19* | *0.41* | *115.66* | - | *12.02* |
| *CV (%)*[2] | *83.0* | *31.7* | - | *6.9* | - | *56.6* | *33.7* | *275.9* | - | *341.1* |

*[1] **, *, ns are, respectively, significant for $p \leq .01$, significant for $p \leq .05$ and not significant (LSD test)*

*[2] Coefficient of variation*

*[3] B-values: -0.5, -0.66 and -0.7 for pigeon bean, field pea and hairy vetch, respectively (Unkovich et al., 2008)*

*Table 35 - δ $^{15}$N, N derived from the atmosphere (Ndfa) and from the soil (Ndfs) in legumes, and transfer of N from legumes to the wheat companion crop under intercropping at **BBCH 89** of wheat in 2010/11. $N_{leg}$ and $N_{wheat}$ are, respectively, N accumulation in total aboveground dry matter of legumes and wheat.*

| **Treatment** | **δ $^{15}$N values** | | | **Ndfa (%)** | **$N_{leg}$ (g N m$^{-2}$)** | **Ndfa (g N m$^{-2}$)** | **Ndfs (g N m$^{-2}$)** | **N transfer (%)** | **$N_{wheat}$ (g N m$^{-2}$)** | **N transfer (g N m$^{-2}$)** |
|---|---|---|---|---|---|---|---|---|---|---|
| | **Legume** | **Wheat** | **Reference** | | | | | | | |
| N0 | - | 4.65 | 4.65 | - | - | | | - | - | |
| PB-DW TIC | - | 4.47 | 4.65 | - | - | | | 3.92 | 5.58 | 0.22 |
| FP-DW TIC | - | 5.89 | 4.65 | - | - | | | -26.72 | 5.76 | -1.54 |
| HV-DW TIC | - | 5.09 | 4.65 | - | - | | | -9.44 | 6.01 | -0.57 |
| PB-DW PIC | 0.14 c | 5.31 | 4.65 | 87.61 ab | 6.77 | 5.93 a | 0.84 | -14.22 | 3.12 | -0.44 |
| FP-DW PIC | 0.94 ab | 5.62 | 4.65 | 69.81 b | 2.50 | 1.75 b | 0.75 | -20.87 | 3.85 | -0.80 |
| HV-DW PIC | -0.39 d | 4.94 | 4.65 | 94.30 a | 4.80 | 4.53 a | 0.27 | -6.18 | 3.73 | -0.23 |
| PB | 0.78 b | - | 4.65 | 75.15 b | 8.06 | 6.06 a | 2.00 | - | - | - |
| FP | 1.13 a | - | 4.65 | 66.24 b | 3.24 | 2.15 b | 1.09 | - | - | - |
| HV | -0.10 cd | - | 4.65 | 88.73 ab | 6.10 | 5.41 a | 0.69 | - | - | - |
| *Significance*[1] | ** | *ns* | - | ** | - | ** | *ns* | *ns* | - | *ns* |
| *LSD* | *0.31* | *2.72* | - | *16.82* | - | *1.96* | *1.71* | *84.89* | - | *2.82* |
| *CV (%)*[2] | *41.2* | *29.8* | - | *11.9* | - | *26.1* | *85.4* | *133.1* | - | *123.4* |

[1] **, *, ns are, respectively, significant for p≤.01, significant for p≤.05 and not significant (LSD test)*

[2] *Coefficient of variation*

[3] *B-values: -0.5, -0.66 and -0.7 for pigeon bean, field pea and hairy vetch, respectively (Unkovich et al., 2008)*

At final harvest (BBCH 89), the values of δ of legumes were significantly affected by treatments in both years (Tables 33 and 35). In the first one (Table 33), the δ value of sole vetch was statistically higher than other legumes, except for bean sole crop and permanent intercropping. Negative values were recorded for the biomass of field pea and hairy vetch under intercropping. In the second year, sole bean was superior to other treatments, except for FP-DW PIC (Table 35). Hairy vetch, both in the mixture and as sole crop, showed negative values.

The $^{15}$N isotope dilution of wheat aerial biomass collected in the different intercropping was affected by treatments only in the first year (Table 33), when the highest value was found for wheat intercropped with pigeon bean (16.78). Averaged over all treatments, delta values of wheat grown under intercropping tended to decrease from early sampling to harvest maturity, whereas the reference crop (N0) did it in the same way only in the second year.

Ndfa% was significantly affected by treatments in both years. In the first one, the highest value was assessed for field pea under intercropping (94.07%), and the lowest for sole vetch (83.13%) (Table 33). In the second year, the highest importance of symbiotic dinitrogen fixation was observed for hairy vetch under intercropping (94.30%), and the lowest for field pea sole crop (66.24%) (Table 35).

Ndfa% did not show a clear trend between stem elongation and harvest, as its values differed depending on years and treatments. Anyway, a reduction in the Ndfa% at harvest in 2010/11 was found in comparison to 2009/10. Only for hairy vetch, intercropped or not, an increase in the contribution of $N_2$-fixation to total N concentration was observed.

Differently from earlier stage, at harvest all the legumes showed higher values of Ndfa % under intercropping than as sole crops, although without the support of statistics.

Pigeon bean, either the treatment, was significantly superior to the other legumes in terms of the weight of N derived from fixation (Ndfa) calculated at harvest in both years (Tables 33 and 35). Anyway, in the second year also hairy vetch showed similar results (Table 35).

A significant contribution of N transferred from legumes to cereal was demonstrated only in 2009/10 (Table 33), when 10% and 14% of total N accumulated by wheat intercropped, respectively, with hairy vetch (all treatments), and pigeon bean (only under temporary intercropping), came from $N_2$-fixation.

Overall, the application of the $^{15}$N natural abundance technique led to acceptable estimation of the Ndfa% of legumes, either the sampling date. On average, the level of symbiotic $N_2$-fixation of legumes was high (around 90% in 2009/10, 80% in 2010/11), suggesting that a deficiency in soil mineral N actually occurred in both years, and also that a good natural population of the specific *Rhizobium* spp. was present in the experimental fields. Similar ranges of values were also reported by other previous studies including field pea (Jensen, 1996; Hauggaard-Nielsen et al., 2001b, 2003; Andersen et al., 2005; Ghaley et al., 2005; Hauggaard-Nielsen et al., 2006; Hauggaard-Nielsen et al., 2008; Bedoussac and Justes, 2009; Hauggaard-Nielsen et al., 2009a; Naudin et al., 2010), faba bean (Fan et al., 2006) and vetch (Kurdali et al., 1996).

Among legumes, only field pea showed a different level of dinitrogen fixation in the two years, producing the highest value in the first one (93%, on average at harvest), and the lowest in the second (68%, on average at harvest). This latter was perfectly in line with that estimated by Hauggaard-Nielsen et al. (2009a) for spring-sown field pea grown in South Italy, under dry conditions (66-73%). The poorest establishment of the crop in 2010/11, in addition to the water stress experienced in spring, may be the two possible main reasons behind this finding.

Although not significantly, our results clearly showed that intercropping increased the final Ndfa%, whatever the legume. This result is consistent with those of most part of literature (Brophy et al., 1987; Jensen, 1996; Kurdali et al., 1996; Hauggaard-Nielsen et al., 2001b, 2003; Chu et al., 2004; Andersen et al., 2005; Ghaley et al., 2005; Fan et al., 2006; Hauggaard-Nielsen et al., 2006; Hauggaard-Nielsen et al., 2008; Bedoussac and Justes, 2009; Hauggaard-Nielsen et al., 2009a; Naudin et al., 2010). According to Fujita et al. (1992), the main reason behind this phenomenon is that, under conditions of co-growth with cereals, legumes usually experience soil inorganic N deficiency, due to the higher affinity of the cereals for the element, and hence this increase the rate of $N_2$-fixation. At early development, this affinity mainly relies on the architecture of root system, with the cereals characterized by long and wide fasciculated roots, able to take up N also relatively deep in the soil. Additionally, at late stages (from stem elongation onwards), cereals have also a higher demand for the element compared to legumes (Corre-Hellou et al., 2006; Corre-Hellou et al., 2007).

Still, not only the rate of $N_2$-fixation is relevant in determining the total amount of N fixed by legumes per area unit (Ndfa). Indeed, the contribution of dry matter production of legumes is more important than the percentage of Ndfa, as for instance explained by Fan et al. (2006). Our data are in agreement with this statement, as also in our experiment the highest Ndfa values were found in the treatments most productive in terms of aerial biomass.

Naudin et al. (2010) discussed this issue in the light of the elucidation of the effects of fertilization with inorganic N on the performances of intercropped legumes. In their experiments on pea-wheat intercropping, the application of mineral N fertilizers actually reduced the total amount of N fixed from the atmosphere (Ndfa) of legumes. Still, this detriment was not due to a reduction of the $N_2$-fixation rate, but rather to a decrease of the total dry matter produced by the intercropped pea. Pea growth was limited by the increased growth of the wheat caused by fertilization, which led to shading and high competition for solar radiation, water and other resources. The consequent reduction in photosynthates supply to the nodules, necessary to fuel their formation and growth, was then the responsible of the reduction in the $N_2$-fixation rate (Fujita et al., 1992). Given these things, the authors concluded that fertilization with inorganic N was not detrimental for symbiotic dinitrogen fixation *per se*.

Concerning the potential benefits for wheat intercropped with $N_2$-fixing legumes, our results did not fully support that a direct transfer of fixed N from legume to wheat occurred. Only in rare cases, a 4-13% transfer was observed in temporary intercropping with bean or vetch at harvest, whereas estimations performed at early stages were inconsistent. Technically, this result was mainly due to the high values of $\delta^{15}N$ measured in wheat under intercropping in both years, leading to the conclusion that for intercropped wheat, whatever the intercropping strategy, N derived from the soil was more important than N from atmosphere. In agreement with basic literature on this topic (see for instance Ofori and Stern, 1987), the main reason behind this fact is the high competition for resources suffered from wheat at early stages, under all intercropping treatments. This competition, amplified by the additive design and also by the peculiar spatial arrangement of the intercrops (i.e. alternate rows), may have led to a higher ability of wheat in the taking up of mineral N in the soil. Conversely, intercropped legumes may have been pushed to increase N availability through intensification of $N_2$-fixation rate.

Using a different sole wheat reference crop (e.g. N40, N80, N120, or N160) would not have led to better results, as in both years sole wheat showed values of $\delta^{15}N$ of its biomass steadily decreasing at increasing rates of mineral N fertilization (data not shown). This surprising finding was also obtained by Bedoussac and Justes (2009), who even measured negative $\delta^{15}N$ values for fertilized wheat. A low $^{15}N$:$^{14}N$ ratio for synthetical mineral fertilizers is supposed to be the result of $^{15}N$ dilution occurred during the production processes (Bedoussac and Justes, 2009).

Anyway, consistently with us, many other previous studies did not find significant transfer of N between legumes and cereals intercropped. Brophy et al. (1987) reported a N transfer of only 15-17% of total Ndfa of fodder legumes to the companion grass, and this happened only within 20 cm of row distance, in function of the seeding density of the component crops. Jensen (1996) did not demonstrate a significant transfer of N from pea to barley even though applying a $^{15}$N enrichment technique, which should lead to lower variability in measurements than natural abundance. The same was for a pea-vetch intercropping under dry conditions reported by Kurdali et al. (1996). Mariotti et al. (2012) simply supposed the occurrence of a transfer of N for a forage wheat-pigeon bean intercropping in the same conditions than ours, but without the support of any measurements.

In his key paper, Stern (1993) brought solid reasons for this uncertainty of N-transfer under intercropping, linking it to the huge number of environmental and management variables potentially able to affect the fate of fixed N in the systems.

As previously mentioned in this manuscript (see chapter 3.1.3), Di Miceli et al. (2009) stated that the increase in N content observed in the biomass and grain of wheat under temporary intercropping might be due to other factors than a direct N transfer, such as, for instance, the stimulation of soil organic matter mineralization and the suppression of weeds, both caused by the mechanical incorporation of the legume into the soil.

To complete the picture of the dynamics characterizing the growth of the three legumes under each treatment, in Tables 36 and 37 data on legume plant height measured over the season in the two years of the experiment are shown.

Pigeon bean was clearly the highest legume among the three species, with an upright growth even higher than 1 meter in the first year. In the second one, the mean plant height of pigeon bean was almost halved, due to the shorter season (Table 37). Anyway, over all years, pigeon bean overgrew the companion wheat plants under permanent intercropping at all sampling dates, except for BBCH 14 in year 1. Our results clearly confirmed those of other studies on field beans (Haymes and Lee, 1999; Tosti and Guiducci, 2010; Mariotti et al., 2012), which clearly stated the occurrence of shading early during tillering stage of wheat.

Pea and vetch had a different behavior. Their mean plant height was more constant over years, and lower on average than pigeon bean. Pea plants never exceeded the height of the companion wheat under permanent intercropping in both years, whereas vetch did it only occasionally (i.e. at BBCH 59 and 69) in 2009/10 (Table 36).

Both hairy vetch and field pea showed a maximum peak of plant height around the heading stage of wheat, and then the plant height started to decrease due to lodging. Intercropping did not affect the rate of lodging in any species, as also observed by Bedoussac and Justes (2010) for field pea.

Conversely, intercropping with wheat generally increased the height of legumes (Tables 36 and 37), with differences among species. For pigeon bean, an increase in the mean plant height was observed under intercropping only at the beginning of its growth (BBCH 14 and 30 of wheat), and then the pure crop became comparable or even higher. For pea and vetch this increase held on for longer (until BBCH 59 or 69 of wheat), and then was not still observed due to lodging.

Obviously, this different behavior of legumes under intercropping than sole crop can be explained by the interspecific competition with wheat for resources, occurring early in the season, but also by intra-specific competition for light due to the higher plant density than sole crops.

*Table 36 - Plant height (cm) at canopy level of legumes grown under sole- (PB, FP, HV) or intercropping (PB-DW PIC, PB-DW TIC, FP-DW PIC, FP-DW TIC, HV-DW PIC, HV-DW TIC) in 2009/10. Within each column, data followed by different letters, are significantly different*

| **Treatment** | **BBCH 14†** | **BBCH 30†** | **BBCH 59‡** | **BBCH 69‡** | **BBCH 75‡** | **BBCH 89‡** |
|---|---|---|---|---|---|---|
| PB | 13.83 b | 47.00 | 100.50 | 140.33 a | 143.00 | 143.00 |
| PB-DW PIC | 19.00 a | 55.67 | 107.83 | 135.17 b | 135.67 | 135.67 |
| PB-DW TIC | 17.00 a | 40.00 | - | - | - | - |
| *$P_{.05}$* | *0.03* | *0.24* | *0.28* | *0.02* | *0.49* | *0.49* |
| FP | 7.25 b | 19.50 | 40.00 | 34.67 | 13.08 | 13.08 |
| FP-DW PIC | 9.08 a | 28.33 | 50.50 | 32.50 | 12.83 | 12.83 |
| FP-DW TIC | 7.92 ab | 23.00 | - | - | - | - |
| *$P_{.05}$* | *0.03* | *0.13* | *0.21* | *0.76* | *0.92* | *0.92* |
| HV | 6.50 | 18.67 b | 52.00 b | 54.33 | 22.79 | 22.79 |
| HV-DW PIC | 7.67 | 28.17 a | 65.33 a | 64.67 | 26.25 | 26.25 |
| HV-DW TIC | 7.92 | 31.83 a | - | - | - | - |
| *$P_{.05}$* | *0.31* | *0.00* | *0.01* | *0.29* | *0.39* | *0.39* |

*[1] **, *, ns are, respectively, significant for p≤.01, significant for p≤.05 and not significant (LSD test)*

*[2] Coefficient of variation*

*† Treatments analyzed under one-way ANOVA (Fisher's protected LSD test)*

*‡ Paired comparison t-test performed with treatments concerning the same legume species (i.e. PB vs PB-DW PIC; FP vs FP-DW PIC, HV vs HV-DW PIC)*

*Table 37 - Plant height (cm) at canopy level of legumes grown under sole- (PB, FP, HV) or inter-cropping (PB-DW PIC, PB-DW TIC, FP-DW PIC, FP-DW TIC, HV-DW PIC, HV-DW TIC) in 2010/11. Within each column, data followed by different letters, are significantly different*

| **Treatment** | **BBCH 14†** | **BBCH 30†** | **BBCH 59‡** | **BBCH 69‡** | **BBCH 75‡** | **BBCH 89‡** |
|---|---|---|---|---|---|---|
| PB | 27.33 | 50.67 | 71.67 | 71.67 | 76.83 | 76.83 |
| PB-DW PIC | 32.00 | 49.50 | 73.33 | 77.83 | 75.83 | 75.83 |
| PB-DW TIC | 33.33 | 49.08 | - | - | - | - |
| *$P_{.05}$* | *0.05* | *0.88* | *0.73* | *0.30* | *0.56* | *0.56* |
| FP | 11.00 | 19.67 | 28.67 | 25.00 | 13.83 | 13.83 |
| FP-DW PIC | 12.67 | 18.33 | 22.00 | 25.33 | 13.50 | 13.50 |
| FP-DW TIC | 11.33 | 19.92 | - | - | - | - |
| *$P_{.05}$* | *0.54* | *0.76* | *0.13* | *0.90* | *0.88* | *0.88* |
| HV | 9.00 | 16.33 | 38.00 | 39.67 | 33.17 | 33.17 |
| HV-DW PIC | 9.33 | 18.50 | 40.33 | 36.83 | 35.17 | 35.17 |
| HV-DW TIC | 10.67 | 17.92 | - | - | - | - |
| *$P_{.05}$* | *0.54* | *0.73* | *0.22* | *0.51* | *0.80* | *0.80* |

*[1] **, *, ns are, respectively, significant for p≤.01, significant for p≤.05 and not significant (LSD test)*

*[2] Coefficient of variation*

*† Treatments analyzed under one-way ANOVA (Fisher's protected LSD test)*

*‡ Paired comparison t-test performed with treatments concerning the same legume species (i.e. PB vs PB-DW PIC; FP vs FP-DW PIC, HV vs HV-DW PIC)*

### *3.1.6. Cumulative dry matter production and NP accumulation of crops*

Cumulative total aboveground dry matter production and total N accumulations of intercropping (PB-DW TIC, FP-DW TIC, HV-DW TIC, PB-DW PIC, FP-DW PIC, HV-DW PIC), sole wheat (N0, N40, N80, N120, N160) and sole legumes (PB, FP, HV) for all sampling dates in the two years of the experiment are shown in Tables 38, 39 (dry matter production), and 40, 41 (N accumulation). The respective data on phosphorus accumulated in total biomass of crops are reported in the Appendix 1.

In both years, total aboveground dry matter production of crops was significantly affected by treatments in all sampling dates (Tables 38 and 39).

Permanent intercropping between bean and wheat determined the highest values of total crop biomass at harvest (BBCH 89) in both years. In 2009/10 only pigeon bean sole crop produced a statistically equal dry matter (Table 38), whereas in 2010/11 its value was comparable to those of N80, N120, N160 (Table 39). This evidence suggests that in the second year the importance of wheat as determinant of total crop biomass was higher compared to bean.

The behavior of FP-DW PIC in the two years was different. Whereas in 2009/10 field pea-durum wheat permanent intercropping did not produced significantly higher biomass than sole pea, sole wheat unfertilized (N0) and durum wheat temporarily intercropped with pea (Table 38), in 2010/11 permanent intercropping showed values superior to all its references (Table 39).

For permanent intercropping between wheat and vetch, performances were more stable over years. In year 1, the total biomass produced at harvest by HV-DW PIC was significantly higher only than N0, whilst in year 2 also sole vetch produced lower than intercropping. In both years, no significant differences in terms of total biomass were observed between the two strategies of intercropping.

In agreement with literature (Jensen, 1996; Kurdali et al., 1996; Hauggaard-Nielsen et al., 2001a; Ghaley et al., 2005; Hauggaard-Nielsen et al., 2006; Andersen et al., 2007; Gunes et al., 2007; Bedoussac and Justes, 2009; Hauggaard-Nielsen et al., 2009a; Tosti and Guiducci, 2010; Pelzer et al., 2012),our results clearly show that, averaged over all treatments, permanent intercropping was able to produce more aerial biomass than both legume and wheat sole crops, or at least comparable to the most productive sole crop, with differences depending on species *x* season interactions.

Pigeon bean, field pea and hairy vetch, in this order, represented 76%, 32% and 39% of total dry matter produced at harvest by permanent intercropping in the first year; and 61%, 36% and 38% in 2010/11. Apparently, pea and vetch were less sensitive to the different weather conditions occurred in the two years of the experiment, whilst, on the contrary, pigeon bean growth was strongly decreased in 2010/11.

In both years, the behavior of the different treatments of permanent intercropping was quite stable over sampling dates (Tables 38 and 39).

*Table 38 - Cumulative aboveground dry matter production (g m$^{-2}$) of crops (wheat + legumes) under different treatments in 2009/10. Data expressed as means ± SD (n = 3). Within each column, data followed by different letters are significantly different*

| Treatment | BBCH 30 | BBCH 59 | BBCH 69 | BBCH 75 | BBCH 89 |
|---|---|---|---|---|---|
| N0 | 94.17 ± 19.84 de | 206.69 ± 82.75 c | 272.11 ± 91.75 f | 342.26 ± 120.31 f | 361.48 ± 135.90 g |
| N40 | 176.02 ± 6.28 abc | 342.99 ± 104.31 abc | 559.89 ± 237.88 abcd | 593.96 ± 227.68 cde | 625.68 ± 234.04 def |
| N80 | 192.91 ± 21.81 abc | 413.13 ± 160.10 abc | 647.82 ± 140.54 abc | 723.40 ± 78.01 bcd | 762.80 ± 97.57 cde |
| N120 | 200.54 ± 29.40 abc | 574.44 ± 34.30 a | 678.30 ± 120.28 ab | 785.89 ± 110.54 bc | 821.85 ± 120.61 bcd |
| N160 | 206.77 ± 55.92 abc | 448.01 ± 77.12 abc | 730.22 ± 87.81 a | 852.94 ± 80.50 b | 895.47 ± 92.32 bc |
| PB-DW TIC | 224.33 ± 30.81 ab | 334.94 ± 21.81 abc | 363.83 ± 19.07 def | 515.22 ± 37.93 def | 538.95 ± 44.17 efg |
| FP-DW TIC | 159.66 ± 57.01 bcd | 275.68 ± 48.76 bc | 346.42 ± 75.33 ef | 475.05 ± 83.80 ef | 482.44 ± 82.41 fg |
| HV-DW TIC | 225.05 ± 74.39 ab | 280.61 ± 26.68 bc | 379.82 ± 34.64 def | 525.63 ± 39.95 def | 552.15 ± 37.95 efg |
| PB-DW PIC | 212.66 ± 13.61 abc | 418.14 ± 92.24 abc | 537.93 ± 57.51 abcde | 869.42 ± 172.79 ab | 1052.83 ± 208.61 ab |
| FP-DW PIC | 145.02 ± 34.51 cd | 341.88 ± 108.62 abc | 457.15 ± 151.65 cdef | 533.05 ± 115.43 def | 590.16 ± 121.27 defg |
| HV-DW PIC | 224.47 ± 17.04 ab | 398.89 ± 57.42 abc | 497.05 ± 128.00 bcde | 551.63 ± 156.69 def | 620.40 ± 167.72 def |
| PB | 228.00 ± 42.51 a | 476.00 ± 58.62 ab | 639.00 ± 106.66 abc | 1099.33 ± 216.82 a | 1171.70 ± 190.80 a |
| FP | 68.67 ± 58.14 e | 209.00 ± 176.60 c | 346.66 ± 304.37 ef | 457.34 ± 376.34 ef | 521.11 ± 417.67 efg |
| HV | 156.33 ± 54.54 cd | 258.33 ± 59.18 bc | 291.00 ± 61.88 f | 353.33 ± 71.58 f | 375.14 ± 74.39 fg |
| *Significance[1]* | ** | ** | ** | ** | ** |
| *LSD* | *68.00* | *136.63* | *200.82* | *233.97* | *253.22* |
| *CV (%)[2]* | *22.6* | *22.9* | *24.8* | *22.5* | *22.5* |

*[1] **, *, ns are, respectively, significant for p≤.01, significant for p≤.05 and not significant (LSD test)*

*[2] Coefficient of variation*

*Table 39 - Cumulative aboveground dry matter production (g $m^{-2}$) of crops (wheat + legumes) under different treatments in 2010/11. Data expressed as means ± SD (n = 3). Within each column, data followed by different letters are significantly different*

| **Treatment** | **BBCH 30** | **BBCH 59** | **BBCH 69** | **BBCH 75** | **BBCH 89** |
|---|---|---|---|---|---|
| N0 | 47.67 ± 6.11 d | 204.00 ± 107.72 de | 234.33 ± 97.27 de | 318.00 ± 106.45 fgh | 377.12 ± 49.30 e |
| N40 | 59.00 ± 2.65 cd | 267.34 ± 31.75 bcde | 375.33 ± 50.56 b | 490.67 ± 38.89 abcd | 533.77 ± 67.39 bc |
| N80 | 56.33 ± 12.10 cd | 308.00 ± 49.43 bc | 364.00 ± 42.67 b | 517.67 ± 61.24 abc | 558.47 ± 25.28 abc |
| N120 | 55.00 ± 12.53 cd | 333.67 ± 26.01 b | 391.33 ± 45.71 b | 526.66 ± 82.28 abc | 570.92 ± 43.50 ab |
| N160 | 64.00 ± 12.53 cd | 294.33 ± 54.31 bcd | 352.67 ± 68.52 bc | 579.00 ± 60.56 ab | 665.27 ± 39.60 a |
| PB-DW TIC | 222.00 ± 69.20 a | 187.67 ± 33.31 e | 208.00 ± 36.51 e | 338.33 ± 50.50 efg | 378.10 ± 80.22 e |
| FP-DW TIC | 128.00 ± 25.87 b | 203.67 ± 41.61 cde | 250.67 ± 45.80 cde | 382.33 ± 108.09 def | 418.03 ± 88.69 de |
| HV-DW TIC | 94.67 ± 41.77 bcd | 188.66 ± 87.87 e | 236.34 ± 59.94 de | 350.00 ± 63.51 efg | 450.32 ± 43.06 cde |
| PB-DW PIC | 217.34 ± 25.15 a | 470.66 ± 56.15 a | 536.00 ± 64.84 a | 583.34 ± 34.24 a | 596.71 ± 34.49 ab |
| FP-DW PIC | 126.00 ± 59.15 b | 304.99 ± 52.52 bcd | 377.33 ± 45.80 b | 453.34 ± 108.09 bcde | 545.82 ± 47.43 bc |
| HV-DW PIC | 101.66 ± 23.01 bc | 296.66 ± 38.53 bcd | 368.34 ± 59.94 b | 449.66 ± 63.51 cde | 501.38 ± 100.17 bcd |
| PB | 190.00 ± 11.36 a | 330.66 ± 53.63 b | 336.00 ± 61.00 bcd | 342.33 ± 66.61 efg | 388.33 ± 112.19 de |
| FP | 92.67 ± 35.12 bcd | 167.67 ± 95.39 e | 177.66 ± 81.46 e | 193.67 ± 82.03 h | 210.00 ± 75.61 f |
| HV | 61.00 ± 12.49 cd | 209.00 ± 36.86 cde | 238.00 ± 54.37 de | 249.34 ± 56.70 gh | 253.00 ± 54.45 f |
| *Significance*[1] | ** | ** | ** | ** | ** |
| *LSD* | *48.20* | *103.75* | *112.36* | *129.28* | *113.71* |
| *CV (%)*[2] | *26.5* | *23.0* | *21.1* | *18.7* | *14.7* |

*[1] **, *, ns are, respectively, significant for p≤.01, significant for p≤.05 and not significant (LSD test)*

*[2] Coefficient of variation*

Data collected at BBCH 30 in both years confirmed the predominance of pigeon bean under intercropping, with a proportion of bean in the total biomass of 44% in 2009/10, and 82% in 2010/11, values which absolutely exceeded the ones expected according to seed ratio (i.e. 16%). These findings fully support our hypothesis of an early interspecific competition between pigeon bean and durum wheat, as also observed by Benincasa et al. (2012), and also reveal a higher growth rate of bean in comparison to wheat at early stages, maybe due to the larger size of seeds of the legume and the consequently better and faster crop establishment (Benincasa et al., 2012).

For pea and vetch, the proportion in the mixture at the first sampling date was 15% and 61%, (field pea), 38% and 53% (hairy vetch), respectively in year 1 and year 2.

Field pea was less represented in the mix in the first year, whereas its proportion reached a value 3 times higher than seed ratio (i.e. 20%) in the second year. Hairy vetch proportion in the two years at BBCH 30 was more consistent with its seed ratio (30%).

Data collected at the other sampling dates in the two years globally confirms the general trend above described. Except for pea in 2009/10, all the legumes grew more and faster than wheat at early stages, and then their relative importance increased over time in the first year (Table 38), whilst decreased in the second year, due to the increased importance of wheat (Table 39).

Data on N accumulated in total aboveground biomass of crops in the two years (Tables 40 and 41) generally are in line with dry matter production, confirming what observed for each component crop.

Nevertheless, the contribution of legume to total N accumulation in crop biomass at harvest was more important than as seen for dry matter. At harvest maturity (BBCH 89) in year 1, N from pigeon bean, field pea, and hairy vetch accounted for 95%, 56% and 51% of total N, respectively. In the second year, the contribution of legumes was lower (68%, 39% and 56%, respectively, for pigeon bean, field pea and hairy vetch), due to the higher relative importance of wheat.

N accumulation at harvest significantly correlated in both years with dry matter production, but with important differences. Whereas in the first year the regression coefficient was quite high ($R^2$ = 88%), meaning a strong relationship between crop biomass productivity and N accumulation, in the second year it was appreciably lower ($R^2$ = 56%), due to the fact that a higher proportion of wheat than in the previous year was observed. Actually, the higher was the contribution of wheat to the level of total biomass, the higher the relative importance of N concentration in total crop biomass. As in the second year the good performances of intercropping were mainly due to a good presence of wheat, N accumulations were strongly influenced also by N concentration, which, in turn was increased by the presence of legumes in the mixture.

Permanent intercropping between pigeon bean and wheat was again the treatment with the highest amount of N accumulated in plant tissues (Tables 40 and 41). Differently from data on dry matter production, N accumulated in total biomass of PB-DW PIC at harvest in year 2 did not differ from sole bean crop.

For field pea-wheat intercropping, in year 1 N accumulations were also higher than the control, unlike the data on dry matter. In the second year, the values of FP-DW PIC did not differ from FP-DW TIC, and neither did they from N0.

Finally, permanent intercropping between vetch and wheat was not statistically different from temporary intercropping in the second year, contrarily to what happened for dry matter data.

*Table 40 - Cumulative N accumulation in aboveground dry matter (g $m^{-2}$) of crops (wheat + legumes) under different treatments in 2009/10. Data expressed as means ± SD (n = 3). Within each column, data followed by different letters are significantly different*

| Treatment | BBCH 30 | BBCH 59 | BBCH 69 | BBCH 75 | BBCH 89 |
|---|---|---|---|---|---|
| N0 | 1.02 ± 0.20 e | 1.48 ± 0.57 e | 1.53 ± 0.49 f | 2.51 ± 1.20 e | 2.67 ± 1.26 f |
| N40 | 2.30 ± 0.15 de | 2.62 ± 0.93 de | 3.00 ± 1.48 ef | 3.93 ± 2.08 de | 4.73 ± 2.22 def |
| N80 | 2.32 ± 0.05 de | 4.55 ± 1.28 bcd | 4.65 ± 1.02 cdef | 6.31 ± 0.90 bcde | 7.02 ± 1.49 bcdef |
| N120 | 2.56 ± 0.16 d | 4.71 ± 0.55 bcd | 5.33 ± 1.42 cde | 7.37 ± 1.03 bcde | 8.51 ± 1.69 bcde |
| N160 | 3.42 ± 0.47 cd | 6.62 ± 2.27 b | 8.19 ± 1.60 bc | 9.73 ± 3.22 bc | 10.70 ± 2.29 b |
| PB-DW TIC | 4.81 ± 0.56 bc | 3.06 ± 0.24 cde | 3.22 ± 0.21 def | 4.99 ± 0.77 cde | 5.75 ± 0.79 cdef |
| FP-DW TIC | 2.48 ± 1.52 d | 2.46 ± 0.39 de | 2.72 ± 1.60 ef | 3.93 ± 1.52 de | 4.25 ± 0.95 ef |
| HV-DW TIC | 4.72 ± 0.79 bc | 3.01 ± 0.35 cde | 3.05 ± 0.36 ef | 5.10 ± 0.89 bcde | 5.74 ± 0.68 cdef |
| PB-DW PIC | 5.00 ± 0.63 b | 10.09 ± 2.39 a | 10.91 ± 0.36 ab | 20.08 ± 2.03 a | 23.47 ± 3.77 a |
| FP-DW PIC | 1.93 ± 0.75 de | 4.25 ± 2.10 bcde | 4.77 ± 2.09 cdef | 6.77 ± 2.56 bcde | 8.68 ± 2.85 bcde |
| HV-DW PIC | 4.55 ± 0.05 bc | 6.55 ± 1.19 b | 6.99 ± 1.84 cd | 7.36 ± 2.50 bcde | 9.20 ± 3.42 bcd |
| PB | 6.84 ± 1.38 a | 11.65 ± 1.28 a | 14.47 ± 2.93 a | 23.94 ± 5.54 a | 26.01 ± 3.50 a |
| FP | 2.14 ± 2.20 de | 5.19 ± 4.74 bc | 6.59 ± 6.81 cde | 9.48 ± 9.08 b | 9.74 ± 8.50 bc |
| HV | 5.24 ± 1.18 b | 6.83 ± 1.67 b | 7.54 ± 1.61 bc | 7.76 ± 1.53 bcd | 9.12 ± 1.89 bcde |
| *Significance*[1] | ** | ** | ** | ** | ** |
| *LSD* | *1.52* | *2.95* | *3.75* | *5.10* | *4.95* |
| *CV (%)*[2] | *25.7* | *33.5* | *37.4* | *35.4* | *30.4* |

*[1] **, *, ns are, respectively, significant for $p \leq .01$, significant for $p \leq .05$ and not significant (LSD test)*

*[2] Coefficient of variation*

*Table 41 - Cumulative N accumulation in aboveground dry matter (g $m^{-2}$) of crops (wheat + legumes) under different treatments in 2010/11. Data expressed as means ± SD (n = 3). Within each column, data followed by different letters are significantly different*

| Treatment | BBCH 30 | BBCH 59 | BBCH 69 | BBCH 75 | BBCH 89 |
|---|---|---|---|---|---|
| N0 | 0.89 ± 0.15 c | 2.57 ± 1.06 efg | 2.61 ± 1.17 f | 3.43 ± 1.30 ef | 4.08 ± 0.48 ef |
| N40 | 1.48 ± 0.06 bc | 3.25 ± 0.13 defg | 3.91 ± 0.46 def | 5.00 ± 0.10 cdef | 5.86 ± 0.93 cde |
| N80 | 1.48 ± 0.44 bc | 3.88 ± 0.97 de | 4.00 ± 1.09 def | 5.22 ± 0.67 cde | 6.32 ± 0.21 bcde |
| N120 | 1.44 ± 0.44 bc | 4.10 ± 0.64 cd | 4.17 ± 0.73 def | 5.67 ± 0.97 cd | 6.78 ± 0.42 bcd |
| N160 | 1.81 ± 0.49 bc | 3.98 ± 0.61 de | 4.00 ± 1.09 def | 6.49 ± 0.87 bcd | 8.09 ± 0.62 abc |
| PB-DW TIC | 6.35 ± 2.19 a | 2.03 ± 0.55 g | 2.42 ± 0.88 f | 4.43 ± 1.17 def | 5.58 ± 2.01 de |
| FP-DW TIC | 2.66 ± 0.67 b | 2.60 ± 0.72 defg | 2.78 ± 0.70 ef | 4.69 ± 1.13 def | 5.76 ± 1.31 de |
| HV-DW TIC | 2.44 ± 1.11 b | 2.68 ± 0.76 defg | 2.95 ± 1.14 ef | 4.39 ± 1.56 def | 6.01 ± 1.09 cde |
| PB-DW PIC | 6.13 ± 0.72 a | 8.02 ± 1.20 a | 8.84 ± 1.05 a | 9.33 ± 0.66 a | 9.89 ± 1.63 a |
| FP-DW PIC | 2.66 ± 1.15 b | 3.81 ± 0.72 def | 4.52 ± 0.70 cde | 5.20 ± 1.13 cde | 6.35 ± 0.55 bcde |
| HV-DW PIC | 2.63 ± 0.70 b | 5.76 ± 0.76 b | 6.06 ± 1.14 bc | 7.06 ± 1.56 bc | 8.53 ± 1.94 ab |
| PB | 6.10 ± 0.34 a | 6.44 ± 1.09 b | 7.37 ± 1.06 a | 7.74 ± 1.52 ab | 8.06 ± 2.48 abc |
| FP | 1.96 ± 0.93 bc | 2.23 ± 1.28 fg | 2.57 ± 1.36 cde | 3.09 ± 1.37 f | 3.24 ± 1.14 f |
| HV | 1.86 ± 0.14 bc | 5.51 ± 1.33 bc | 5.61 ± 1.39 bc | 5.68 ± 1.43 cd | 6.10 ± 1.45 cde |
| *Significance*[1] | ** | ** | ** | ** | ** |
| *LSD* | *1.31* | *1.59* | *1.83* | *2.05* | *2.26* |
| *CV (%)*[2] | *27.4* | *23.4* | *24.6* | *22.2* | *20.8* |

*[1] **, *, ns are, respectively, significant for $p \leq .01$, significant for $p \leq .05$ and not significant (LSD test)*

*[2] Coefficient of variation*

Overall, data on total crop aerial biomass production and N accumulations suggest that, although their detrimental effect on single component crop, and above all on wheat yield, permanent intercropping, as a whole, are more efficient than temporary intercropping and sole crops in the use of environmental resources.

To test whether this impression is true or not, and to highlight the dynamics of competition between species intercropped, an in-depth analysis has been performed by calculating competition indexes for each intercropping.

### 3.1.7. Competitive interactions between component crops under intercropping

Land Equivalent Ratio (LER), Relative Neighbour Effect (RNE) and Aggressivity Index of permanent intercropping total aboveground dry matter production and N accumulation at harvest (BBCH 89) in 2009/10 and 2010/11 are shown in Figures 27 and 28, respectively.

*Figure 27 - Land Equivalent Ratio (LER) of crops (wheat+legume), Relative Neighbour Effect (RNE) and Aggressivity Index of wheat computed for permanent intercropping treatments at **BBCH 89** of wheat in 2009/10. Indexes computed on the basis of both aboveground total dry matter (see charts a, c and e) and N accumulation in total aboveground dry matter (see charts b, d and f) are shown. Data are plotted in function of different reference wheat pure crops (N0, N40, N80, N120, N160, respectively).*

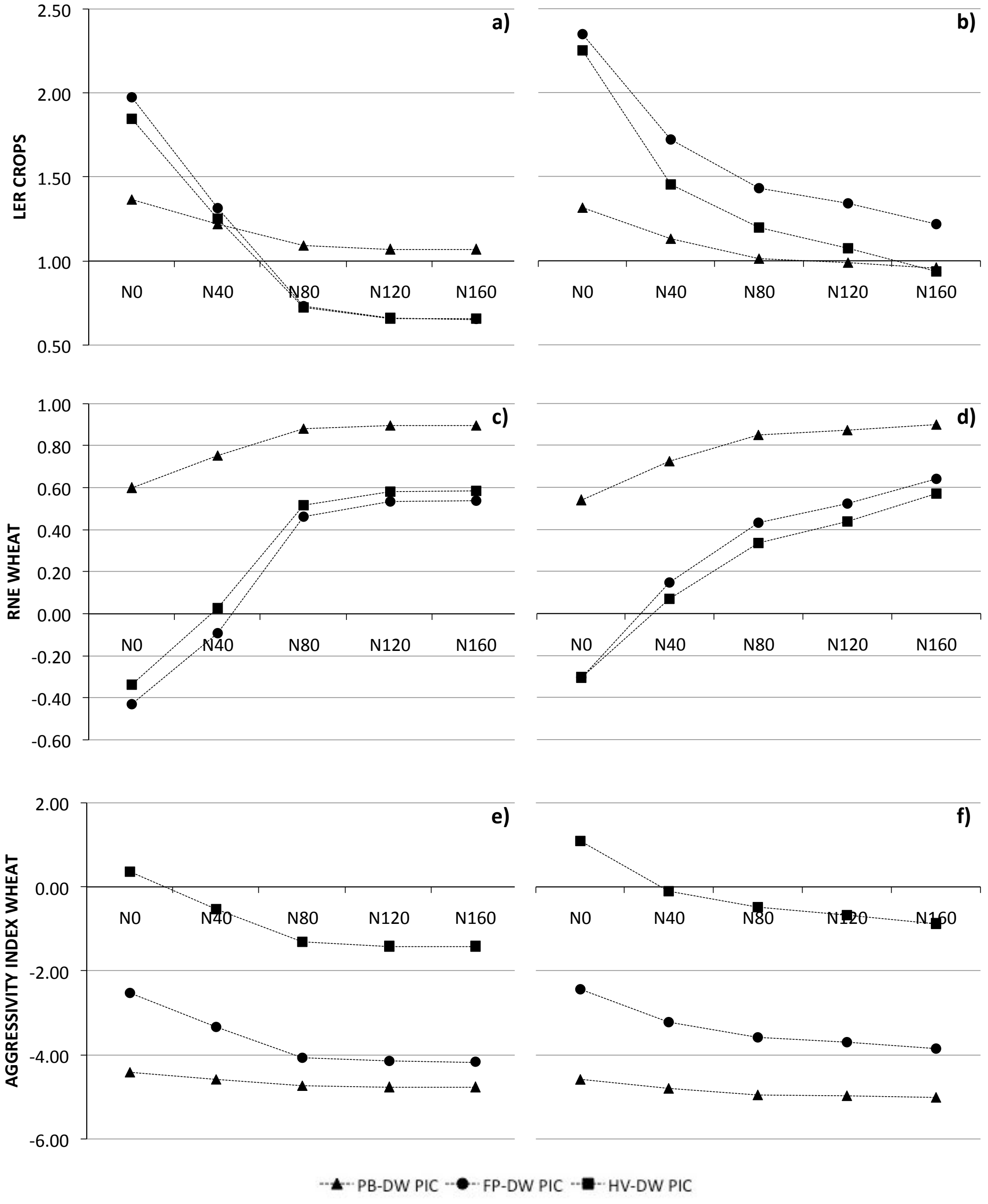
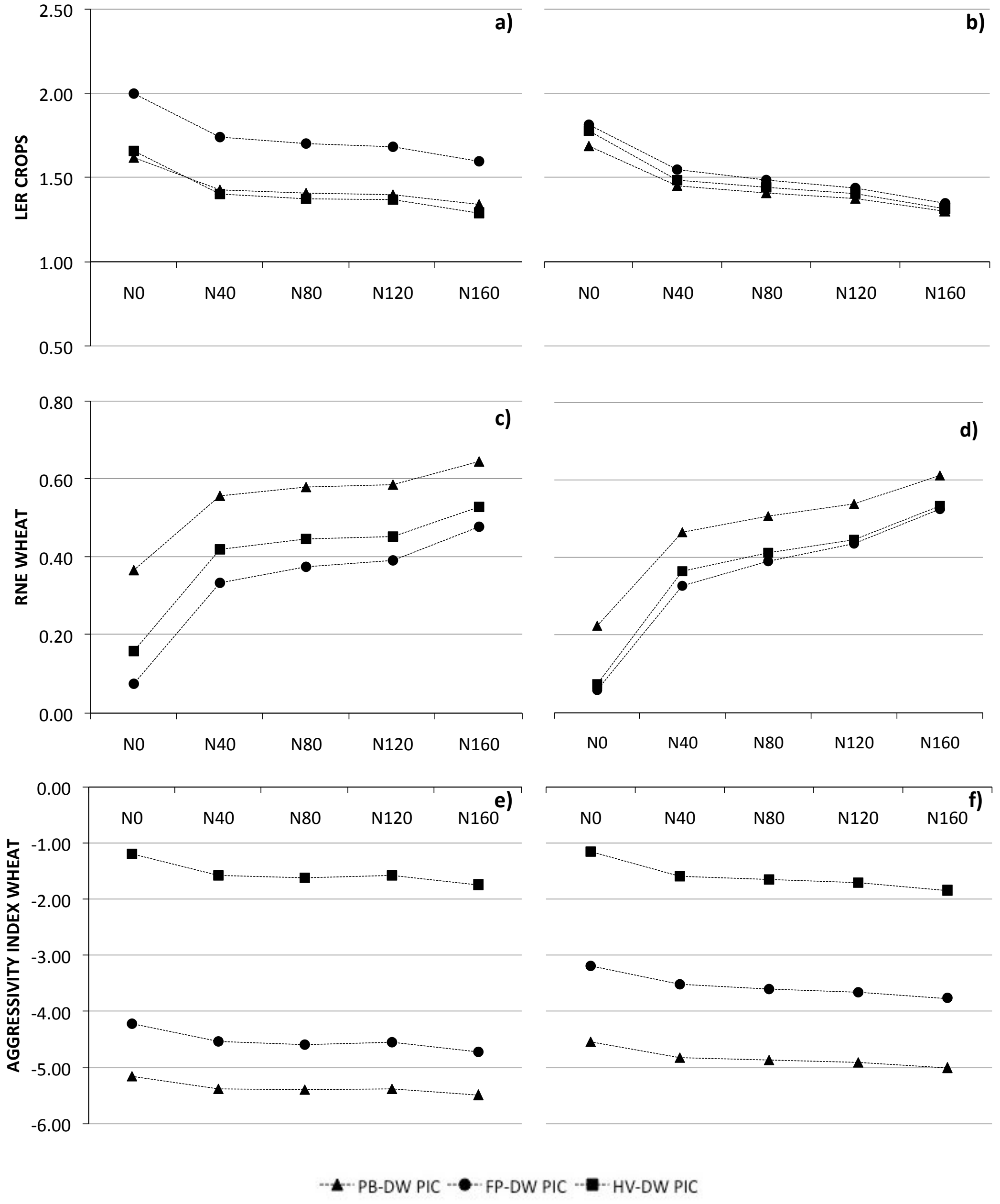


*Figure 28 - Land Equivalent Ratio (LER) of crops (wheat+legume), Relative Neighbour Effect (RNE) and Agggressivity Index of wheat computed for permanent intercropping treatments at BBCH 89 of wheat in 2010/11. Indexes computed on the basis of both aboveground total dry matter (see charts a, c and e) and N accumulation in total aboveground dry matter (see charts b, d and f) are shown. Data are plotted in function of different reference wheat pure crops (N0, N40, N80, N120, N160, respectively).*



The analysis of competition indexes at harvest for the two considered parameters (total aerial biomass of the crops and total N accumulations of the crops) shows similar trends in the two years of the experiment, relative to the reference wheat crop (N0, N40, N80, N120, or N160) used for calculations.

Obviously, LER decreased when passing from N0 to fertilized sole wheat, i.e. weighting the performance of intercropping on the basis of the progressively more productive treatments. The higher rate of decline was observed in the first year compared to the second one.

In 2010/11, permanent intercropping, averaged over all treatments, revealed LER higher than unit whatever the reference wheat sole crop, both for dry matter production and N accumulations (Figure 28a and -b). Whereas for N accumulations (Figure 28b) a substantial equivalence of treatments occurred, for dry biomass production (Figure 28a) intercropping between wheat and field pea resulted in a constantly higher LER than PB- and HV-DW PIC, which did not distinguish from each other. This evidence simply confirms that field pea was the legume most able to grow in complementarity with wheat for a long period, such as under permanent, rather than temporary intercropping.

Data from the first year partially confirm this argument. Intercropping with pea resulted again in the highest LER values, either the treatment, for total N accumulation (Figure 27b). Also intercropping with hairy vetch determined high LER values, close to FP-DW PIC, but only when calculated with reference to N0, then it progressively decreased at a steady rate from N40 to N160, when it showed a LER<1. Whatever the reference wheat crop, intercropping between wheat and pigeon bean had the lowest LER, which was close to 1 until N160, when it became even lower than 1, like HV-DW PIC.

For dry matter production, FP- and HV-DW PIC showed the highest LER when compared to N0. If compared to N40, all intercropping showed similar LER between 1 and 1.5, but when the comparison occurred with sole wheat fertilized at higher N rates (N80, N!20 , N160) only PB-DW PIC maintained a LER>1.

These evidences suggest that in 2009/10 the LER of the mixture was mainly determined by the performance of the wheat in FP- and HV-DW PIC, for which a low contribution to total biomass from the legume was assessed (partial LER of hairy vetch and field pea, respectively, of 0.24 and 0.19). For these treatments, the calculation of LER with reference to sole wheat crops at increasing level of yield (i.e. from N0 to N160) readily determined significant decreases in the partial LER of the wheat (from 1.79 at N0 to 0.46 at N160 for FP-DW PIC; from 1.60 at N0 to 0.41 at N160, for HV-DW PIC) and then in the total resource use efficiency. On the contrary, for pigeon bean the performance of the mixture was mostly due to the high biomass produced by the legume (partial LER of 0.97), which was higher than that of the wheat under sole crops, and hence also the LER was almost constant, irrespective of the reference wheat used for the calculation (partial LER of wheat between 0.40 at N0 and 0.10 at N160).

On the other hand, in the second year the contribution of each legume to the respective mixture was high for all species (partial LER of 0.99, 1.07, 0.82, respectively, for pigeon bean, field pea and hairy vetch), due to the short duration of the cycle, which negatively affected also the growth of legumes under sole crop. The partial LER of wheat, which produced a lower total biomass than in the first year also in sole crops, resulted more constant over reference sole wheat than in the first year. Consequently, also total LER of mixtures was more stable over reference wheat crops.

The pattern of the other indexes (RNE and Aggressivity Index) fully confirmed this finding (FIgures 27 and 28) and their interpretation allows to highlight the dynamic of competition within each mixture.

In the first year, RNE for dry matter and N accumulation was negative, meaning facilitation, only for FP- and HV-DW PIC with reference to N0, then it became close to 0 at N40 and positive, meaning competition, at higher N rates (Figure 27c and -d). Intercropping with pigeon bean always produced extremely positive RNE, revealing a strong competition irrespective of the sole wheat reference.

The Aggressivity Index of wheat in 2009/10 confirmed that, in this context of competition, wheat was the dominated crop, and the legumes the dominant ones. Only when intercropped with vetch in comparison to N0, durum wheat showed positive value of the index (Figures 27e and -f).

In the second year, over all reference wheat and treatments, RNE was always positive, meaning competition between component crops of mixtures, and Aggressivity Index was always negative, revealing that wheat was definitively the dominated crop (Figures 28c to 28f). Wheat was less dominated by hairy vetch (Figures 28e and -f), and the competition was lower when wheat was intercropped with vetch and pea than with bean (Figures 28c and -d).

The analysis of the three competition indexes for total dry matter production and total N accumulations of crop at stem elongation of wheat (BBCH 30) reveals that in both years of the experiment the performances and the dynamics of interspecific competition of the mixtures observed at final harvest were already established at earlier stages. The pattern of the three indexes, averaged over all treatments and reference wheat crops, surprisingly matched that delineated at harvest (Figures 29 and 30).

*Figure 29 - Land Equivalent Ratio (LER) of crops (wheat+legume), Relative Neighbour Effect (RNE) and Agggressivity Index of wheat computed for permanent and temporary intercropping treatments at **BBCH 30** of wheat in **2009/10**. Indexes computed on the basis of both aboveground total dry matter (see charts a, c and e) and N accumulation in total aboveground dry matter (see charts b, d and f) are shown. Data are plotted in function of different reference wheat pure crops (N0, N40, N80, N120, N160, respectively).*

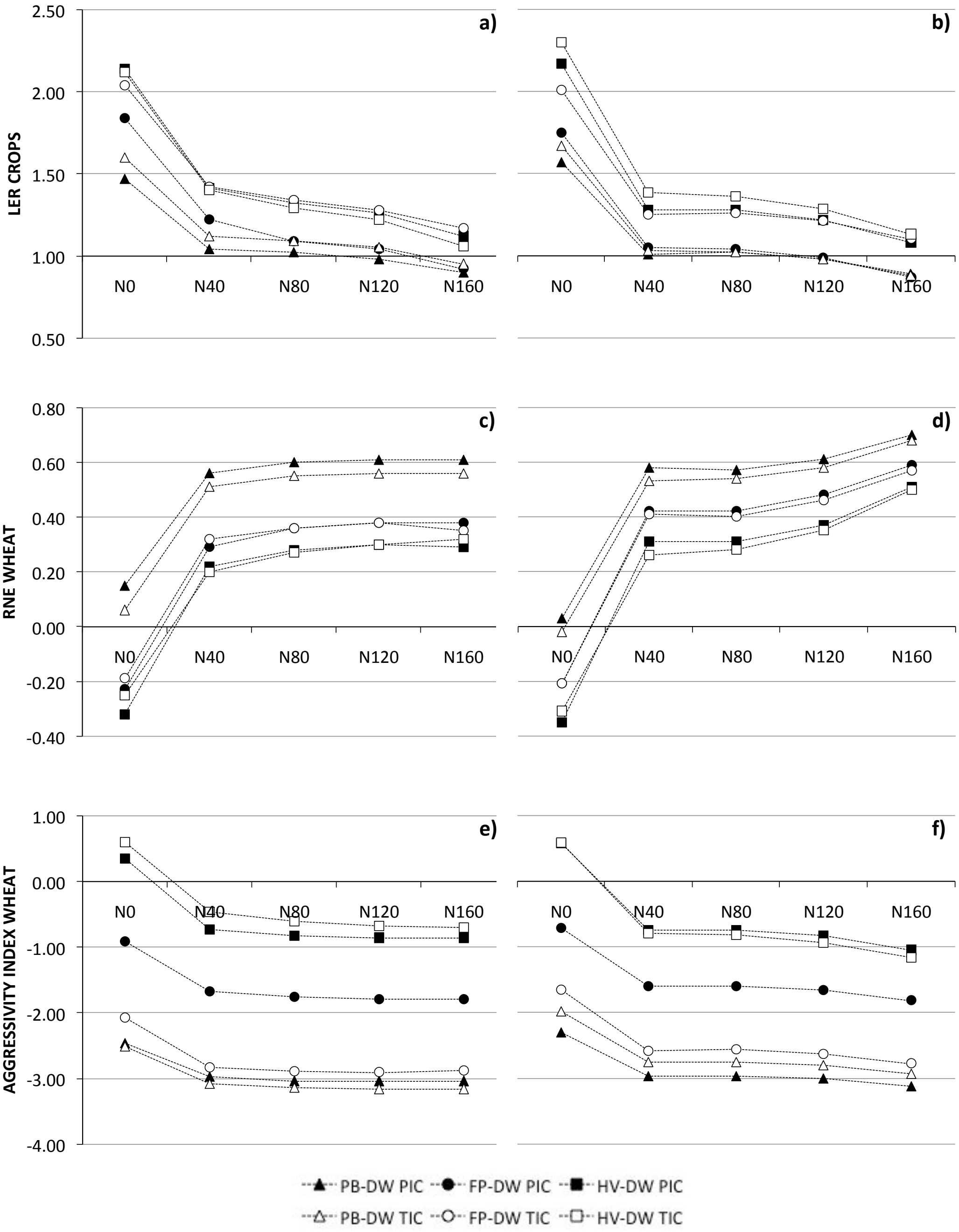
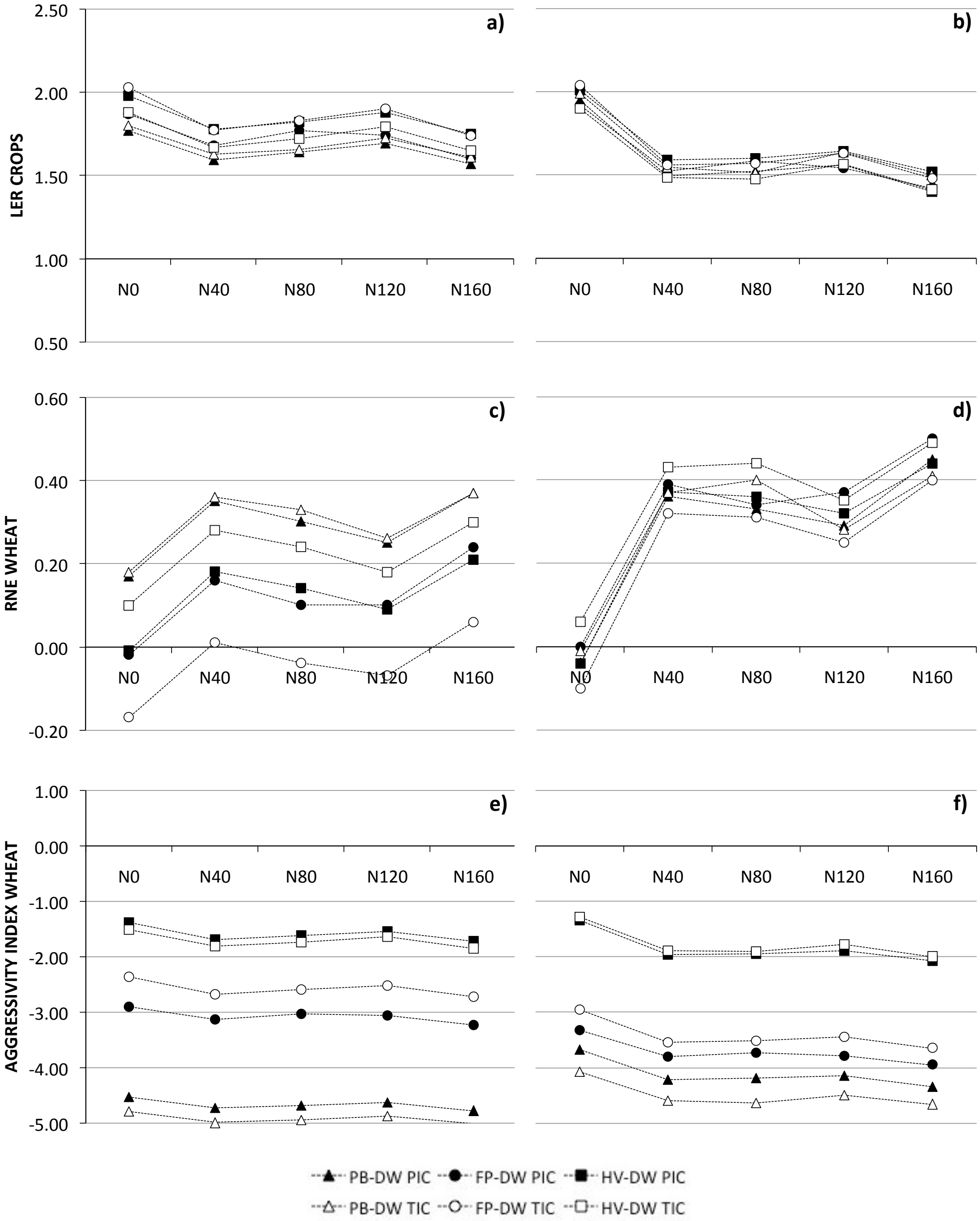


*Figure 30 - Land Equivalent Ratio (LER) of crops (wheat+legume), Relative Neighbour Effect (RNE) and Agggressivity Index of wheat computed for permanent and temporary intercropping treatments at* ***BBCH 30*** *of wheat in 2010/11. Indexes computed on the basis of both aboveground total dry matter (see charts a, c and e) and N accumulation in total aboveground dry matter (see charts b, d and f) are shown. Data are plotted in function of different reference wheat pure crops (N0, N40, N80, N120, N160, respectively).*



Globally, our results confirm the higher resource use efficiency of permanent intercropping compared to sole crops, as well pointed out by the majority of the studies on this topic (Jensen, 1996; Kurdali et al., 1996; Hauggaard-Nielsen et al., 2001b, a; Chu et al., 2004; Andersen et al., 2005; Ghaley et al., 2005; Hauggaard-Nielsen et al., 2006; Andersen et al., 2007; Gunes et al., 2007; Hauggaard-Nielsen et al., 2008; Bedoussac and Justes, 2009; Hauggaard-Nielsen et al., 2009a; Tosti et al., 2010).

LER was steadily high in the second year, when the contribution of legumes to total dry matter and N accumulations was higher. Nevertheless, the total LER value of mixtures was decreased when the legume became too much competitive and aggressive, as shown for pigeon bean.

Moreover, our findings also relate the final resource use efficiency of intercropping to the early dynamics of competition or facilitation between the component crops. In the case of intercropping with pigeon bean, actually the least efficient treatment in terms of LER in both years, our data clearly show that a strong competition for resources already took place at stem elongation of wheat. According to that, it would have been better to perform an additional sampling of biomass between crop emergence and wheat tillering to investigate the real dynamics of this competition, as also suggested by Benincasa et al. (2012).

In our conditions, intercropping had detrimental effect on the growth of legumes in the first year, when all partial LER for dry matter production of legumes, except for pigeon bean, revealed very low values. In the second year, when the shorter duration of the cycle negatively affected also performance of pure stands, the partial LER of legumes was higher (close to 0.90, on average) and, in the case of field pea, even >1, meaning a better performance than under sole crop. The negative effect of intercropping on biomass production of legumes was also demonstrated by Andersen et al. (2005) and Hauggaard-Nielsen et al. (2001b).

Concerning N accumulations at harvest, the partial LER of legumes was higher than for dry matter only in some cases (for FP-DW PIC in year 1, for HV-DW PIC in both year 1 and 2). For pigeon bean the partial LER for N was always lower than for dry matter, meaning a higher importance of biomass than N concentration.

Moreover, total LER of FP- and HV-DW PIC in 2009/10 showed values higher than for dry matter (Figure 27b). Following Ghosh et al. (2009), this supports the hypothesis that in the first year N was the resource more used by intercropping and, hence, that it was not the most important limiting factor for intercropping, as also obtained by Mariotti et al. (2012) for pigeon bean-durum wheat intercropping in similar conditions.

For wheat, we observed partial LER > 1 only in 2009/10 for FP- and HV-DW PIC at harvest when compared to N0 and N40. The same treatments in the second year showed values slightly inferior to 1. Nevertheless, these findings are extremely valuable for the aim of this research. Actually, they suggest that, even under extremely competitive intercropping schemes, such as under permanent intercropping, the biomass production of wheat could be higher than sole wheat unfertilized or fertilized with 40 units of N $ha^{-1}$, that is a level of fertilization in the range of that commonly adopted also by organic or low-input farmers. A higher resource use efficiency of intercropping at low levels of N fertilization of wheat was also demonstrated by other studies (Andersen et al., 2005; Ghaley et al., 2005; Bedoussac and Justes, 2009), in which also the mixture was fertilized at different rates.

The behavior of the wheat under intercropping with pigeon bean was absolutely different from the others, resulting in partial LER between 0.10 and 0.40 in year 1, and between 0.35 and 0.63 in year 2. For this treatment, our results clearly demonstrated the predominance of bean over wheat, as also highlighted by the values of RNE and Aggressivity Index. Legume was the dominant crop also in the experiment carried out by Andersen et al. (2007), who identified in the low N content in the soil the main determinant of this evidence.

On the other hand, other studies carried out in conditions similar to ours and with adequate N supply found still the same predominance of pigeon bean (Tosti and Guiducci, 2010; Mariotti et al., 2012). The authors concluded that this was primarily due to shading, rather than stimulation of $N_2$-fixation of the legume. Shading occurred since early development stages, in particular during tillering, due to the higher plant height of bean compared to wheat, an evidence observed also in our experiment (chapter 3.1.4).

### *3.1.8. Weed aboveground dry matter production and competition with crops for N and space*

The ability of intercropping to suppress weeds was evaluated, first, through the assessment of the aboveground dry matter production of weeds collected within crop samples in the two years at each sampling date (Tables 42 and 43).

On average, at final harvest weed pressure was 30% lower in the second year than the first (135.91 g $m^{-2}$ *vs* 192.82 g $m^{-2}$, averaged over all treatments at harvest, respectively for year 2 and year 1). In both years, wheat sole crop fertilized with the highest rates of N (N120 and N160) showed the highest values of weed dry matter at harvest (Tables 42 and 43). The other treatments had a different behavior in the two years.

In year 1 (Table 42), intercropping determined a significant decrease in weed total biomass at BBCH 89. Temporary intercropping, whatever the treatment, resulted in a mean dry matter of weeds of 104.37 g $m^{-2}$, whereas permanent intercropping performed even better (although not significantly), reducing by 50% more the weed presence (50.92 g $m^{-2}$, on average). Among intercropping, treatments including hairy vetch were the least effective in terms of weed suppression, whilst with pigeon bean were the most. In comparison to intercropping, N0 resulted in 116% and 350% higher weed biomass than, respectively, TIC and PIC. Similar weed detriments were observed also when intercropping was compared to sole wheat fertilized with ammonium nitrate.

In the plots with sole legumes, a mean value of 137 g $m^{-2}$ of weed dry matter came out at harvest. This value was, respectively, 31% higher than TIC treatments, and 175% higher than PIC treatments. The highest presence of weeds in legumes was recorded in field pea plots (255.61 g $m^{-2}$), the lowest in pigeon bean (20.05 g $m^{-2}$).

*Table 42 - Aboveground dry matter production (g $m^{-2}$) of weeds collected in 2009/10. Values are means ± SD (n=3). Within each column, data followed by different letters are significantly different*

| Treatment | BBCH 30 | BBCH 59 | BBCH 69 | BBCH 75 | BBCH 89 |
|---|---|---|---|---|---|
| N0 | 13.67 ± 13.43 cde | 49.67 ± 45.00 bc | 56.33 ± 17.62 bc | 116.00 ± 165.42 bcde | 225.83 ± 131.32 ab |
| N40 | 14.67 ± 14.50 cde | 32.00 ± 19.92 cd | 61.33 ± 27.79 bc | 135.67 ± 66.73 abcde | 169.91 ± 54.18 abc |
| N80 | 27.00 ± 13.00 abc | 74.00 ± 32.91 ab | 123.33 ± 86.63 ab | 204.67 ± 111.36 abcd | 217.84 ± 72.78 ab |
| N120 | 25.00 ± 24.33 ab | 92.33 ± 31.39 a | 171.33 ± 108.19 a | 212.00 ± 80.13 abc | 237.48 ± 155.93 ab |
| N160 | 42.33 ± 13.32 ab | 99.00 ± 28.16 a | 132.33 ± 107.95 ab | 242.67 ± 92.59 ab | 258.45 ± 45.14 a |
| PB-DW TIC | 3.00 ± 1.00 e | 1.67 ± 2.89 d | 23.67 ± 17.62 c | 54.00 ± 74.51 e | 79.02 ± 10.88 cde |
| FP-DW TIC | 8.67 ± 3.22 de | 9.67 ± 9.07 d | 10.67 ± 6.51 c | 38.33 ± 31.63 e | 95.45 ± 46.07 cde |
| HV-DW TIC | 4.67 ± 1.16 e | 4.67 ± 6.43 d | 27.00 ± 20.66 c | 75.67 ± 51.23 de | 138.64 ± 40.93 bcd |
| PB-DW PIC | 12.33 ± 10.02 cde | 14.00 ± 11.53 cd | 15.33 ± 13.58 c | 17.33 ± 21.39 e | 20.77 ± 14.95 e |
| FP-DW PIC | 10.00 ± 6.25 cde | 15.67 ± 19.86 cd | 31.33 ± 37.17 c | 45.33 ± 38.11 e | 58.46 ± 44.14 de |
| HV-DW PIC | 5.00 ± 7.00 e | 5.67 ± 2.08 d | 9.67 ± 2.52 c | 17.67 ± 7.37 e | 73.52 ± 16.51 cde |
| PB | 9.00 ± 4.36 de | 10.00 ± 8.66 d | 12.67 ± 11.37 c | 15.00 ± 7.21 e | 20.05 ± 15.62 e |
| FP | 44.67 ± 15.31 a | 76.33 ± 14.84 ab | 128.67 ± 7.02 ab | 250.33 ± 174.36 a | 255.61 ± 87.66 a |
| HV | 35.67 ± 18.61 ab | 50.00 ± 46.60 bc | 51.33 ± 64.73 bc | 94.67 ± 80.16 cde | 134.42 ± 119.03 bcd |
| *Significance[1]* | ** | ** | ** | ** | ** |
| *LSD* | *17.98* | *38.51* | *89.54* | *134.21* | *109.41* |
| *CV (%)[2]* | *58.7* | *60.1* | *87.4* | *73.7* | *46.0* |

*[1] **, *, ns are, respectively, significant for p≤.01, significant for p≤.05 and not significant (LSD test)*

*[2] Coefficient of variation*

*Table 43 - Aboveground dry matter production (g $m^{-2}$) of weeds collected in 2010/11. Values are means ± SD (n=3). Within each column, data followed by different letters are significantly different*

| **Treatment** | **BBCH 30** | **BBCH 59** | **BBCH 69** | **BBCH 75** | **BBCH 89** |
|---|---|---|---|---|---|
| N0 | 8.00 ± 5.20 | 37.33 ± 4.04 cde | 43.67 ± 6.03 cde | 117.33 ± 91.51 abc | 124.67 ± 27.43 de |
| N40 | 13.33 ± 10.41 | 52.33 ± 30.99 bcde | 81.00 ± 62.95 abcd | 108.33 ± 119.18 bc | 158.33 ± 27.93 cde |
| N80 | 23.33 ± 14.01 | 82.67 ± 43.84 abc | 102.00 ± 71.71 abcd | 190.00 ± 43.55 ab | 275.67 ± 108.08 ab |
| N120 | 17.00 ± 11.36 | 67.33 ± 36.75 abcd | 121.67 ± 50.06 a | 180.00 ± 9.85 ab | 311.33 ± 80.36 a |
| N160 | 17.67 ± 20.11 | 112.67 ± 80.06 a | 121.00 ± 71.46 a | 142.67 ± 111.91 abc | 298.33 ± 16.04 a |
| PB-DW TIC | 5.67 ± 4.04 | 1.33 ± 2.31 e | 0.00 e | 0.00 d | 6.67 ± 6.51 f |
| FP-DW TIC | 5.67 ± 3.51 | 0.00 e | 0.00 e | 0.00 d | 14.67 ± 18.18 f |
| HV-DW TIC | 7.67 ± 4.04 | 0.00 e | 0.00 e | 0.00 d | 21.67 ± 9.61 f |
| PB-DW PIC | 11.00 ± 9.64 | 18.00 ± 6.24 de | 33.00 ± 2.65 de | 66.33 ± 14.57 cd | 73.33 ± 11.93 ef |
| FP-DW PIC | 6.33 ± 3.79 | 30.67 ± 16.62 cde | 40.33 ± 9.45 cde | 74.67 ± 27.65 cd | 87.33 ± 47.72 ef |
| HV-DW PIC | 9.33 ± 2.08 | 22.67 ± 8.39 e | 48.00 ± 26.06 bcde | 65.67 ± 31.09 cd | 72.00 ± 22.91 ef |
| PB | 19.00 ± 10.00 | 63.33 ± 23.86 abcd | 73.33 ± 17.93 abcd | 132.00 ± 11.14 cd | 200.33 ± 37.82 bcd |
| FP | 18.33 ± 8.14 | 95.33 ± 34.21 ab | 116.33 ± 29.16 ab | 205.00 ± 16.09 ab | 217.67 ± 55.87 abcd |
| HV | 17.67 ± 12.58 | 69.67 ± 55.87 abcd | 103.00 ± 79.17 abc | 214.00 ± 110.24 a | 220.33 ± 125.82 abc |
| *Significance[1]* | *ns* | ** | ** | ** | ** |
| *LSD* | *16.57* | *55.33* | *69.76* | *101.43* | *94.19* |
| *CV (%)[2]* | *76.9* | *70.6* | *65.9* | *56.6* | *37.7* |

*[1] **, *, ns are, respectively, significant for $p \leq .01$, significant for $p \leq .05$ and not significant (LSD test)*

*[2] Coefficient of variation*

In year 2 (Table 43), the depletion of weed biomass under intercropping compared to sole crops of wheat and legumes was confirmed.

Averaged over all treatments, weeds produced at harvest 43.01 and 77.55 g $m^{-2}$ of dry matter, respectively, under temporary and permanent intercropping, without significant differences again. The trend was completely opposite to that observed in 2009/10, when permanent intercropping suppress more the weeds than temporary. The different sowing date in the two years might be the main reason for this evidence. In the second year, the late sowing excluded winter-germinating weeds from the experimental field, and hence the incorporation of legumes into the soil under temporary intercropping significantly reduced all the apparent weed flora, as shown by the null values of weed biomass for TIC treatments until harvest (Table 43). On the contrary, in the first year, after the termination of pulses under TIC treatments, new spring-germinating weeds emerged, as shown by increasing dry matter of weeds collected under all TIC treatments from BBCH 59 onwards (Table 42). The most frequent and abundant species of that kind was *Picris echioides* L., which was spread almost over the entire field. The differences among the intercropping treatments were irrelevant.

The weed biomass produced under N0 (124.67 g $m^{-2}$) and, on average, by all fertilized wheat sole crop (221.33 g $m^{-2}$) was, respectively lower than and comparable to their own values observed in the year before. Anyway, all wheat sole crops suppress much less the weeds than intercropped. For TIC treatments, the difference from sole wheat at harvest was even significant (Table 43).

For sole legumes, the presence of weeds at harvest was 55% higher in 2010/11 than in 2009/10, but on the other hand the differences among species were strongly reduced (10% difference at most).

The competitive ability of intercropping against weeds was expressed differently in the two years. Looking at the trend of the dry matter production of weeds over time in 2009/10, a very early (at BBCH 30 of wheat) advantage of intercropping compared to the other treatments was observed. This might have been probably due to the larger row distance under sole crops than intercropping, which increased the proportion of bare soil available for weeds to colonize. Oppositely, in the second year, a clear suppression of weeds under intercropping was assessed only at late stages, from heading onwards. This was due to the shorter temporal distance between the sowing and the first sampling (80 days) than in the year before (137 days), occurred because of the late sowing of the second year. The consequently shorter duration of co-growth determined thus a delay in the manifestation of the competition against weeds of intercropping.

Competition with weeds for soil nitrogen was also assessed, by the measurement of N concentration and accumulation in aboveground dry matter of weeds collected within each plot in both years at each sampling date (Tables 44 and 45).

Also tables on phosphorus concentration and accumulation in weed dry matter were produced and enclosed in the Appendix 1.

*Table 44 - N concentration -Nconc- (g kg$^{-1}$) and N accumulation -Nacc- (g m$^{-2}$) in weed aboveground dry matter in 2009/10. Values are means ± SD (n=3). Within each column, data followed by different letters are significantly different*

| **Treatment** | **BBCH 30** | | **BBCH 59** | | **BBCH 69** | | **BBCH 75** | | **BBCH 89** | |
|---|---|---|---|---|---|---|---|---|---|---|
| | **Nconc** | **Nacc** | **Nconc** | **Nacc** | **Nconc** | **Nacc** | **Nconc** | **Nacc** | **Nconc** | **Nacc** |
| N0 | 15.37 ± 6.35 | 0.21 ± 0.15def | 10.57 ± 0.49 g | 0.53 ± 0.44 de | 10.07 ± 0.76 | 0.57 ± 0.14 cd | 16.70 ± 7.34 bcd | 1.94 ± 1.32 bcde | 13.12 ± 1.08 cd | 2.96 ± 1.44 abcd |
| N40 | 10.57 ± 9.56 | 0.16 ± 0.27 def | 16.23 ± 3.01 defg | 0.52 ± 0.39 de | 10.13 ± 0.21 | 0.62 ± 0.28 cd | 9.63 ± 4.65 e | 1.31 ± 0.21 cde | 13.29 ± 2.77 cd | 2.26 ± 1.13 abcd |
| N80 | 13.97 ± 2.67 | 0.38 ± 0.14 bcd | 22.87 ± 0.40 abcd | 1.69 ± 0.76 abc | 13.63 ± 3.31 | 1.68 ± 1.51 abc | 9.07 ± 0.25 e | 1.86 ± 1.03 abc | 9.72 ± 2.98 d | 2.12 ± 0.29 abcde |
| N120 | 13.70 ± 2.43 | 0.34 ± 0.29 cde | 22.57 ± 5.20 abcd | 2.08 ± 1.06 ab | 12.73 ± 0.86 | 2.18 ± 1.52 ab | 10.87 ± 1.21 de | 2.30 ± 0.69 ab | 10.56 ± 2.97 d | 2.51 ± 1.85 abc |
| N160 | 14.17 ± 2.95 | 0.60 ± 0.24 a | 25.37 ± 1.24 abc | 2.51 ± 0.62 a | 20.37 ± 2.96 | 2.70 ± 2.13 a | 11.27 ± 3.27 de | 2.73 ± 1.02 a | 12.41 ± 3.10 cd | 3.21 ± 0.51 a |
| PB-DW TIC | 12.50 ± 2.95 | 0.04 ± 0.02 f | 31.60 ± 0.00 a | 0.05 ± 0.00 e | 15.67 ± 1.85 | 0.37 ± 0.25 cd | 15.27 ± 4.60 bcde | 0.82 ± 0.70 de | 18.12 ± 2.74 abc | 1.43 ± 0.10 cdef |
| FP-DW TIC | 13.90 ± 1.39 | 0.12 ± 0.05 ef | 12.45 ± 0.35 fg | 0.12 ± 0.06 de | 19.67 ± 1.53 | 0.21 ± 0.11 cd | 16.73 ± 2.86 bcd | 0.64 ± 0.46 de | 13.52 ± 3.01 cd | 1.29 ± 0.60 cdef |
| HV-DW TIC | 17.07 ± 2.16 | 0.08 ± 0.02 f | 28.20 ± 13.58 ab | 0.13 ± 0.10 de | 14.23 ± 1.17 | 0.38 ± 0.31 cd | 12.73 ± 0.93 cde | 0.96 ± 0.56 cde | 14.56 ± 2.19 cd | 2.02 ± 0.29 abcde |
| PB-DW PIC | 14.10 ± 4.62 | 0.17 ± 0.10 def | 18.87 ± 1.63 cdef | 0.26 ± 0.24 de | 18.37 ± 1.46 | 0.28 ± 0.27 cd | 19.77 ± 2.40 ab | 0.34 ± 0.38 e | 21.82 ± 12.29 a | 0.45 ± 0.19 f |
| FP-DW PIC | 15.33 ± 0.97 | 0.15 ± 0.09 def | 14.65 ± 1.34 efg | 0.23 ± 0.27 de | 11.97 ± 0.06 | 0.38 ± 0.45 cd | 18.30 ± 7.52 abc | 0.83 ± 0.50 de | 15.57 ± 1.60 bcd | 0.91 ± 0.60 f |
| HV-DW PIC | 10.13 ± 8.96 | 0.05 ± 0.12 f | 15.75 ± 1.34 defg | 0.09 ± 0.02 e | 12.63 ± 11.39 | 0.12 ± 0.09 d | 17.57 ± 6.34 bcd | 0.31 ± 0.06 cde | 16.49 ± 3.42 abcd | 1.21 ± 0.03 ef |
| PB | 15.43 ± 3.90 | 0.14 ± 0.04 def | 23.00 ± 2.69 abcd | 0.23 ± 0.04 de | 13.1 ± 11.55 | 0.17 ± 0.21 cd | 24.63 ± 0.67 a | 0.37 ± 0.17 e | 23.25 ± 3.40 a | 0.47 ± 0.38 f |
| FP | 13.13 ± 2.38 | 0.59 ± 0.09 ab | 17.93 ± 4.84 def | 1.37 ± 0.35 bcd | 12.30 ± 4.94 | 1.58 ± 0.73 abcd | 11.67 ± 4.19 abc | 2.92 ± 1.41 a | 10.10 ± 3.04 d | 2.58 ± 0.64 abcd |
| HV | 15.23 ± 3.35 | 0.54 ± 0.20 abc | 20.80 ± 0.27 bcde | 1.04 ± 0.98 cde | 21.97 ± 1.10 | 1.13 ± 1.34 bcd | 17.37 ± 2.17 bcd | 1.64 ± 1.25 abcd | 13.92 ± 1.27 cd | 1.87 ± 1.48 bcde |
| *Significance[1]* | *ns* | **** | **** | **** | *ns* | *** | **** | **** | *** | **** |
| *LSD* | *8.15* | *0.23* | *11.53* | *1.65* | *8.18* | *1.60* | *7.00* | *1.12* | *7.11* | *12.7* |
| *CV (%)[2]* | *34.9* | *55.0* | *19.9* | *59.8* | *33.0* | *106.2* | *27.6* | *55.5* | *28.7* | *43.1* |

[1] ***, *, ns are, respectively, significant for p≤.01, significant for p≤.05 and not significant (LSD test)*

[2] *Coefficient of variation*

*Table 45 - N concentration -Nconc- (g kg$^{-1}$) and N accumulation -Nacc- (g m$^{-2}$) in weed aboveground dry matter in 2010/11. Values are means ± SD (n=3). Within each column, data followed by different letters are significantly different*

| **Treatment** | **BBCH 30** | | **BBCH 59** | | **BBCH 69** | | **BBCH 75** | | **BBCH 89** | |
|---|---|---|---|---|---|---|---|---|---|---|
| | **Nconc** | **Nacc** | **Nconc** | **Nacc** | **Nconc** | **Nacc** | **Nconc** | **Nacc** | **Nconc** | **Nacc** |
| N0 | 22.80 ± 0.87 de | 0.18 ± 0.12 | 21.00 ± 0.17 | 0.78 ± 0.09 | 22.33 ± 5.59 | 0.98 ± 0.15 | 16.33 ± 1.03 cd | 1.92 ± 1.33 bc | 18.37 ± 0.45 bcd | 2.29 ± 0.51 cde |
| N40 | 24.53 ± 4.50 bcd | 0.33 ± 0.29 | 19.03 ± 3.16 | 1.00 ± 0.64 | 21.23 ± 6.97 | 1.72 ± 1.30 | 19.33 ± 0.57 abc | 2.09 ± 2.21 abc | 17.07 ± 0.40 de | 2.70 ± 0.46 cd |
| N80 | 28.57 ± 2.71 ab | 0.67 ± 0.34 | 21.03 ± 0.47 | 1.74 ± 0.77 | 20.33 ± 2.32 | 2.07 ± 1.74 | 20.93 ± 1.70 a | 3.98 ± 1.20 a | 17.90 ± 0.56 cd | 4.93 ± 1.85 ab |
| N120 | 28.37 ± 0.85 abc | 0.48 ± 0.32 | 19.20 ± 1.41 | 1.29 ± 0.77 | 17.80 ± 1.95 | 2.17 ± 1.02 | 20.57 ± 3.01 a | 3.70 ± 0.55 ab | 20.20 ± 0.92 bc | 6.29 ± 1.40 a |
| N160 | 31.63 ± 3.01 a | 0.56 ± 0.56 | 18.93 ± 3.15 | 2.13 ± 1.34 | 18.73 ± 1.03 | 2.27 ± 1.18 | 20.13 ± 1.32 ab | 2.87 ± 2.01 abc | 20.37 ± 1.31 b | 6.08 ± 0.61 a |
| PB-DW TIC | 22.97 ± 2.48 de | 0.13 ± 0.11 | 15.30 ± 0.00 | 0.02 ± 0.00 | - | - | - | - | 18.25 ± 0.35 bcd | 0.12 ± 0.07 f |
| FP-DW TIC | 23.37 ± 2.56 cde | 0.13 ± 0.07 | - | - | - | - | - | - | 25.65 ± 4.74 a | 0.38 ± 0.56 ef |
| HV-DW TIC | 18.60 ± 5.05 e | 0.14 ± 0.11 | - | - | - | - | - | - | 20.93 ± 2.65 b | 0.45 ± 0.14 ef |
| PB-DW PIC | 22.93 ± 2.72 de | 0.25 ± 0.26 | 19.03 ± 2.27 | 0.34 ± 0.10 | 18.77 ± 3.88 | 0.62 ± 0.16 | 16.00 ± 2.96 cd | 1.06 ± 0.08 c | 16.30 ± 0.66 de | 1.20 ± 0.16 def |
| FP-DW PIC | 18.83 ± 2.65 e | 0.12 ± 0.08 | 16.77 ± 2.39 | 0.51 ± 0.32 | 15.97 ± 5.68 | 0.64 ± 0.38 | 13.50 ± 3.75 d | 1.01 ± 0.59 c | 13.57 ± 1.34 fg | 1.19 ± 0.61 def |
| HV-DW PIC | 21.47 ±4.88 de | 0.20 ± 0.01 | 18.47 ± 2.64 | 0.42 ± 0.18 | 17.53 ± 1.35 | 0.84 ± 0.41 | 16.67 ± 1.07 bcd | 1.09 ± 0.59 abc | 16.13 ± 1.16 de | 1.16 ± 0.31 def |
| PB | 25.83 ± 2.59 bcd | 0.49 ± 0.23 | 19.40 ± 2.82 | 1.23 ± 0.29 | 20.00 ± 6.86 | 1.47 ± 0.57 | 16.63 ± 1.36 bcd | 2.20 ± 0.27 abc | 15.20 ± 0.72 efg | 3.05 ± 0.56 c |
| FP | 21.77 ± 0.97 de | 0.40 ± 0.17 | 17.87 ± 0.59 | 1.70 ± 0.63 | 17.33 ± 4.46 | 2.02 ± 0.37 | 13.40 ± 0.80 d | 2.75 ± 0.31 abc | 13.23 ± 1.56 g | 2.88 ± 1.06 c |
| HV | 21.53 ± 0.67 de | 0.38 ± 0.26 | 22.50 ± 4.02 | 1.57 ± 1.04 | 19.90 ± 2.70 | 2.05 ± 1.73 | 16.63 ± 1.95 bcd | 3.56 ± 1.75 ab | 15.97 ± 0.90 def | 3.52 ± 2.24 bc |
| *Significance*[1] | ** | *ns* | *ns* | *ns* | *ns* | *ns* | ** | * | ** | ** |
| *LSD* | *5.12* | *0.42* | *7.43* | *1.99* | *7.82* | *1.67* | *3.56* | *2.02* | *3.01* | *2.08* |
| *CV (%)*[2] | *12.8* | *88.2* | *13.1* | *61.1* | *24.1* | *63.7* | *12.1* | *50.1* | *8.3* | *37.0* |

[1] **, *, ns are, respectively, significant for $p \leq .01$, significant for $p \leq .05$ and not significant (LSD test)

[2] Coefficient of variation

Data from the two years revealed a tendency to higher N concentration in weeds grown close to sole legumes and intercrops than in sole wheat plots (Tables 44 and 45). Anyway, this increase in N content of weeds was always associated to a proportional decrease in dry matter production. This evidence was clearly demonstrated at harvest in both years. In 2009/10 (Table 44), temporary intercropping (15.40 g N kg$^{-1}$ d.m., on average) and permanent intercropping (17.96 g N kg$^{-1}$ d.m., on average) resulted in higher values than the control (13.12 g N kg$^{-1}$ d.m.) and the fertilized sole wheat (11.45 g N kg$^{-1}$ d.m., on average). Then, a clear effect of the legume species was also observed, with pigeon bean determining the statistically highest values in all the treatments where it was included in (PB, PB-DW TIC, PB-DW TIC). And pigeon bean was actually the treatment determining the lowest level of weeds in the crops (Table 42). In 2010/11 (Table 45), weed N concentration at harvest was 18.37 g N kg$^{-1}$ d.m. for N0, 18.89 for fertilized sole wheat (averaged over all treatments), and 14.80 for sole legumes, over all species. Weeds collected under temporary and permanent intercropping treatments contained, on average, 21.61 and 15.33 g N kg$^{-1}$ d.m., respectively. The highest N concentration was observed for weeds collected in FP-DW TIC (25.65 g N kg$^{-1}$ d.m.). Again, the treatments lower in weed biomass showed the higher N concentration levels.

In both years, data on N accumulation in weed dry matter were mostly determined by the dry matter component, rather by N concentration (Tables 44 and 45).

Also soil cover, assessed with visual method, was considered as a parameter possibly helpful to understand the competition between crops and weeds under intercropping and sole crops. Data on visual soil cover of wheat, legumes and weeds at harvest (BBCH 89) in the two years are reported in Table 46. Weed cover was in line in both years with the dry matter production (Tables 42 and 43), with high values of soil cover in correspondence of high values of weed biomass. And so it was for each crop.

To investigate the soil cover provided by temporary and permanent intercropping over sampling time in the two years, the Soil Visual Cover Ratio (SVCR) was computed and plotted in Figure 31. With reference to the best performing sole crop, temporarily intercropped wheat covered the soil almost always lower in the two years. Only at BBCH 14 and 30, when also the companion legume was present, the SVCR was close to 1, which means equality to sole crop. Permanent intercropping covered soil generally better than sole crops (SVCR > 1), whatever the treatment. Still, at late stages, the differences between the two strategies of intercropping tended to decrease in magnitude, due to the late competition between component crops compared to pure stands. In the cases of HV- and FP-DW PIC in 2010/11 (Figure 31d and -f) negative values of SVCR were even achieved, meaning a worse soil cover than the best sole crop, which was vetch alone, for HV-DW PIC, and wheat alone under N160 for FP-DW PIC (Table 46). For pigeon bean-durum wheat permanent intercropping a positive value of SVCR was noticed even at harvest, due to the good contribution of wheat too (Figure 31b).

*Table 46 - Soil cover (%) of wheat, legumes, weeds and soil at BBCH 89 of wheat in 2009/10 and 2010/11.*

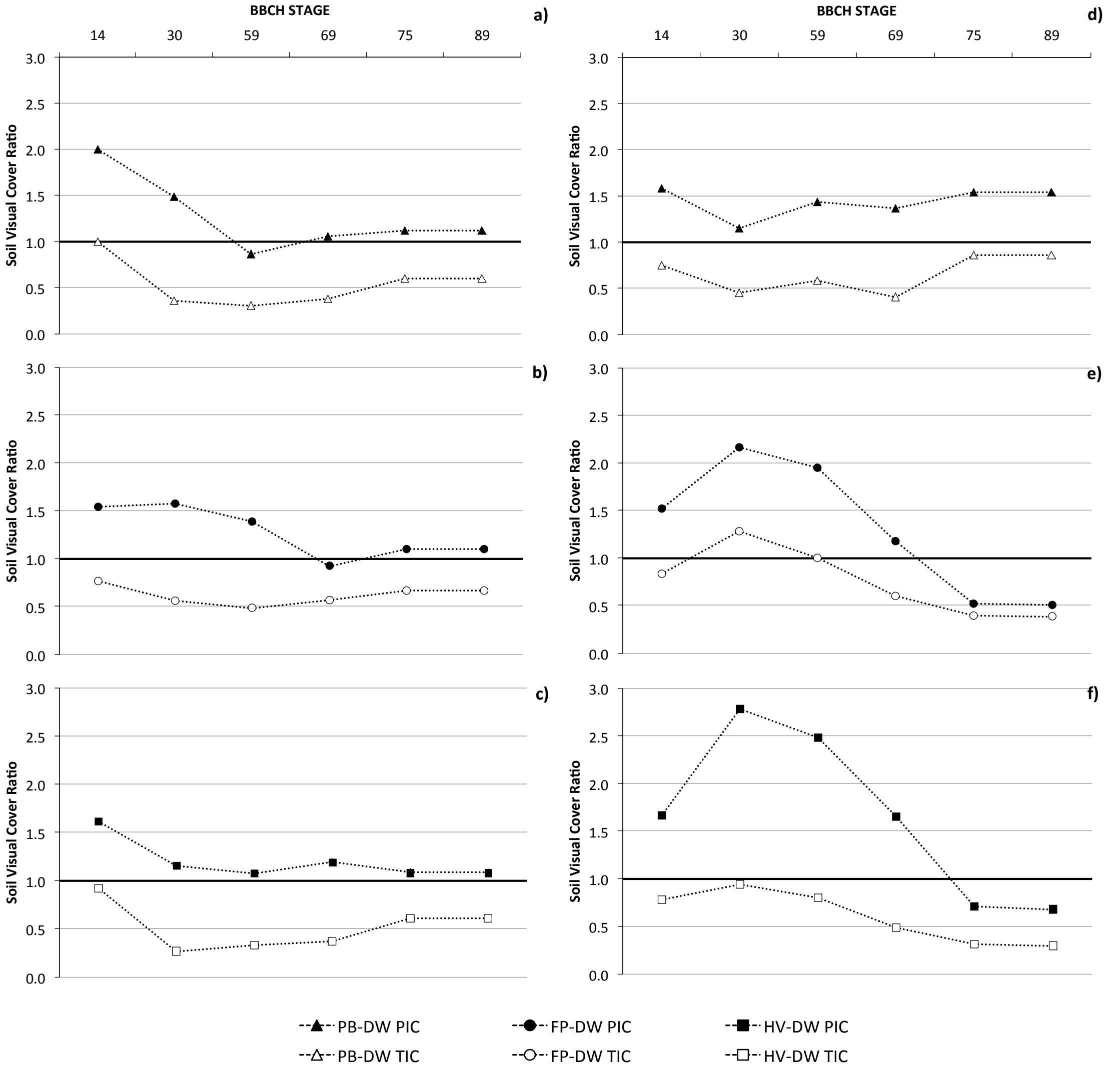
| **Treatment** | **2009/10** | | | | **2010/11** | | | |
|---|---|---|---|---|---|---|---|---|
| | **Wheat** | **Legume** | **Weeds** | **Bare Soil** | **Wheat** | **Legume** | **Weeds** | **Bare Soil** |
| N0 | 41.67 b | - | 45.00 bc | 18.33 | 31.00 bcde | - | 40.00 defg | 35.00 b |
| N40 | 46.67 ab | - | 35.00 bcd | 23.33 | 35.00 ab | - | 60.00 bcde | 18.33 bcde |
| N80 | 51.67 ab | - | 48.33 abc | 10.00 | 34.00 abc | - | 85.00 ab | 4.33 de |
| N120 | 55.00 ab | - | 50.67 ab | 8.33 | 33.33 bcd | - | 95.83 a | 14.33 cde |
| N160 | 60.00 a | - | 55.00 ab | 8.33 | 38.33 bcd | - | 76.67 abc | 13.33 cde |
| PB-DW TIC | 46.67 ab | - | 41.67 bc | 16.67 | 33.00 bcd | - | 8.00 g | 67.00 a |
| FP-DW TIC | 40.00 b | - | 43.33 bc | 21.67 | 32.67 bcd | - | 9.33 g | 67.33 a |
| HV-DW TIC | 46.67 ab | - | 35.00 bcd | 23.33 | 28.33 e | - | 15.33 fg | 71.67 a |
| PB-DW PIC | 14.00 c | 73.33 a | 10.00 e | 16.67 | 29.00 de | 30.00 b | 36.67 efg | 16.67 bcd |
| FP-DW PIC | 41.67 b | 24.17 b | 30.00 cd | 13.33 | 30.00 cde | 13.33 c | 36.67 efg | 26.00 bc |
| HV-DW PIC | 20.00 c | 63.33 a | 18.33 de | 11.67 | 30.00 cde | 35.00 b | 38.33 efg | 13.33 bcde |
| PB | - | 78.33 a | 11.67 e | 18.33 | - | 37.33 b | 73.33 abcde | 7.67 cde |
| FP | - | 28.33 b | 68.33 a | 11.67 | - | 11.67 c | 76.67 abc | 22.33 bc |
| HV | - | 76.67 a | 38.33 bc | 5.00 | - | 66.67 a | 51.67 cdef | 1.67 e |
| *Significance*[1] | ** | ** | ** | *ns* | ** | ** | ** | ** |
| *LSD*[3] | *(10.25)* | *(13.68)* | *(12.28)* | *(11.92)* | *(2.70)* | *(11.18)* | *(26.97)* | *(18.72)* |
| *CV (%)*[2] | *15.1* | *15.3* | *19.6* | *33.0* | *4.6* | *18.3* | *34.0* | *40.6* |

*[1] **, *, ns are, respectively, significant for $p \leq .01$, significant for $p \leq .05$ and not significant (LSD test)*

*[2] Coefficient of variation*

*[3] Under brackets values transformed*

*Figure 31- Visual Soil Cover Ratio of total crops (wheat + legume) grown under intercropping in 2009/10 (fig. a, b and c) and in 2010/11 (fig. d, e, f). x-axis (y = 1) is the maximum soil cover value among pure crops (wheat -all N fertilization rates- and legume - PB for PB-DW PIC and PB-DW TIC; FP for FP-DW PIC and FP-DW TIC; HV for HV-DW PIC and HV-DW TIC).*



Weed control is a crucial point for crop management, and particularly under organic farming. Intercropping is well documented to be an effective tool also for weed control (Bulson et al., 1997; Hauggaard-Nielsen et al., 2001a; Poggio, 2005; Hauggaard-Nielsen et al., 2006; Hauggaard-Nielsen et al., 2008; Corre-Hellou et al., 2011), although few exceptions (Pridham and Entz, 2008).

Our results are fully consistent with the literature. In our conditions, the competitive ability of intercropping against weeds mainly relied upon their efficiency in capturing a share of available resources greater than weeds. Space was the most important resource contended by plants at early stages of their establishment, whereas soil N and solar radiation became more and more important at further growth stages. Unlike permanent intercropping, for temporary intercropping the peculiar management strategy reduced the importance of free soil colonization by crops at early growth stages. The mechanical termination of the legume within temporary intercropping treatments actually acted as a direct weed control against already emerged weed flora, but also stimulated the rush of seed germination in spring. Consequently, the effectiveness of weed suppression under this particular kind of intercropping relied more upon the advantage in growth gained by the crop compared to new emerging weeds.

For permanent intercropping, the high rate of weed suppression observed in our experiment in both years is absolutely consistent with literature, where this strategy of intercropping is reported to allow a relevant control of weeds, over all several strategies and management options.

In an important study carried out by pooling data coming from a network of experiments across Western Europe, Corre-Hellou et al. (2011) found that neither the design of intercropping (i.e. additive or replacement) nor the seed ratio of component crops significantly altered the weed suppression of a spring pea-wheat intercropping. The observed reduction in weed biomass at maturity was extremely important, especially if compared to the sole pea plots, where weeds accounted for a three time higher biomass.

According to the study of Hauggaard-Nielsen et al. (2008), even the species of legumes was perceived as able to affect weed suppression under intercropping. In that research, the authors tested three different mixtures between barley and one of three legumes (pea, bean and lupin), very much differing in physiology and growth habit.

Conversely, seed density of component crops was reported by Hauggaard-Nielsen et al. (2006) to achieve a different rate of weed suppression in a spring pea-barley intercropping. In particular an increase in the seed density of pea was perceived as correlated with a relevant increase in weed suppression.

In the case of legumes characterized by prostrate habit rather than by upright growth, such as pea and vetch, the main determinant of their competitive ability against weeds can be identified in the rapidity of their growth and their efficiency in the colonization of free soil. Dry matter production at intermediate growth stages can be consequently a direct measure of weed suppression ability of these species. For instance, Poggio et al. (2005) related the low dry matter produced by weeds growing in a mixture of pea and barley to the level of crop productivity. The role of the legume was perceived as pivotal in order to bring to maximum level the weed suppressiveness of intercropping, whereas for the cereal a steady ability to compete with other species was basically undertaken.

Hauggaard-Nielsen et al. (2001a) confirmed the pivotal role played by the legumes, highlighting also the occurrence of an interesting mechanism of indirect competition driven by affinity for nitrogen. In this study, weeds grown in pea-barley intercropping showed a lower accumulation of N in their dry matter than under pea sole crop. This was mainly due to the fact that pea stimulated the growth of barley, and hence increased its sink ability for soil N, which consequently was not taken up by weeds, leading to low weed biomass and N accumulation.

In our experiment, a lower N accumulation in weed dry matter in intercropping than wheat and legume sole crop was observed too. Still, in our conditions we found also a higher concentration of N in the tissues of weed plants under intercropping than sole crops. This was not due, as expected, to a transfer of N from legumes to weeds growing in close proximity to them, but rather to a higher proportion of N in weed dry matter reversely related to the decrease in dry matter of weeds. The same finding was obtained also by Bulson et al. (1997), who reported an increasing value of N concentration in weeds collected in the plots of a wheat-bean intercropping at increasing seed density of the legume. Conversely, N accumulations in weed biomass decreased at increasing presence of bean, revealing the occurrence of a competition for, rather than a sharing of resources over the plant community.

### *3.1.9. Aboveground dry matter production and N accumulation in the whole plant community*

In Tables 47 and 48 data on the total aboveground dry matter production and N accumulation of the total plant community (crops + weeds) assessed in the two years at different phenological stages of wheat are reported.

Compared to separated data of total crops and weeds, these data do not change the understanding of the performance of different treatments in terms of resource use efficiency.

For dry matter production, as well as for N accumulation, a significant correlation with original data on total crops was observed, leading to the same conclusions drawn for crops and weed analysis.

*Table 47 - Aboveground dry matter production -DW- (g m$^{-2}$) and N accumulation -Nacc- (g m$^{-2}$) of total plant community (crops + weeds) in 2009/10. Values are means ± SD (n=3). Within each column, data followed by different letters are significantly different*

| **Treatment** | **BBCH 30** | | **BBCH 59** | | **BBCH 69** | | **BBCH 75** | | **BBCH 89** | |
|---|---|---|---|---|---|---|---|---|---|---|
| | **DW** | **Nacc** | **DW** | **Nacc** | **DW** | **Nacc** | **DW** | **Nacc** | **DW** | **Nacc** |
| N0 | 107.84 ± 6.81 d | 1.23 ± 0.06 f | 256.36 ± 42.04 f | 2.01 ± 0.20 f | 328.44 ± 75.79 f | 2.10 ± 0.37 f | 458.26 ± 84.32 d | 4.45 ± 0.65 d | 587.31 ± 115.10 de | 5.63 ± 1.29 d |
| N40 | 190.69 ± 10.21 abc | 2.46 ± 0.18 def | 374.99 ± 101.50 cdef | 3.14 ± 0.95 ef | 621.22 ± 221.80 bcde | 3.62 ± 1.31 def | 729.63 ± 187.15 bc | 5.24 ± 1.87 cd | 795.59 ± 182.06 bcd | 6.99 ± 1.21 cd |
| N80 | 219.91 ± 33.45 abc | 2.70 ± 0.17 def | 487.13 ± 182.77 bc | 6.24 ± 1.95 cde | 771.15 ± 206.00 abc | 6.33 ± 2.28 cdef | 928.07 ± 172.22 ab | 8.17 ± 1.94 bcd | 980.64 ± 164.86 abc | 9.14 ± 1.73 bcd |
| N120 | 225.54 ± 34.93 abc | 2.90 ± 0.31 de | 666.77 ± 59.57 a | 6.79 ± 1.60 cd | 849.63 ± 163.33 ab | 7.51 ± 2.16 bcd | 997.89 ± 189.00 a | 9.67 ± 1.69 bc | 1059.33 ± 242.25 ab | 11.02 ± 3.35 bcd |
| N160 | 249.10 ± 68.35 a | 4.02 ± 0.70 cd | 547.01 ± 71.50 ab | 9.13 ± 1.77 abc | 862.55 ± 75.19 a | 10.89 ± 1.51 sb | 1095.61 ± 96.01 a | 12.46 ± 4.16 b | 1153.92 ± 103.94 a | 13.91 ± 2.78 b |
| PB-DW TIC | 227.33 ± 30.45 ab | 4.85 ± 0.57 bc | 336.61 ± 19.14 def | 3.11 ± 0.15 ef | 387.50 ± 7.21 f | 3.59 ± 0.07 def | 569.22 ± 82.01 cd | 5.81 ± 0.80 cd | 617.97 ± 39.47 de | 7.18 ± 0.85 cd |
| FP-DW TIC | 168.33 ± 57.87 bcd | 2.60 ± 1.52 def | 285.35 ± 45.17 ef | 2.58 ± 0.31 f | 357.09 ± 77.42 f | 2.93 ± 1.62 ef | 513.38 ± 52.21 a | 4.57 ± 1.08 cd | 577.89 ± 69.12 de | 5.54 ± 0.80 cd |
| HV-DW TIC | 229.72 ± 75.54 ab | 4.80 ± 0.81 bc | 285.28 ± 30.17 ef | 3.14 ± 0.42 ef | 406.82 ± 55.33 ef | 3.43 ± 0.67 def | 601.30 ± 89.00 cd | 6.06 ± 1.46 cd | 690.79 ± 76.98 de | 7.76 ± 0.92 cd |
| PB-DW PIC | 224.99 ± 21.79 abc | 5.17 ± 0.64 bc | 432.14 ± 103.66 bcd | 10.35 ± 2.62 ab | 553.26 ± 46.44 cdef | 11.19 ± 1.86 ab | 886.75 ± 189.49 ab | 20.42 ± 2.41 a | 1073.60 ± 221.88 ab | 23.92 ± 3.95 a |
| FP-DW PIC | 155.02 ± 36.66 cd | 2.08 ± 0.82 ef | 357.55 ± 93.52 cdef | 4.48 ± 1.85 def | 488.48 ± 171.19 def | 5.15 ± 2.27 cdef | 578.38 ± 81.33 cd | 7.60 ± 2.33 bcd | 648.62 ± 84.44 de | 9.59 ± 2.30 bcd |
| HV-DW PIC | 229.47 ± 17.01 ab | 4.60 ± 0.08 bc | 404.56 ± 55.37 bcde | 6.64 ± 1.13 cd | 506.72 ± 130.50 def | 7.11 ± 1.76 bcde | 569.30 ± 149.84 cd | 7.67 ± 2.47 bcd | 693.92 ± 178.06 de | 10.41 ± 3.45 bcd |
| PB | 237.00 ± 41.22 ab | 6.98 ± 1.35 a | 486.00 ± 67.09 bc | 11.88 ± 1.47 a | 651.67 ± 116.80 abcd | 14.64 ± 3.14 a | 1114.33 ± 222.15 a | 24.31 ± 5.68 a | 1191.75 ± 203.62 a | 26.48 ± 3.80 a |
| FP | 113.34 ± 44.41 d | 2.73 ± 2.12 de | 285.33 ± 162.26 def | 6.56 ± 4.84 bcd | 475.33 ± 308.14 def | 8.17 ± 7.48 bc | 707.67 ± 281.40 cd | 12.40 ± 8.04 b | 776.72 ± 343.19 cde | 12.32 ± 8.51 bc |
| HV | 192.00 ± 66.78 abc | 5.78 ± 1.34 ab | 308.33 ± 105.62 def | 7.87 ± 2.62 bc | 342.33 ± 124.58 f | 8.67 ± 2.92 bc | 448.00 ± 133.63 d | 9.40 ± 2.59 c | 509.56 ± 189.12 e | 10.99 ± 3.12 bcd |
| *Significance*[1] | ** | ** | ** | ** | ** | ** | ** | ** | ** | ** |
| *LSD* | *71.26* | *1.58* | *146.98* | *3.24* | *232.79* | *4.45* | *232.22* | *5.10* | *282.38* | *5.51* |
| *CV (%)*[2] | *21.5* | *24.9* | *22.2* | *32.1* | *25.5* | *38.6* | *19.0* | *31.0* | *20.7* | *28.7* |

*[1] **, *, ns are, respectively, significant for p≤.01, significant for p≤.05 and not significant (LSD test)*

*[2] Coefficient of variation*

Table 48 - Aboveground dry matter production -DW- (g m$^{-2}$) and N accumulation -Nacc- (g m$^{-2}$) of total plant community (crops + weeds) in 2010/11. Values are means ± SD (n=3). Within each column, data followed by different letters are significantly different

| Treatment | BBCH 30 | | BBCH 59 | | BBCH 69 | | BBCH 75 | | BBCH 89 | |
|---|---|---|---|---|---|---|---|---|---|---|
| | DW | Nacc | DW | Nacc | DW | Nacc | DW | Nacc | DW | Nacc |
| N0 | 55.67 ± 10.60 d | 1.07 ± 0.26 c | 241.33 ± 111.34 de | 3.35 ± 1.15 fg | 278.00 ± 97.53 ef | 3.59 ± 1.27 fgh | 435.33 ± 197.63 cdef | 5.35 ± 2.63 d | 501.79 ± 77.31 defg | 6.37 ± 0.98 e |
| N40 | 72.33 ± 7.77 cd | 1.81 ± 0.30 bc | 319.67 ± 21.57 bcd | 4.25 ± 0.62 def | 456.33 ± 15.28 abc | 5.63 ± 0.84 def | 599.00 ± 82.27 abc | 7.09 ± 2.23 bcd | 692.1 ± 59.81 bc | 8.56 ± 0.92 cde |
| N80 | 79.66 ± 4.04 cd | 2.15 ± 0.11 bc | 390.67 ± 24.68 abc | 5.62 ± 0.44 cde | 466.00 ± 31.05 abc | 6.07 ± 0.69 cde | 707.67 ± 18.93 a | 9.20 ± 0.54 ab | 834.14 ± 84.79 ab | 11.25 ± 1.66 bc |
| N120 | 72.00 ± 18.52 cd | 1.92 ± 0.62 bc | 401.00 ± 46.89 ab | 5.39 ± 1.15 cde | 513.00 ± 87.47 ab | 6.34 ± 1.63 cde | 706.66 ± 74.19 a | 9.37 ± 1.24 ab | 882.25 ± 123.31 a | 13.07 ± 1.78 ab |
| N160 | 81.67 ± 32.25 cd | 2.37 ± 1.01 bc | 407.00 ± 125.05 ab | 6.11 ± 1.81 bcd | 473.67 ± 137.32 ab | 6.27 ± 2.26 cde | 721.67 ± 168.78 a | 9.36 ± 2.76 ab | 963.6 ± 50.86 a | 14.17 ± 0.79 a |
| PB-DW TIC | 227.67 ± 70.51 a | 6.48 ± 2.20 a | 189.00 ± 32.74 e | 2.05 ± 0.54 g | 208.00 ± 36.51 f | 2.42 ± 0.86 h | 338.33 ± 50.50 f | 4.43 ± 1.17 d | 384.77 ± 103.12 g | 5.70 ± 2.13 e |
| FP-DW TIC | 133.67 ± 25.15 b | 2.79 ± 0.68 b | 203.67 ± 52.52 e | 2.60 ± 0.72 fg | 250.67 ± 45.80 ef | 2.78 ± 0.70 h | 382.33 ± 108.09 def | 4.69 ± 1.13 d | 432.70 ± 52.05 fg | 6.14 ± 1.68 e |
| HV-DW TIC | 102.34 ± 42.16 bcd | 2.58 ± 1.14 b | 188.66 ± 38.53 e | 2.68 ± 0.76 fg | 236.34 ± 59.94 ef | 2.95 ± 1.14 gh | 350.00 ± 63.51 ef | 4.39 ± 1.56 d | 471.99 ± 31.53 efg | 6.46 ± 1.23 e |
| PB-DW PIC | 228.34 ± 31.53 a | 6.38 ± 0.90 a | 488.66 ± 52.18 a | 8.36 ± 1.11 a | 569.00 ± 67.10 a | 9.46 ± 1.21 a | 649.67 ± 47.52 ab | 10.39 ± 0.72 a | 670.04 ± 31.53 c | 11.09 ± 1.55 bc |
| FP-DW PIC | 132.33 ± 55.37 b | 2.78 ± 1.07 b | 335.66 ± 79.43 bcd | 4.32 ± 0.82 def | 417.66 ± 68.00 bcd | 5.16 ± 0.63 defg | 528.01 ± 44.00 bcd | 6.21 ± 0.13 cd | 633.15 ± 35.95 cd | 7.54 ± 0.34 de |
| HV-DW PIC | 110.99 ± 25.00 bc | 2.83 ± 0.71 b | 319.33 ± 31.47 bcd | 6.18 ± 0.64 bc | 416.34 ± 74.00 bcd | 6.90 ± 1.23 bcd | 515.33 ± 119.78 bcde | 8.15 ± 2.08 abc | 573.38 ± 84.11 cdef | 9.69 ± 1.75 cd |
| PB | 209.00 ± 7.00 a | 6.59 ± 0.35 a | 393.99 ± 65.28 abc | 7.67 ± 1.28 ab | 409.33 ± 45.72 bcd | 8.84 ± 0.53 ab | 474.33 ± 55.54 cdef | 9.94 ± 1.32 d | 588.66 ± 135.11 cde | 11.11 ± 2.92 bc |
| FP | 111.00 ± 34.39 bc | 2.36 ± 0.96 bc | 263.00 ± 107.09 de | 3.93 ± 1.74 ef | 293.99 ± 102.33 def | 4.59 ± 1.27 efgh | 398.67 ± 69.21 def | 5.84 ± 1.07 d | 427.67 ± 59.76 g | 6.12 ± 0.94 e |
| HV | 78.67 ± 2.08 cd | 2.24 ± 0.15 bc | 278.67 ± 24.17 | 7.08 ± 0.71 abc | 341.00 ± 85.06 cde | 7.66 ± 1.90 abc | 463.34 ± 81.21 cdef | 9.24 ± 1.62 d | 473.33 ± efg | 9.62 ± 2.45 cd |
| *Significance* | ** | ** | ** | ** | ** | ** | ** | ** | ** | ** |
| *LSD* | *49.09* | *1.38* | *115.59* | *180.39* | *129.21* | *2.17* | *168.18* | *2.79* | *145.62* | *2.80* |
| *CV (%)*$^{2}$ | *24.2* | *26.0* | *21.8* | *21.7* | *20.2* | *23.0* | *19.3* | *22.5* | *14.2* | *18.4* |

[1] **, *, ns are, respectively, significant for p≤.01, significant for p≤.05 and not significant (LSD test)

[2] Coefficient of variation

### *3.1.10. Residual effect of fertility on the following maize crop*

A potential residual effect of fertility of treatments tested in 2009/10 was studied in 2010/11 on the same original field through the assessment of the dry matter (Table 49) and of the N accumulation (Table 50) of a grain maize crop.

Data clearly showed that a residual effect of fertility did not take place for any of the tested parameters.

*Table 49 - Number of plants ($m^{-2}$), number of ears ($m^{-2}$), dry matter production (t $ha^{-1}$) of different plant components of maize grown in 2010/11 on the first year plots. Within each column, data followed by different letter are significantly different.*

| **Treatment** | **Nr. of plants** | **Nr.of ears** | **Dry Matter Production** | | | | | **Harvest Index (%)** |
|---|---|---|---|---|---|---|---|---|
| | | | **Stubbles** | **Cobs** | **Residues** | **Grain** | **Total** | |
| N0 | 6.67 | 4.83 | 1.70 | 0.32 | 2.02 | 1.10 | 2.12 | 51.89 |
| N40 | 7.83 | 5.83 | 1.88 | 0.31 | 2.19 | 1.16 | 3.35 | 34.63 |
| N80 | 7.83 | 4.67 | 2.20 | 0.25 | 2.45 | 0.99 | 3.44 | 28.78 |
| N120 | 7.50 | 5.33 | 2.16 | 0.31 | 2.47 | 1.26 | 3.73 | 33.78 |
| N160 | 6.17 | 5.00 | 1.90 | 0.35 | 2.25 | 1.31 | 3.56 | 36.80 |
| PB-DW TIC | 7.17 | 5.50 | 2.14 | 0.41 | 2.55 | 1.47 | 4.02 | 36.57 |
| FP-DW TIC | 6.83 | 5.33 | 2.20 | 0.39 | 2.59 | 1.47 | 4.06 | 36.21 |
| HV-DW TIC | 7.00 | 5.00 | 1.98 | 0.35 | 2.33 | 1.14 | 3.47 | 32.85 |
| PB-DW PIC | 6.50 | 4.50 | 1.88 | 0.29 | 2.17 | 1.13 | 3.40 | 33.24 |
| FP-DW PIC | 7.33 | 5.83 | 1.80 | 0.27 | 2.07 | 1.15 | 3.22 | 35.71 |
| HV-DW PIC | 5.17 | 4.00 | 1.53 | 0.30 | 1.83 | 1.11 | 2.94 | 37.76 |
| PB | 6.00 | 5.00 | 1.74 | 0.37 | 2.11 | 1.39 | 3.50 | 39.71 |
| FP | 6.83 | 4.83 | 1.92 | 0.35 | 2.27 | 1.39 | 3.66 | 37.98 |
| HV | 8.17 | 5.83 | 2.13 | 0.32 | 2.45 | 1.22 | 3.67 | 33.24 |
| *Significance[1]* | *ns* | *ns* | *ns* | *ns* | *ns* | *ns* | *ns* | *ns* |
| *LSD* | *3.13* | *3.28* | *1.39* | *0.29* | *1.64* | *1.07* | *2.63* | *11.16* |
| *CV (%)[2]* | *22.0* | *31.2* | *34.9* | *42.9* | *35.1* | *42.2* | *36.5* | *15.6* |

*[1] **, *, ns are, respectively, significant for p≤.01, significant for p≤.05 and not significant (LSD test)*

*[2] Coefficient of variation*

*Table 50 - N concentration (g kg$^{-1}$) and accumulation (kg ha$^{-1}$) in different plant components of maize grown in 2010/11 on the first year plots. Within each column, data followed by different letter are significantly different.*

| Treatment | N concentration (g kg$^{-1}$) | | | N accumulation (kg ha$^{-1}$) | | | | |
|---|---|---|---|---|---|---|---|---|
| | Stubbles | Cobs | Grain | Stubbles | Cobs | Residues | Grain | Total |
| N0 | 3.50 | 6.27 | 13.10 | 5.95 | 2.01 | 7.96 | 14.41 | 22.37 |
| N40 | 3.70 | 8.17 | 14.40 | 6.96 | 2.53 | 9.49 | 16.70 | 26.19 |
| N80 | 4.17 | 8.07 | 13.77 | 9.17 | 2.02 | 11.19 | 13.63 | 24.82 |
| N120 | 4.63 | 7.07 | 14.80 | 10.00 | 2.19 | 12.19 | 18.65 | 30.84 |
| N160 | 3.93 | 7.33 | 14.40 | 7.47 | 2.57 | 10.04 | 18.86 | 28.90 |
| PB-DW TIC | 5.30 | 5.47 | 14.00 | 11.34 | 2.24 | 13.58 | 20.58 | 34.16 |
| FP-DW TIC | 6.40 | 6.93 | 14.43 | 14.08 | 2.70 | 16.78 | 21.21 | 37.99 |
| HV-DW TIC | 4.70 | 7.45 | 13.25 | 9.31 | 2.61 | 11.92 | 15.11 | 27.03 |
| PB-DW PIC | 4.37 | 10.03 | 16.27 | 8.22 | 2.91 | 11.13 | 18.39 | 29.52 |
| FP-DW PIC | 4.67 | 7.60 | 15.53 | 8.41 | 2.05 | 10.46 | 17.86 | 28.32 |
| HV-DW PIC | 5.53 | 7.17 | 16.07 | 8.46 | 2.15 | 10.61 | 17.84 | 28.45 |
| PB | 4.57 | 7.17 | 14.53 | 7.95 | 2.65 | 10.60 | 20.20 | 30.80 |
| FP | 4.40 | 6.73 | 14.33 | 8.45 | 2.36 | 10.81 | 19.92 | 30.73 |
| HV | 4.73 | 7.70 | 15.30 | 10.07 | 2.46 | 12.53 | 18.67 | 31.20 |
| *Significance*[1] | *ns* | *ns* | *ns* | *ns* | *ns* | *ns* | *ns* | *ns* |
| *LSD* | *3.29* | *4.02* | *3.43* | *7.89* | *1.87* | *9.19* | *14.92* | *22.13* |
| *CV (%)*[2] | *34.7* | *26.5* | *11.4* | *43.5* | *40.0* | *40.3* | *40.8* | *37.3* |

*[1] **, *, ns are, respectively, significant for $p \leq .01$, significant for $p \leq .05$ and not significant (LSD test)*

*[2] Coefficient of variation*

Our results on maize were consistent with those on final dry matter and N yield of wheat in 2009/10, which revealed an overuse of N under intercropping, exceeding the amount supplied by legumes in many cases. Furthermore, the adverse weather condition in the year after, characterized by frequent rainfall during fall-winter, did not allow to seed a winter crop, which would have been better to match our requirements (i.e. a fast growing catch crop). Conversely, the growth of a spring crop after a very rainy winter did not permit to observe a potential N sparing following legumes and intercropping. First, this was because the risk of significant nitrate leaching was not averted due to the absence of a crop in the winter. Second, also an interaction between residual N and N newly formed from soil organic matter mineralization, peaking at this latitude in fall and spring, might have taken place.

Nonetheless, to our knowledge none of the papers in the literature which dealt with this issue reported different findings. For instance, Hauggaard-Nielsen et al. (2009b) did not observe any significant effect neither on soil mineral N content, nor on the productivity of a rye crop following a pea-barley intercropping under organic conditions. In a former lysimeter study with the same crop sequence, Hauggaard-Nielsen et al. (2003) did not find any significant effect on the following crop (i.e. rye), nor on nitrate leaching during fallow after rye harvest. Consistently, Szumigalski and Van Acker (2006) reported a higher level of soil nitrates in plots following a sole crop of pea, rather than the pea-wheat intercropping.

### *3.1.10. Management options to improve resource use efficiency of intercropping*

Overall, in our conditions both permanent and temporary intercropping showed possibility to improve their efficiency, by fine-tuning crop management. Nonetheless, as well argued by Fukai and Trenbath (1993), manipulating only the component crop with the lower proportion in the mixture may not lead to great effect on the best performing species, and hence on the whole intercropping, due to the complex interspecific and intraspecific interactions. Therefore, the best way to enhance the efficiency of an inefficient intercropping should be to rearrange the whole system, rather than its single components.

Midmore (1993) discussed in-depth many different options to intensify the ecological efficiency of intercropping, identifying the most important ones in: i) N fertilization; ii) differentiation in sowing date of component crops; iii) irrigation; iv) spatial arrangement of crops.

Fukai and Trenbath (1993) underlined also the importance of an appropriate choice of crop genotype. For instance, the selection of growth-determined, not vigorous cultivars of the dominating species, may be helpful to increase the presence of the dominated crop, especially under additive design of intercropping. In our conditions, the results of mixtures clearly put the light on the little competitive ability of wheat, over all intercropping treatments. Therefore, the use of tall, fast-growing wheat varieties, different from the one used in the experiment (namely, cv. *Claudio*), would probably have been able to reduce the aggressivity of legumes, as also obtained by Tosti and Guiducci (2010) with cv. *Cappelli*, a traditional tall wheat variety. On the other hand, a different cultivar choice would have been also recommendable for pigeon bean, as cv. *Torre Lama Scuro* demonstrated to be too much vigorous, tall and fast-growing to be intercropped with wheat in our environment.

Launay et al. (2009) tested through modeling some other management options in order to enhance the performances of a pea-barley intercrop. Concerning sowing, the authors tested the effect of an earlier sowing date for barley than pea, with the aim to explore potential benefits for the establishment of the cereal. Their results suggest that this option can be effective, but with differences in function of the environment.

A temporal differentiation of sowing of the different strategies of intercropping may be worthy also in our conditions, as our results clearly showed the occurrence of an early growth detriment of intercropped wheat. For temporary intercropping, an early contemporary sowing of legumes and wheat would have possibly helped to increase the biomass production of the legume at the end of the winter, and hence to maximize the amount of N returned to the soil through the mineralization of the legume biomass ploughed under. In this eventuality, also an early termination of legumes would have taken place, in order to reduce the duration of co-growth and thus the competition for wheat. Anyway, this combination of things is not very easy to put into practice, as in our climate conditions both early fall and late winter are periods with peaks of rainfall, and hence fields are hardly accessible to machines.

Interseeding of legumes late in the season in established wheat crop (the so called “relay intercropping”) may be an other suitable option to decrease the early interspecific competition between wheat and legumes, as well as to enhance facilitation and complementary growth in the pivotal stage for wheat grain yield and quality, i.e. from end of tillering onwards. Good results with this technique were achieved by several studies (Thiessen Martens et al., 2001; Amossé et al., 2013b). Contrastingly, Blaser et al. (2011) reported also the risk of decrease in legume growth due to the shading caused by the greater development of the winter cereal.

For our experiment purposes, this option would have been suitable for permanent intercropping, and especially for wheat-bean intercropping, in order to reduce early interspecific competition and enhance late facilitation. To reduce the presence of winter-germinating weeds in the inter-row space in absence of the legume until interseeding, mechanical weeding operations (e.g. tine harrowing) would have been also considered.

Launay et al. (2009) examined also the option of manipulating seeding density and seed ratio of intercrops. These two aspects influence the choice of intercropping design (i.e. replacement or additive) and might have been extremely important also in our conditions in order to increase the facilitation and to reduce the competition between species in mixture. In their work, Launay et al. (2009) reported that these options were not as viable as expected, due to their strong dependence on environmental conditions. A weak effect of seed ratio on the performances of the intercropping, and specifically a mixture of vetch and winter cereals (wheat, oats, barley and triticale), was also found by Dhima et al. (2007). Unconsistently, Bulson et al. (1997) found a significant increase in the LER of a wheat-bean intercropping system under organic farming conditions, when both the component crops were sown at 75% of their full seeding rate.

Interestingly, Hauggaard-Nielsen et al. (2006) studied the combination of different seeding densities (from 50% to 200% the recommended density) with 2 seeding ratios (1/3 legume:2/3 cereal vs 2/3 legume:1/3 cereal) on a field pea-spring barley intercrop. Results underlined the importance of high seeding density (200% of recommended value) for the legume, which significantly increased its proportion in the mixture more than proportionally respect to the specific seed ratio. On the other hand, an increased proportion of the legume in the mixture also negatively reflected on the value of soil N uptake of intercropping.

In our experiment, the adoption of an additive design with both species sown at full seed rate resulted in an excessive inter-specific competition for the most limiting factors (i.e. radiation, space and nitrogen), as also explained by Fukai and Trenbath (1993). Also intra-specific competition, imposed by the intra-row plant density twice that normal, probably played a key role, as supported by the small number of tillers per plant showed by wheat in the two years of the experiment. In the light of our results, a shift to a replacement design would have been valuable, even though some important previous studies did not report any significant improvements from changing the design of intercropping (Hauggaard-Nielsen et al., 2009a; Benincasa et al., 2012).

Regarding seed density, an increase would have been worthy for field pea, which showed actually the lower presence in the tested mixtures. Conversely, for pigeon bean a strong decrease in its seeding density would have been even better. For hairy vetch the technique used in the experiment was quite effective, although a decrease in the seeding density would have been helpful to reduce intra-specific competition. Still, whatever the legume, also a reduction in wheat plant density would have been advisable, in order to drastically reduce intra-specific competition of wheat and to stimulate the production of secondary tillers.

All these impressions are confirmed by the results obtained for pigeon bean by Mariotti et al. (2012), for hairy vetch by Tosti et al. (2010), and for field pea by Hauggaard-Nielsen et al. (2006).

Additionally, interspecific competition can be also manipulated through the adoption of a different spatial design of intercropping, aimed to reduce or enhance the level of inter-specific interactions, depending on the actual complementarity of component crops.

Eskandari et al. (2011) tested, without significant results, the effect of three different spatial arrangements of a wheat-bean intercropping (alternate row intercrop; within row intercrop; mixed intercrop). In same conditions as ours, Mariotti et al. (2012) assessed the effect of two row ratios for a durum wheat-pigeon bean intercropping for silage. The highest row ratio for wheat (2 wheat : 1 bean) succeeded to increase wheat proportion in the mixture from 0.34 to 0.41. In our experiment, this option would have been suitable also for permanent intercropping, and particularly for wheat intercropped with pigeon bean.

Finally, N fertilization may be another technical option, potentially able to reduce the disadvantages suffered by wheat from the peculiar conditions of intercropping (i.e. high crop density, shading, etc.). In the literature, several studies dealt with the effect of additional provision of N to legume-cereal intercropping, with focus on total dry matter production, total N accumulations, and effects on symbiotic $N_2$-fixation (Jensen, 1996; Andersen et al., 2005; Ghaley et al., 2005; Bedoussac and Justes, 2009; Naudin et al., 2010; Mariotti et al., 2012; Pelzer et al., 2012). Summarizing, their results clearly demonstrated that the early application of moderate amount of mineral N to intercropping generally favored the cereal component in terms of dry matter production and N accumulations, resulting in more efficient use of resources of whole intercropping and higher complementarity between components if compared to unfertilized intercrops. On the other hand, the application of external inputs of N into the system may also generate deleterious effects on legume nodulation and symbiotic $N_2$-fixation, as also demonstrated by previous studies (Herridge et al., 1984; Peoples et al., 1995b; Jensen, 1996; Andersen et al., 2005; Bedoussac and Justes, 2009), leading to a lower relative importance of the legume in the mixture, and hence limiting benefits coming from co-growth of cereals and legumes.

This option has doubtful advantages if applied to the context of organic farming, where synthetic mineral fertilizer are banned, and the application of N through organic fertilizers could fail to improve intercropping efficiency because of the slow and variable availability for the plants of this form of N. Moreover, additional expensive inputs of N to the system may negatively reduce the economical viability of intercropping, also affected by the costs for crop establishment and for legume management, higher than for pure crops.

## 3.2. Lysimeter Experiment

The results of wheat performances under the different treatments in the first season will be discussed first, then the main findings on the effect of the treatments on N mineralization and leaching through the analysis of N leached content in drainage water will be reported. All the results will be discussed separately for each sampling date (e.g. for crop performance) or period (e.g. for N content in drainage water), and globally at the end, through the analysis of the apparent N budget.

### *3.2.1. Four-leaves stage of wheat (BBCH 14)*

At the first sampling date, occurred at the 4-leave stage of wheat, no significant differences were observed in terms of dry matter production of wheat grown under different treatments (Table 51). Anyway, the analysis of plant height revealed higher mean values for wheat fertilized with the organic fertilizer, whatever the number of applications.

The evidence of a higher availability of nitrogen for wheat fertilized with dried blood was confirmed by data on N concentration and N accumulations in the dry matter of wheat at the same date (Table 52).

The highest concentration of N in wheat leaves what found for N80 ORG (33.13 mg $g^{-1}$), which was significantly superior than all the other treatments, except for N40+40 ORG (29.17 mg $g^{-1}$). As a result, N accumulated in the dry matter showed the same trend as for N concentration, with N80 ORG showing the highest values (4.64 g $plant^{-1}$). The remaining treatments did not differ from each other.

Wheat under permanent intercropping with bean showed a slight increase in dry matter production and plant height compared to the N0.

*Table 51 - Dry matter weight, number of ears/pods and plant height of wheat and pigeon bean (under PB -DW TIC) at 4-leave stage of wheat (**BBCH 14**) in 2009/10. Within each column, data followed by different letters are significantly different.*

| BBCH 14 | | | | | | |
|---|---|---|---|---|---|---|
| **Treatment** | **Dry matter production ($g\ plant^{-1}$)** | | | | | **Plant height ($cm$)[3]** |
| | **Leaves[3]** | **Stems** | **Ears/Pods** | **Grain** | **Total Biomass[3]** | |
| N0 | 0.09 | - | - | - | 0.09 | 13.33 b |
| N40+40 ORG | 0.13 | - | - | - | 0.13 | 18.75 a |
| N80 MIN | 0.10 | - | - | - | 0.10 | 13.08 b |
| N 80 ORG | 0.14 | - | - | - | 0.14 | 18.33 a |
| PB-DW TIC | | | | | | |
| *Wheat* | 0.10 | - | - | - | 0.10 | 13.58 b |
| *Pigeon bean* | (0.46) | - | - | - | (0.46) | (11.17) |
| *Total* | (0.56) | - | - | - | (0.56) | - |
| *Significance[1]* | *ns* | - | - | - | *ns* | ** |
| *LSD* | *0.04* | - | - | - | *0.04* | *2.32* |
| *CV (%)[2]* | *20.2* | - | - | - | *20.2* | *8.3* |

*[1] **, *, ns are, respectively, significant for p≤.01, significant for p≤.05 and not significant (LSD test)*

*[2] Coefficient of variation*

*[3] Between brackets, data excluded from the ANOVA*

*Table 52 - N concentration ($mg\ g^{-1}$) and accumulation ($mg\ plant^{-1}$) in different plant components of wheat and pigeon bean (under PB -DW TIC) at 4-leave stage of wheat (**BBCH 14**) in 2009/10. Within each column, data followed by different letters are significantly different.*

| BBCH 14 | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|
| **Treatment** | **N concentration ($mg\ g^{-1}$)[3]** | | | | **N accumulation ($mg\ plant^{-1}$) [3]** | | | | |
| | **Leaves** | **Stems** | **Ears/ Pods** | **Grain** | **Leaves** | **Stems** | **Ears/ Pods** | **Grain** | **Total** |
| N0 | 25.03 c | - | - | - | 2.25 c | - | - | - | 2.25 c |
| N40+40 ORG | 29.17 ab | - | - | - | 3.79 ab | - | - | - | 3.79 ab |
| N80 MIN | 24.80 c | - | - | - | 2.48 c | - | - | - | 2.48 c |
| N 80 ORG | 33.13 a | - | - | - | 4.64 a | - | - | - | 4.64 a |
| PB-DW TIC | | | | | | | | | |
| *Wheat* | 25.30 bc | - | - | - | 2.53 bc | - | - | - | 2.53 bc |
| *Pigeon bean* | (3.47) | - | - | - | (1.60) | - | - | - | (1.60) |
| *Total* | - | - | - | - | (4.13) | - | - | - | (4.13) |
| *Significance[1]* | ** | - | - | - | ** | - | - | - | ** |
| *LSD* | *3.97* | - | - | - | *1.30* | - | - | - | *1.30* |
| *CV (%)[2]* | *7.9* | - | - | - | *22.5* | - | - | - | *22.5* |

*[1] **, *, ns are, respectively, significant for p≤.01, significant for p≤.05 and not significant (LSD test)*

*[2] Coefficient of variation*

*[3] Between brackets, data excluded from the ANOVA*

### *3.2.2. Stem elongation stage of wheat (BBCH 30)*

At stem elongation stage of wheat (BBCH30), treatments produced significant differences both for dry matter production of leaves and mean plant height (Table 53).

*Table 53 - Dry matter weight, number of ears/pods and plant height of wheat and pigeon bean (under PB-DW TIC) at end of stem elongation stage of wheat (**BBCH 30**) in 2009/10. Within each column, data followed by different letters are significantly different.*

| **BBCH 30** | | | | | | |
|---|---|---|---|---|---|---|
| **Treatment** | **Dry matter production (g plant$^{-1}$)** | | | | | **Plant height (cm)$^3$** |
| | **Leaves$^3$** | **Stems** | **Ears/Pods** | **Grain** | **Total Biomass$^3$** | |
| N0 | 0.27 c | - | - | - | 0.27 c | 29.83 c |
| N40+40 ORG | 0.89 a | - | - | - | 0.89 ab | 46.33 a |
| N80 MIN | 0.69 ab | - | - | - | 0.69 ab | 40.17 ab |
| N 80 ORG | 0.86 a | - | - | - | 0.86 a | 43.83 a |
| PB-DW TIC | | | | | | |
| *Wheat* | 0.48 bc | - | - | - | 0.48 bc | 31.43 bc |
| *Pigeon bean* | (2.14) | - | - | - | (2.14) | (31.50) |
| Total | (2.62) | - | - | - | (2.62) | - |
| *Significance$^1$* | ** | - | - | - | ** | * |
| *LSD* | *0.30* | - | - | - | *0.30* | *10.01* |
| *CV (%)$^2$* | *26.0* | - | - | - | *26.0* | *14.4* |

*[1] **, *, ns are, respectively, significant for p≤.01, significant for p≤.05 and not significant (LSD test)*
*[2] Coefficient of variation*
*[3] Between brackets, data excluded from the ANOVA*

N40+40 ORG and N80 ORG were again the treatments with the highest wheat dry matter, but also NMIN started to differentiate from the control (N0). Wheat under intercropping revealed for the two parameters values between fertilized treatments and the control. There was no significant difference between PB-DW TIC and N80 MIN which have received at that moment only half rate of N. Intercropped wheat showed the same plant height than the companion pigeon bean.

Concerning N concentration (Table 54), it is noteworthy that wheat grown under intercropping (14.07 mg g$^{-1}$) resulted in a value statistically comparable to that of N80MIN (17.07 mg g$^{-1}$) and also higher than the control (10.27 mg g$^{-1}$). This fact suggests that, irrespective of the lower dry matter production compared to sole wheat fertilized with dried blood, the availability of N for PB-DW TIC and N80 MIN was higher.

For wheat fertilized with ammonium nitrate, this was quite obvious, due to the faster availability of this form of N compared to the organic form. Still, for intercropped wheat this was not only the result of a higher proportion of N in the biomass, caused by the low dry matter production, as demonstrated by the different behavior of the N0. The competition for soil N between wheat and bean under intercropping might have stimulated the symbiotic fixation of dinitrogen from the atmosphere in the legume, leading to a higher availability of soil mineral N for the wheat, compared to other treatments.

The level of N accumulation in wheat plants, resulting from the combination of N concentration and dry matter production, did not show any significant differences among treatments (Table 54).

Immediately before incorporation into the soil, pigeon bean accumulated 66.98 mg N per plant (Table 54).

*Table 54 - N concentration (mg $g^{-1}$) and accumulation (mg $plant^{-1}$) in different plant components of wheat and pigeon bean (under PB -DW TIC) at end of stem elongation stage of wheat (**BBCH 30**) in 2009/10. Within each column, data followed by different letters are significantly different.*

| **BBCH 30** | | | | | | | | |
|---|---|---|---|---|---|---|---|---|
| **Treatment** | **N concentration (mg $g^{-1}$)[3]** | | | | **N accumulation (mg $plant^{-1}$) [3]** | | | |
| | **Leaves** | **Stems** | **Ears/ Pods** | **Grain** | **Leaves** | **Stems** | **Ears/ Pods** | **Grain** | **Total** |
| N0 | 10.27 c | - | - | - | 2.77 | - | - | - | 2.77 |
| N40+40 ORG | 11.87 bc | - | - | - | 10.56 | - | - | - | 10.56 |
| N80 MIN | 17.07 a | - | - | - | 11.78 | - | - | - | 11.78 |
| N 80 ORG | 10.73 bc | - | - | - | 9.23 | - | - | - | 9.23 |
| PB-DW TIC | | | | | | | | | |
| *Wheat* | 14.07 ab | - | - | - | 6.75 | - | - | - | 6.75 |
| *Pigeon bean* | (31.30) | - | - | - | (66.98) | - | - | - | (66.98) |
| *Total* | - | - | - | - | (73.73) | - | - | - | (73.73) |
| *Significance[1]* | ** | - | - | - | *ns* | - | - | - | *ns* |
| *LSD* | *3.50* | - | - | - | *9.85* | - | - | - | *9.85* |
| *CV (%)[2]* | *15.2* | - | - | - | *56.9* | - | - | - | *56.9* |

*[1] **, *, ns are, respectively, significant for p≤.01, significant for p≤.05 and not significant (LSD test)*

*[2] Coefficient of variation*

*[3] Between brackets, data excluded from the ANOVA*

### *3.2.3. End of heading stage of wheat (BBCH 59)*

At wheat heading (BBCH59), the dry matter production of wheat under N80 ORG was superior to intercropped wheat and to the control for all plant components (Table 55).

*Table 55 - Dry matter weight, number of ears/pods and plant height of wheat at end of heading stage of wheat (**BBCH 59**) in 2009/10. Within each column, data followed by different letters are significantly different.*

| BBCH 59 | | | | | | |
|---|---|---|---|---|---|---|
| **Treatment** | **Dry matter production (g plant$^{-1}$)** | | | | | **Plant height (cm)** |
| | **Leaves** | **Stems** | **Ears/Pods** | **Grain** | **Total Biomass** | |
| N0 | 0.13 b | 0.30 c | 0.09 b | - | 0.52 c | 34.25 b |
| N40+40 ORG | 0.32 ab | 0.88 ab | 0.20 ab | - | 1.40 ab | 56.50 a |
| N80 MIN | 0.32 ab | 0.99 a | 0.32 a | - | 1.63 a | 59.83 a |
| N 80 ORG | 0.47 a | 1.15 a | 0.29 a | - | 1.91 a | 60.66 a |
| PB-DW TIC | | | | | | |
| *Wheat* | 0.20 b | 0.51 bc | 0.13 b | - | 0.84 bc | 40.01 b |
| *Pigeon bean* | - | - | - | - | - | - |
| *Total* | 0.20 b | 0.51 bc | 0.13 b | - | 0.84 bc | - |
| *Significance[1]* | * | ** | ** | | ** | ** |
| *LSD* | *0.26* | *0.56* | *0.14* | | *0.93* | *9.95* |
| *CV (%)[2]* | *26.8* | *21.3* | *19.7* | | *21.4* | *5.9* |

*[1] **, *, ns are, respectively, significant for p≤.01, significant for p≤.05 and not significant (LSD test)*
*[2] Coefficient of variation*

After the application of the second rate of ammonium nitrate, wheat under N80MIN showed values of dry matter production higher than N40+40ORG in terms of absolute values. Intercropped wheat, although showing higher absolute values, did not differentiate from the control.

Plant height was significantly increased only by fertilization, whatever the treatment (Table 55).

Only N concentration in wheat leaves what significantly affected by treatments (Table 56). N80 MIN and intercropping revealed again the higher values for N in leaves. Also for other plant components, wheat under temporary intercropping showed the highest values of N concentration, although without the support of statistics.

Due to the low dry matter production, the N accumulation in intercropped wheat were superior to the control only for leaves and total aboveground dry matter (Table 56). Although statistically comparable to N80 ORG, N accumulations in wheat fertilized with ammonium nitrate were the highest.

*Table 56 - N concentration (mg g$^{-1}$) and accumulation (mg plant$^{-1}$) in different plant components of wheat at end of heading stage of wheat (**BBCH 59**) in 2009/10. Within each column, data followed by different letters are significantly different.*

| **BBCH 59** | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|
| **Treatment** | **N concentration (mg g$^{-1}$)** | | | | **N accumulation (mg plant$^{-1}$)** | | | | |
| | **Leaves** | **Stems** | **Ears/ Pods** | **Grain** | **Leaves** | **Stems** | **Ears/ Pods** | **Grain** | **Total** |
| N0 | 7.90 b | 3.40 | 11.40 | - | 1.03 b | 1.02 c | 1.03 d | - | 3.08 c |
| N40+40 ORG | 9.30 ab | 5.20 | 14.60 | - | 2.98 a | 4.59 ab | 2.92 bc | - | 10.49 ab |
| N80 MIN | 11.70 a | 4.70 | 13.40 | - | 3.74 a | 4.64 ab | 4.29 a | - | 12.67 a |
| N 80 ORG | 6.30 b | 4.30 | 12.60 | - | 2.96 a | 4.96 a | 3.65 ab | - | 11.57 a |
| PB-DW TIC | | | | | | | | | |
| *Wheat* | 12.50 a | 5.40 | 16.20 | - | 2.50 a | 2.75 bc | 2.11 cd | - | 7.36 b |
| *Pigeon bean* | - | - | - | - | - | - | - | - | - |
| *Total* | 12.50 a | 5.40 | 16.20 | - | 2.50 a | 2.75 bc | 2.11 cd | - | 7.36 b |
| *Significance[1]* | ** | *ns* | *ns* | - | ** | ** | ** | - | ** |
| *LSD* | *4.53* | *3.32* | *10.66* | - | *1.29* | *2.09* | *1.60* | - | *3.76* |
| *CV (%)[2]* | *14.0* | *21.6* | *23.3* | - | *14.6* | *17.6* | *16.7* | - | *12.4* |

*[1] **, *, ns are, respectively, significant for p≤.01, significant for p≤.05 and not significant (LSD test)*

*[2] Coefficient of variation*

### *3.2.4. End of flowering stage of wheat (BBCH 69)*

At wheat flowering stage (BBCH 69), fertilized sole wheat produced 3.7 and 2 times higher above ground dry matter than the control N0 and intercropped wheat, respectively. Apparently, the second application of dried blood in N40+40 ORG determined a strong effect on the growth of wheat, which produced more than the other treatments (Table 57). PB-DW TIC and N0 were statistically comparable.

As regards plant height, a precise trend among treatments was observed, with fertilized sole wheat showing the highest values (between 69 and 72 cm), followed by intercropped wheat (52 cm) and, then, N (45.5 cm).

Stems and ears of intercropped wheat contained more N than the other treatments (Table 58). In terms of N accumulation, the effect of dry matter component leveled down intercropped wheat, which at the end resulted in total N accumulations intermediate between fertilized wheat and the control.

*Table 57 - Dry matter weight, number of ears/pods and plant height of wheat at end of flowering stage of wheat (**BBCH 69**) in 2009/10. Within each column, data followed by different letters are significantly different.*

| **BBCH 69** | | | | | | |
|---|---|---|---|---|---|---|
| **Treatment** | **Dry matter production (g plant$^{-1}$)** | | | | | **Plant height (cm)** |
| | **Leaves** | **Stems** | **Ears/Pods** | **Grain** | **Total Biomass** | |
| N0 | 0.13 c | 0.31 c | 0.17 b | - | 0.61 b | 45.50 c |
| N40+40 ORG | 0.56 a | 1.28 a | 0.51 a | - | 2.35 a | 69.00 a |
| N80 MIN | 0.36 b | 1.19 a | 0.60 a | - | 2.15 a | 72.00 a |
| N 80 ORG | 0.41 ab | 1.22 a | 0.58 a | - | 2.21 a | 69.89 a |
| PB-DW TIC | | | | | | |
| *Wheat* | 0.19 c | 0.67 b | 0.24 b | - | 1.10 b | 52.02 b |
| *Pigeon bean* | - | - | - | - | - | - |
| *Total* | 0.19 c | 0.67 b | 0.24 b | - | 1.10 b | 52.02 b |
| *Significance*[1] | ** | ** | ** | - | ** | ** |
| *LSD* | *0.22* | *0.41 ab* | *0.32* | - | *0.81* | *6.05* |
| *CV (%)*[2] | *20.7* | *13.1* | *22.2* | - | *14.5* | *2.9* |

*[1] **, *, ns are, respectively, significant for p≤.01, significant for p≤.05 and not significant (LSD test)*

*[2] Coefficient of variation*

*Table 58 - N concentration (mg g$^{-1}$) and accumulation (mg plant$^{-1}$) in different plant components of wheat at end of flowering stage of wheat (**BBCH 69**) in 2009/10. Within each column, data followed by different letters are significantly different.*

| **BBCH 69** | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|
| **Treatment** | **N concentration (mg g$^{-1}$)** | | | | **N accumulation (mg plant$^{-1}$)** | | | | |
| | **Leaves** | **Stems** | **Ears/ Pods** | **Grain** | **Leaves** | **Stems** | **Ears/ Pods** | **Grain** | **Total** |
| N0 | 3.90 | 2.80 b | 10.90 b | - | 0.51 c | 0.86 b | 1.85 c | - | 3.22 c |
| N40+40 ORG | 4.70 | 2.30 b | 11.80 b | - | 2.63 a | 2.95 a | 6.02 ab | - | 11.60 a |
| N80 MIN | 4.70 | 2.70 b | 13.80 b | - | 1.69 b | 3.23 a | 8.28 a | - | 13.20 a |
| N 80 ORG | 3.90 | 2.30 b | 13.20 b | - | 1.60 b | 2.81 a | 7.66 a | - | 12.07 a |
| PB-DW TIC | | | | | | | | | |
| *Wheat* | 4.30 | 4.50 a | 17.60 a | - | 0.82 c | 3.02 a | 4.22 b | - | 8.06 b |
| *Pigeon bean* | - | - | - | - | - | - | - | - | - |
| *Total* | - | - | - | - | 0.82 c | 3.02 a | 4.22 b | - | 8.06 b |
| *Significance*[1] | *ns* | ** | * | - | ** | ** | ** | - | ** |
| *LSD* | *1.19* | *1.37* | *6.43* | - | *0.67* | *1.50* | *3.13* | - | *3.55* |
| *CV (%)*[2] | *8.4* | *13.4* | *13.8* | - | *15.1* | *16.7* | *16.2* | - | *10.9* |

*[1] **, *, ns are, respectively, significant for p≤.01, significant for p≤.05 and not significant (LSD test)*

*[2] Coefficient of variation*

### *3.2.5. Milky ripening stage of wheat (BBCH 75)*

At BBCH 75 the data on dry matter production of wheat confirmed the trend observed in the former stage (Table 59).

*Table 59 - Dry matter weight, number of ears/pods and plant height of wheat at medium milky ripening stage of wheat (**BBCH 75**) in 2009/10. Within each column, data followed by different letters are significantly different.*

| **BBCH 75** | | | | | | |
|---|---|---|---|---|---|---|
| **Treatment** | **Dry matter production (g plant$^{-1}$)** | | | | | **Plant height (cm)** |
| | **Leaves** | **Stems** | **Ears/Pods** | **Grain** | **Total Biomass** | |
| N0 | 0.09 c | 0.26 b | 0.30 c | - | 0.65 b | 47.25 b |
| N40+40 ORG | 0.30 ab | 1.01 a | 1.31 a | - | 2.62 a | 72.05 a |
| N80 MIN | 0.30 ab | 1.00 a | 0.99 ab | - | 2.29 a | 72.57 a |
| N 80 ORG | 0.41 a | 1.01 a | 0.90 b | - | 2.32 a | 70.30 a |
| PB-DW TIC | | | | | | |
| *Wheat* | 0.19 bc | 0.48 b | 0.54 c | - | 1.21 b | 52.37 b |
| *Pigeon bean* | - | - | - | - | - | - |
| *Total* | 0.19 bc | 0.48 b | 0.54 c | - | 1.21 b | 52.37 b |
| *Significance[1]* | ** | ** | ** | - | ** | ** |
| *LSD* | *0.17* | *0.49* | *0.42* | - | *0.89* | *11.18* |
| *CV (%)[2]* | *19.5* | *19.5* | *16.5* | - | *15.0* | *5.3* |

*[1] **, *, ns are, respectively, significant for p≤.01, significant for p≤.05 and not significant (LSD test)*
*[2] Coefficient of variation*

Also for N concentration (Table 60) the performance of the treatments was similar to that at the end of flowering stage. Intercropped wheat showed again the highest values for N concentration in leaves, stems and ears.

Total N accumulations in wheat followed this order: N80MIN, N80 ORG and N40+40 ORG, PB-DW TIC, N0.

Again, for N accumulations, the high N concentration of wheat plants under N80 MIN compensated for the lower level of dry matter than under N40+40 ORG. And the same was observed for intercropped wheat compared to N0.

*Table 60 - N concentration (mg g$^{-1}$) and accumulation (mg plant$^{-1}$) in different plant components of wheat at medium milky ripening stage of wheat (**BBCH 75**) in 2009/10. Within each column, data followed by different letters are significantly different.*

| **BBCH 75** | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|
| **Treatment** | **N concentration (mg g$^{-1}$)** | | | | **N accumulation (mg plant$^{-1}$)** | | | | |
| | **Leaves** | **Stems** | **Ears/ Pods** | **Grain** | **Leaves** | **Stems** | **Ears/ Pods** | **Grain** | **Total** |
| N0 | 3.50 c | 2.70 b | 8.00 b | - | 0.32 c | 0.71 d | 2.40 c | - | 3.43 d |
| N40+40 ORG | 3.60 bc | 2.20 b | 7.90 b | - | 1.08 b | 2.22 bc | 10.35 a | - | 13.65 ab |
| N80 MIN | 5.40 b | 2.70 b | 9.90 b | - | 1.62 ab | 2.69 ab | 9.80 a | - | 14.11 a |
| N 80 ORG | 4.40 bc | 2.90 b | 8.80 b | - | 1.80 a | 2.94 a | 7.92 b | - | 12.66 b |
| PB-DW TIC | | | | | | | | | |
| *Wheat* | 7.40 a | 4.00 a | 12.50 a | - | 1.41 b | 1.92 c | 6.75 b | - | 10.08 c |
| *Pigeon bean* | - | - | - | - | - | - | - | - | - |
| *Total* | 7.40 a | 4.00 a | 12.50 a | - | 1.41 b | 1.92 c | 6.75 b | - | 10.08 c |
| *Significance[1]* | ** | ** | * | | ** | ** | ** | - | ** |
| *LSD* | *2.79* | *1.28* | *2.94* | | *0.74* | *0.59* | *3.02* | - | *3.30* |
| *CV (%)[2]* | *16.1* | *12.6* | *9.0* | | *16.7* | *8.3* | *12.5* | - | *9.2* |

*[1] **, *, ns are, respectively, significant for $p \leq .01$, significant for $p \leq .05$ and not significant (LSD test)*

*[2] Coefficient of variation*

### *3.2.6. Harvest maturity stage of wheat (BBCH 89)*

At harvest maturity there were few differences among treatments in the dry matter of different plant components of wheat (Table 61). In terms of grain yield, wheat under intercropping was statistically comparable to fertilized wheat, averaged over all treatments, but also to N0, which was in turn lower than fertilized wheat.

A very similar trend was observed also for the other plant components, except for leaves, for which theN40+40 ORG over-yielded all the other treatments.

In terms of total biomass, fertilized wheat, whatever the treatment, produced more than three-time higher than N0, and 93% more than intercropped wheat. N0 and intercropped wheat were comparable.

Although not significantly, a trend of a higher number of ear per plant was found in intercropped wheat.

Plant height was statistically superior in fertilized wheat than remaining treatments.

N concentration (Table 62) was significantly increased in intercropped wheat, whatever the plant components, whereas for the other treatments results did not came up with relevant differences.

*Table 61 - Dry matter weight, number of ears/pods and plant height of wheat at harvest maturity stage (**BBCH 89**) in 2009/10. Within each column, data followed by different letters are significantly different.*

| **BBCH 89** | | | | | | | |
|---|---|---|---|---|---|---|---|
| **Treatment** | **Dry matter production (g plant$^{-1}$)** | | | | | **Number of ears (plant$^{-1}$)** | **Plant height (cm)** |
| | **Leaves** | **Stems** | **Ears/Pods** | **Grain** | **Total Biomass** | | |
| N0 | 0.10 d | 0.26 b | 0.12 b | 0.34 b | 0.82 b | 1.34 | 47.25 b |
| N40+40 ORG | 0.70 a | 0.91 a | 0.36 a | 0.97 a | 2.94 a | 1.33 | 72.05 a |
| N80 MIN | 0.45 b | 0.95 a | 0.35 a | 0.90 a | 2.65 a | 1.00 | 72.57 a |
| N 80 ORG | 0.51 b | 0.85 a | 0.26 a | 0.89 a | 2.51 a | 1.33 | 70.30 a |
| PB-DW TIC | | | | | | | |
| *Wheat* | 0.26 c | 0.37 b | 0.21 ab | 0.56 ab | 1.40 b | 1.89 | 52.37 b |
| *Pigeon bean* | - | - | - | - | - | - | - |
| *Total* | 0.26 c | 0.37 b | 0.21 ab | 0.56 ab | 1.40 b | 1.89 | 52.37 b |
| *Significance[1]* | **** | **** | *** | **** | **** | *ns* | **** |
| *LSD* | *0.21* | *0.32* | *0.19* | *0.51 b* | *1.14* | *0.91* | *11.18* |
| *CV (%)[2]* | *16.7* | *14.6* | *22.3* | *21.2* | *16.9* | *19.6* | *5.3* |

*[1] **, *, ns are, respectively, significant for $p \leq .01$, significant for $p \leq .05$ and not significant (LSD test)*

*[2] Coefficient of variation*

The increase in N concentration under intercropping did not completely compensate for dry matter depletion. Only for grain component (Table 62), PB-DW PIC was statistically comparable to the best performing treatments, i.e. all the three fertilized wheat treatments.

Due to the high relative contribution of grain to total dry matter (harvest index was 40%), also for total N accumulations intercropped wheat showed results comparable to fertilized sole wheat (Table 62), and superior to the control. N0 resulted in extremely low level of N content in the plants, revealing a clear deficiency of N, compared to the other treatments.

*Table 62 - N concentration (mg g$^{-1}$) and accumulation (mg plant$^{-1}$) in different plant components of wheat at harvest maturity stage (**BBCH 89**) in 2009/10. Within each column, data followed by different letters are significantly different.*

| **BBCH 89** | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|
| **Treatment** | **N concentration (mg g$^{-1}$)** | | | | **N accumulation (mg plant$^{-1}$)** | | | | |
| | **Leaves** | **Stems** | **Ears/ Pods** | **Grain** | **Leaves** | **Stems** | **Ears/ Pods** | **Grain** | **Total** |
| N0 | 3.30 b | 2.50 | 0.90 b | 11.80 b | 0.33 c | 0.65 b | 0.11 c | 4.01 b | 5.10 b |
| N40+40 ORG | 3.60 b | 2.20 | 1.00 b | 12.00 b | 2.52 a | 2.00 ab | 0.36 a | 11.64 a | 16.52 a |
| N80 MIN | 3.80 b | 2.70 | 1.00 b | 12.50 b | 1.71 b | 2.57 a | 0.35 a | 11.25 a | 15.88 a |
| N 80 ORG | 3.30 b | 3.10 | 1.10 b | 12.20 b | 1.68 b | 2.65 a | 0.29 b | 10.86 a | 15.48 a |
| PB-DW TIC | | | | | | | | | |
| *Wheat* | 6.10 a | 2.80 | 1.30 a | 17.80 a | 1.59 b | 1.02 b | 0.27 b | 9.97 a | 12.85 a |
| *Pigeon bean* | - | - | - | - | - | - | - | - | - |
| *Total* | 6.10 a | 2.80 | 1.30 a | 17.80 a | 1.59 b | 1.02 b | 0.27 b | 9.97 a | 12.85 a |
| *Significance[1]* | ** | *ns* | * | ** | ** | ** | ** | ** | ** |
| *LSD* | *1.10* | *2.72* | *0.39* | *3.55* | *0.93* | *1.59* | *0.13* | *2.81* | *5.54* |
| *CV (%)[2]* | *7.9* | *29.6* | *10.5* | *7.8* | *18.6* | *26.6* | *14.8* | *16.9* | *12.6* |

*[1] **, *, ns are, respectively, significant for p≤.01, significant for p≤.05 and not significant (LSD test)*

*[2] Coefficient of variation*

### *3.2.7. Wheat dry matter production and N accumulation over the growing cycle*

The time courses of total dry matter production and N accumulations of wheat grown under the different treatments are plotted in Figure 32 and 33, respectively.

From Figure 32 the existence of three different pattern of biomass production appears clear. All the three sole wheat treatments receiving N fertilizers produced a clearly higher dry matter from heading completion (BBCH 59). Earlier, only N80 ORG revealed a growth rate superior to the others, from BBCH 30 to 59. Late in the season, the pattern followed by wheat under N40+40 ORG clearly differed from the others, with a peak of growth at BBCH 69, and then an increasing dry matter production afterwards at a steady rate.

Wheat under intercropping with bean had a similar growth rate to N80 ORG from BBCH 59 onwards, but at early stages (BBCH 14 and 30) it revealed a very slowly growth, only higher than the control. The big difference between the patterns of N0 and

PB-DW PIC was between BBCH 59 and 69, when the growth rate of intercropped wheat did not change, whereas that of N0 slowed down (Figure 32).

*Figure 32 - Total aboveground dry matter production of durum wheat grown under different treatments in 2009/10.*

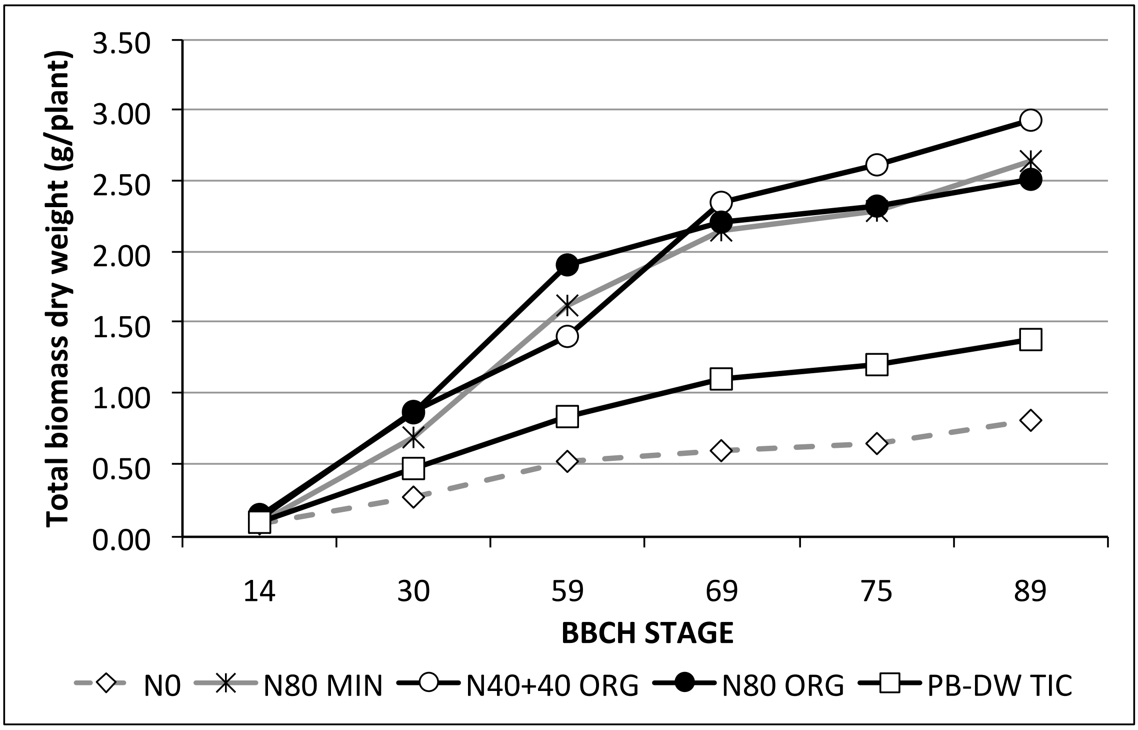
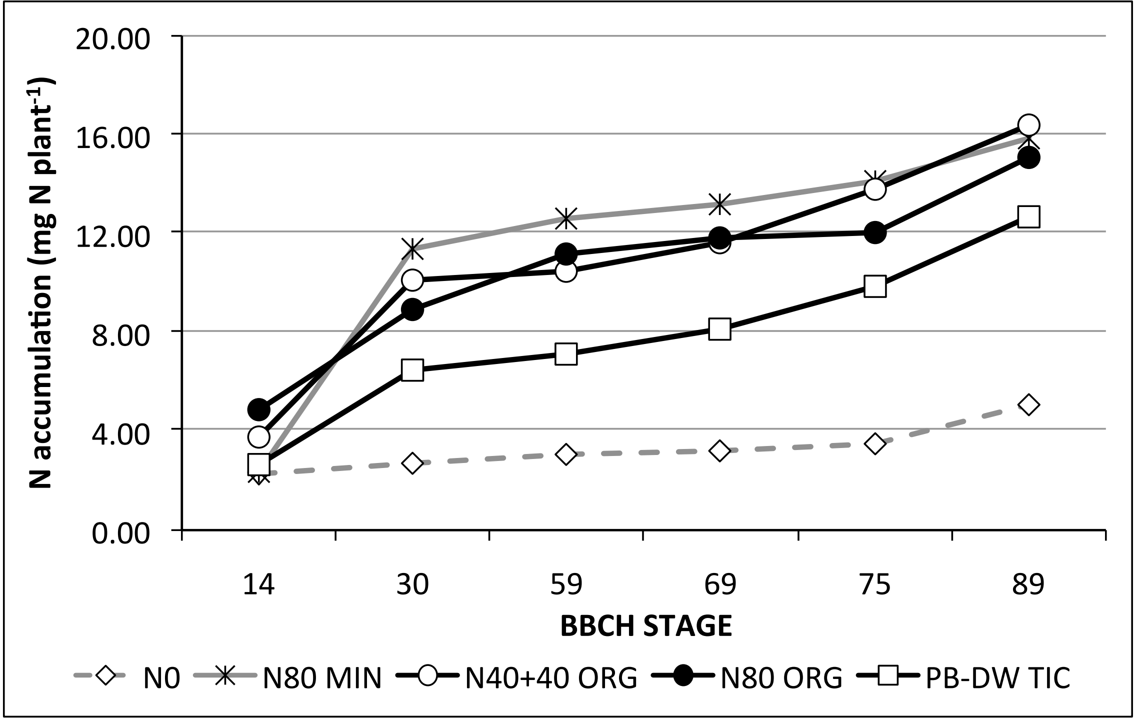


*Figure 33 - N accumulation in total aboveground dry matter of durum wheat grown under different treatments in 2009/10. Error bars are standard deviations (n = 3).*



The time course of N accumulation was partially in line with that of dry matter production (Figure 33). Nonetheless, the effect of the high N concentration in wheat biomass under N80 MIN lifted up the curve of the treatment, which was clearly higher than those of wheat fertilized with dried blood (both N80 ORG ad N40+40 ORG) until milky ripening (BBCH 75). The increasing rate of N accumulation in the two treatments fertilized with blood was similar until end of flowering, then for N40+40 ORG a huge increase was observed between BBCH 69 and 75, and then wheat steadily growth until harvest, when it exceeded the value of N accumulated in wheat under N80 MIN. A similar trend was also observed for the intercropped wheat, whose total N accumulation increased from BBCH 59 at a steady rate comparable to that of N40+40 ORG. This evidence strongly supports the hypothesis of a significant effect of a late peak of mineralization of the organic matter, and also of legume biomass incorporated into the soil in PB-DW TIC, as well as of the second application of dried blood in N40+40 ORG. An increase in the rate of mineralization might have been due to the heavy rainfall occurred in May and June 2009 (see Figure 6), coupled with the medium-high temperature recorded in the same period. As the soil contained in the lysimeter was a sandy type, microbial biomass might have needed more time and perfect combination of weather condition in order to activate and to attack the material supplied to the soil.

It is noteworthy that the increasing rate of N accumulation in the last time frame was very similar among all the treatments.

To our knowledge, very few studies in recent literature dealt with the study of the effect of intercropping between legume and cereals in lysimeters.

The most similar to ours was the paper published by Hauggaard-Nielsen et al. (2003). The authors compared the effect of a spring barley-field pea permanent intercropping with those of the respective sole crops of barley and pea. Their results clearly showed a significant advantage from intercropping than sole crops in terms of LER, as well as in terms of total grain yield and N accumulation, compared to sole barley. Our results only partially agree with theirs, as we did not observed any increase neither in total dry matter production, nor in grain yield under intercropping than sole wheat unfertilized (N0). Still, the comparison between our work and that of Hauggaard-Nielsen et al. (2003) is not fair, as the two researches dealt with different strategies of intercropping (temporary intercropping in our experiment, permanent intercropping in the other case). Anyway, the results from the lysimeter trial confirmed the findings obtained in the field experiment as regards temporary intercropping between durum wheat and pigeon bean. The competition for resources at early stage resulted in the field experiment was here assessed again, and clearly affected the difference in growth level and in N accumulation of intercropped wheat compared to the other treatments.

Differently from the field experiment, in the lysimeters intercropping produced a higher concentration of N in the wheat plants, not only because of the low dry matter production, but also of a higher N availability late in the season. This temporary effect of N sparing was possibly due to the stimulation of the microbial activity caused by the hoeing performed for the incorporation of the bean into the soil at BBCH 30 of wheat.

### *3.2.8. N mineralization and leaching through drainage water*

In Table 63, the cumulative weight of drainage water, the mean concentration of nitrates in the water, and the total amount of N leached in drainage water are reported, with reference to 5 main periods: Period 1 ended at BBCH 14 of wheat; Period 2 ended at BBCH 29 of wheat; Period 3 ended at BBCH 69 of wheat; Period 4 ended at BBCH 89 of wheat; Period 5 was fallow after wheat harvest.

Cumulatively, total nitric nitrogen lost by leaching during crop growth and fallow was 29.83 kg N-$NO_3^-$ $ha^{-1}$ for N0, 36.34 kg N-$NO_3^-$ $ha^{-1}$ for N40+40 ORG, 32.35 kg N-$NO_3^-$ $ha^{-1}$ for N80 MIN, 46.61 kg N-$NO_3^-$ $ha^{-1}$ for N80 ORG and 40.23 kg N-$NO_3^-$ $ha^{-1}$ for PB-DW TIC (Table 63).

Among treatments, in none of the considered periods a significant difference in terms of nitrogen leaching was observed. Also mean N concentration in drainage water was found to be irrespective of the treatments, except for period 1 (from the beginning of the experiment to the 4-leave stage of wheat), when N80 ORG and N40+40 ORG were superior to PB-DW TIC. In periods 2 and 3, the total amount of N losses was very low, averaged over all treatments, mainly due to the low rainfall and the high crop intake.

In the whole period of crop growth (i.e. from period 1 to 4), N80 ORG was found to lose more nitrogen than other treatments (38.50 kg N-$NO_3^-$ $ha^{-1}$ vs 15.69 kg N-$NO_3^-$ $ha^{-1}$, on average). In the fallow period following the harvest of wheat (period 5), the highest values of N leaching were observed for N80 ORG (24.09 kg N-$NO_3^-$ $ha^{-1}$) and intercropping (26.39 kg N-$NO_3^-$ $ha^{-1}$).

*Table 63 - Weight of drained water - Water - (g), mean nitrate concentration - $NO_3^-$ conc - (µg $g^{-1}$) and total N-$NO_3^-$ leached (mg $m^{-2}$) - N leached - at different sampling dates.*

| Treatment | PERIOD 1 | | | PERIOD 2 | | | PERIOD 3 | | | PERIOD 4 | | | PERIOD 5 | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | **Water** | **N conc** | **N-$NO_3^-$ leached** | **Water** | **N conc** | **N-$NO_3^-$ leached** | **Water** | **N conc** | **N-$NO_3^-$ leached** | **Water** | **N conc** | **N-$NO_3^-$ leached** | **Water** | **N conc** | **N-$NO_3^-$ leached** |
| N0 | 348667 | 21.10 bc | 1520.30 | 71877 | 0.00 | 0.00 | 87343 a | 0.00 | 0.00 | 16288 | 0.00 | 0.00 | 531590 a | 13.31 | 1463.27 |
| N40+40 ORG | 294820 | 29.14 ab | 1775.47 | 45945 | 0.00 | 0.00 | 70685 ab | 0.00 | 0.00 | 16685 | 0.00 | 0.00 | 506791 abc | 17.75 | 1859.49 |
| N80 MIN | 331213 | 22.98 bc | 1573.34 | 63863 | 1.80 | 23.76 | 29670 c | 0.00 | 0.00 | 8773 | 0.00 | 0.00 | 490549 bc | 16.16 | 1638.27 |
| N 80 ORG | 311245 | 34.98 a | 2250.11 | 52060 | 0.25 | 2.73 | 59905 b | 0.00 | 0.00 | 18128 | 0.00 | 0.00 | 506855 abc | 22.99 | 2408.65 |
| PB-DW TIC | 343138 | 19.27 c | 1366.95 | 60735 | 0.00 | 0.00 | 73411 ab | 1.07 | 16.31 | 19235 | 0.00 | 0.00 | 510524 ab | 25.01 | 2639.30 |
| *Significanc* | *ns* | **** | *ns* | *ns* | *ns* | *ns* | **** | *ns* | *ns* | *ns* | - | - | **** | *ns* | *ns* |
| *LSD³* | *99883* | *12.08* | *1056.27* | *(0.19)* | *3.20* | *46.33* | *40201* | *2.44* | *30.41* | *13306* | - | - | *35387* | *45.93* | *4763.56* |
| *CV (%)²* | *9.5* | *14.3* | *18.5* | *1.2* | *58.8* | *61.9* | *23.0* | *224.2* | *219.4* | *27.4* | - | - | *2.2* | *71.2* | *70.9* |

*[1] **, *, ns are, respectively, significant for p≤.01, significant for p≤.05 and not significant (LSD test)*

*[2] Coefficient of variation*

*[3] Under brackets values after transformation in $\log_{10}(x)$*

Our results suggested that splitting N fertilization rates in two different applications (see N40+40 ORG and N80 MIN) produced lower N leaching compared to unique application strategy (see N80 ORG). This might have been due to the particular weather conditions observed in the two years of the experiment. The huge amounts of rainfall recorded in fall-winter 2009/10 and 2010/11 might have increased at most the risk of nitrate leaching. Consequently, treatments with a fractionate application of N over the season might have produced lower losses of N.

Moreover, the higher N leaching observed for N80 ORG and PB-DW TIC in the fallow led to draw the conclusion that the two materials, namely dried blood and the dry matter of pigeon bean, were only partially mineralized at the harvest date of wheat in 2010. This fact might have been due to the sandy texture of the soil, related to a low presence and activity of the microbial populations deputed to the mineralization of organic material. The final segments of growth curves of the crops under N80 ORG and PB-DW TIC seemed to confirm this argument, as the increasing rate in wheat dry matter was lower than under N40+40 ORG and N80 MIN (Figure 32), possibly underlining a minor availability of N late in the season in the plots of these two treatments.

Our results confirmed the absence of significant differences in N leaching between intercropping and pure crops reported by Hauggaard-Nielsen et al. (2003) in the only paper recently published on this topic. The authors did not observed leaching at the end of the first crops in the sequence (i.e. pea, barley and pea-barley), due to the high water intake of crops. In the year after, the growth of a catch crop with high affinity for soil nitrates (i.e. rye) actually offset the difference in N leaching among treatments.

Concerning the other treatments, the observed high nitrate leaching in the fallow was in agreement with a soil-less incubation study carried out by Smith and Hadley (1990). The authors found a late peak of mineralization of dried blood, because of the quality of the biomass, characterized by a low C:N ratio.

### *3.2.9. Apparent N budget*

In Table 64, the apparent N budget for the whole period of the experiment is reported.

The amount of N inputs from the rain was estimated in 2.7 g N $m^{-2}$, based on the total weight of water from rain fallen down in the two years and the mean nitrogen content of rain obtained from long-term measurements in the area.

For temporary intercropping between durum wheat and pigeon bean, N from symbiotic $N_2$-fixation accounted for 4.3 g N $m^{-2}$.

Subtracting the amount of $N_{rain}$ from N inputs of treatments other than the control, the amount of net N inputs were obtained. Obviously, the value of total N inputs was lower under PB-DW TIC than fertilized sole wheat.

Among outputs, crop N uptake accounted for 40% of total outputs in N0, 65% in N40+40 ORG, 66% in N80 MIN, 57% in N80 ORG and 56% in PB-DW TIC.
Total gross outputs were higher in wheat fertilized with dried blood, whatever the treatment, followed by N80 MIN, PB-DW TIC and then N0. The outputs of the control underlined values 50% lower than other treatments, meaning that a basic mineralization of soil organic matter naturally occurred.

Apparent gross budget was negative for all the treatments, showing higher outputs than inputs. The budget was more negative for intercropping, and more close to parity for N80 MIN.
Considering also the natural inputs and outputs of N, the net apparent budget was estimated (Table 64). Net budget was positive for N80 MIN (+0.73), close to parity for N40+40 ORG (+0.08), negative for remaining treatments. Wheat-bean intercropping revealed again the most negative value (-2.54).

Our results clearly showed that temporary intercropping consumed more N than provided. This was because N supply and outputs were lower than under organic N fertilization. Still, the ratio between output and input was significantly higher than other treatments, both in terms of total outputs per input unit (2.13) and of crop uptake per input unit (1.19). This evidence supports the hypothesis of a higher efficiency in use of inputs under intercropping than sole crops.
Moreover, in terms of environmental impact, the negative value of the net N balance under intercropping can also be considered good, as it implies a low risk of N losses in the environment.
Our findings were partially in line with those of Hauggaard-Nielsen et al. (2003), who observed a net balance of N close to 0 under pea-barley intercropping, whereas for barley the balance was more negative. The authors of the paper demonstrated also an effect from the type of crop residue management (i.e. incorporation of residues into the soil or residue removal). Restitution of residues to the soil increased the value of N balance under pea sole crop and intercropping. In our case, crop residues were removed from the system in all the treatments, except for pigeon bean under temporary intercropping with wheat. This fact, combined with an asynchrony between

N availability and crop uptake, might have been the main reason behind the higher N leaching under intercropping than other treatments.

In the literature, other works dealt with the residual effect of intercropping on soil nitrates, even though with reference to permanent intercropping and real field conditions.

Hauggaard-Nielsen et al. (2009b) found no significant change in soil mineral N content following an intercropping between barley and field pea, grown under organic conditions in several sites across Western Europe.

In East Scotland, Pappa et al. (2011) found a decrease in nitrate leaching following an intercropping between several species of legumes and cereals compared to sole crops (0.67 kg $N\text{-}NO_3^-$ $ha^{-1}$ following IC *vs* 3.80 kg $N\text{-}NO_3^-$ $ha^{-1}$ under sole crops). In the same paper, authors also reported no significant differences in terms of $N_2O$ emissions among treatments, with this form of N loss being more related to the effect of cultivar than intercropping.

In Canada, Szumigalski and Van Acker (2006) observed higher fall soil nitrate content after pea sole crop than following field pea-wheat intercropping.

In Germany, Urbatzka et al. (2009) found more nitrates in the soil after pea sole crop than pea-cereals intercropping. The level of nitrates in the soil following the sole crop of pea was so high that a catch crop grown after the legume was considered necessary to avoid environmental risks.

Consistently with literature, our results confirmed the importance of the choice of the position of intercropping within crop sequences, in order to keep under certain levels the potential of N losses deriving from asynchronism between N sparing due to legumes and N uptake by component non-legume crops.

*Table 64 - Cumulative N inputs (g N $m^{-2}$), outputs (g N $m^{-2}$) and inputs-outputs (g N $m^{-2}$) under the different treatments over the whole duration of the experiment (2009/11).*

| **N INPUTS** | | | | | |
|---|---|---|---|---|---|
| **Treatment** | **$N_{FERT}$** | **$N_{FIX}$** | **$N_{RAIN}$** | ***TOTAL*** | ***NET TOTAL*** |
| N0 | 0.00 | 0.00 | 2.70 | ***2.70*** | ***0.00*** |
| N40+40 ORG | 8.00 | 0.00 | - | ***8.00*** | ***5.30*** |
| N80 MIN | 8.00 | 0.00 | - | ***8.00*** | ***5.30*** |
| N 80 ORG | 8.00 | 0.00 | - | ***8.00*** | ***5.30*** |
| PB-DW TIC | 0.00 | 4.30 | - | ***4.30*** | ***1.60*** |

| **N OUTPUTS** | | | | | |
|---|---|---|---|---|---|
| **Treatment** | | **$N_{UPT}$** | **$N_{LEACH}$** | **TOTAL** | **NET TOTAL** |
| N0 | | 2.04 | 2.98 | ***5.02*** | ***0.00*** |
| N40+40 ORG | | 6.61 | 3.63 | ***10.24*** | ***5.22*** |
| N80 MIN | | 6.35 | 3.24 | ***9.59*** | ***4.57*** |
| N 80 ORG | | 6.19 | 4.66 | ***10.85*** | ***5.83*** |
| PB-DW TIC | | 5.14 | 4.02 | ***9.16*** | ***4.14*** |

| **N INPUTS-OUTPUTS** | | | |
|---|---|---|---|
| | **Treatment** | **TOTAL** | **NET TOTAL** |
| | N0 | -2.32 | 0.00 |
| | N40+40 ORG | -2.24 | 0.08 |
| | N80 MIN | -1.59 | 0.73 |
| | N 80 ORG | -2.85 | -0.53 |
| | PB-DW TIC | -4.86 | -2.54 |

# 4. Conclusion

Our results confirmed the hypothesis that facilitative temporary intercropping with legume cover crops, particularly with hairy vetch, might be an effective tool for enhancing grain yield and quality of durum wheat grown under organic and low-input conditions. The mutual interactions established during co-growth between wheat and legumes produced benefits especially in terms of translocation of photosynthates to the grain of wheat in the late stage of ripening, resulting in a higher grain quality than for sole wheat. This facilitation have possibly occurred due to the increased availability of mineral N in the rhizosphere as result of the symbiotic $N_2$-fixation of the legume, incorporated into the soil at the beginning of stem elongation of the cereal. Anyway, the application of the technique of $^{15}N$ natural abundance did not allow to assess a significant transfer of fixed N from legume to wheat, due to the high variability of N isotope dilution in the soil, and also to the effect of a number of interfering environmental factors. Therefore, further study on this issue should also integrate $^{15}N$ natural abundance with other techniques, as well as with the measurement of other environmental parameters (i.e. soil microbial biomass and activity, soil moisture) potentially helpful for the interpretation of these phenomena.

Nevertheless, the performance of intercropped wheat was compared to that of sole wheat grown with unusual wide-row arrangement, which resulted in high intra-specific competition in-the row among wheat plants, and in a high weed pressure between the rows. For future developments of research on facilitative intercropping, the peculiar effect of seeding density and spatial arrangement should be evaluated in addition.

The higher N content of grain of wheat intercropped with legumes than sole crop clearly demonstrated that N was the real limiting factor of wheat growth, and also that the facilitation provided by the legumes mainly regarded N. Nevertheless, an interspecific competition for this element, as well as for other basic resources (i.e. space, radiation) was demonstrated to occur in all intercropping treatments since very early stage of crop development, resulting in a growth depletion of both crops and in the stimulation of biological $N_2$-fixation in the legumes. In our work, we mainly focused on the phenological stages considered most important for the N nutrition of wheat, neglecting at the same time that early competition might have been equally important. Further research is needed to study in-depth the dynamics of interspecific competition occurring from emergence to tillering and to test the most effective managerial options to overcome these constraints. Early application of mineral N fertilizers in low-input conditions might be a viable solution, in order to facilitate the growth of wheat during early stages. Still, its efficacy might also be evaluated from the point of view of its side impact on fossil-fuel resource use and GHG emissions.

Weed suppression was another goal clearly targeted by temporary intercropping, mainly due to the mechanical incorporation of the legume into the soil, which acted as a mechanical weed control operation. Nonetheless, in our experiment we observed also the presence of weeds along the wheat rows also in temporary intercropping plots after the incorporation of legumes into the soil. This suggests that wild flora might have acted opportunistically colonizing all the space left free by the crops. To increase the effect of weed suppression by intercropping, a suitable option might be the growth of wheat and legume genotypes specifically bred to be grown under intercropping conditions, including in breeding programs all the traits potentially conferring a weed suppression ability to the crops.

Anyway, the mechanization of facilitative temporary intercrop itself remain one of the main constraints on the diffusion of the technique in commercial farms. In Italy, wheat is largely grown on the heavy soils characterizing the hilly internal area of the country. Hoeing, in such conditions, might not be suitable and other machines have to be further developed and tested in order to make practicable the extension of the technique. Alternatively, the broadcast undersowing of legumes in the established wheat crop at the end of winter (relay intercropping) might be a possible alternative that is worthy to be tested in conditions of good soil water availability in the spring season. In such circumstance, the main focus of experimental work might be on the reduction of the detrimental effect of wheat predominance on legumes.

From an ecological point of view, our research clearly demonstrated that permanent intercropping might be globally more efficient than temporary in terms of environmental resource uses. In this sense, permanent intercropping between wheat and pulses can be considered more able than temporary intercropping to contribute to the mitigation of climate change, provided that proper agronomic management might allow to overcome the constraints of the technique. In our research, the early competition for resources between legumes and wheat excessively depleted the niches, with significant disadvantages for the growth of wheat, thus negatively affecting the global performance of intercropping for the remaining parts of the cycle. A proper management of the main aspects of intercropping as a whole, aiming to increase the presence of wheat in the mixture and the complementarity of resource use, might lead to significantly better results, also from the point of view of marketable products. The complexity of interactions highlighted in this kind of intercropping, where the duration of co-growth is much longer than under temporary intercropping, understands that further specific research is needed on this specific topic.

Concerning the profitability of intercropping, the main advantages of the technique arose from the combination of the yields of both intercrops rather than from the enhancement of only one of them. Thus, both crop products should be sold on the market, in order to achieve a good return for farmers. Whereas for some of the legumes object of this study (i.e. pigeon bean and pea) a post-harvest separation of grain from wheat might be more easy to practice, due to the difference in grain size, for hairy vetch this process might be too expensive. In addition, the simultaneous harvest of both intercrops with combine machines might be difficult to perform, due to the huge amount of plant biomass produced by intercropping, and also to the lodging of intercrops which was observed also in some of treatments compared in this work.

Also in terms of the effect of intercropping on residual soil fertility, our research did not find clear results, mainly due to adverse weather conditions, for the field experiment, and due to the particular soil texture, for the lysimeter trial. In any case, we analyzed data from only one year, which were at the end not sufficient to elucidate the dynamics of N in the whole cropping system, suggesting that this issue might be studied in the future with reference to several years and also differing soil types. Due to the huge number of factors potentially affecting the fate of fixed N when it enters the soil system, study under controlled environment and the simultaneous use of modeling might help in the short term to select the most promising managerial options to reduce the losses of N in cropping systems where intercropping are inserted in. In addition, the role of position of intercropping in the whole crop sequence might play a relevant role as regards to the impacts on N cycle, and hereby the inclusion of this topic is worthy to be further elucidated.

Finally, in our research weather conditions varied significantly from year to year. On one hand, this might have increased variability and reduced the solidity of outcomes, which in some cases differed substantially from one year to another, but on the other, the stability of main trends observed in the different years suggests that the agroecological approach behind intercropping might really be the key to achieve stability of food production in a context of climate change. To validate this impression, longer series of data might be necessary.

# References

Altieri, M. A. (1999). The ecological role of biodiversity in agroecosystems. *Agriculture, Ecosystems & Environment* 74(1-3): 19-31.

Alzueta, I., Abeledo, L. G., Mignone, C. M. and Miralles, D. J. (2012). Differences between wheat and barley in leaf and tillering coordination under contrasting nitrogen and sulfur conditions. *European Journal of Agronomy* 41(0): 92-102.

Amossé, C., Jeuffroy, M. H., Celette, F. and David, C. (2013a). Relay-intercropped forage legumes help to control weeds in organic grain production. *European Journal of Agronomy*.

Amossé, C., Jeuffroy, M. H. and David, C. (2013b). Relay intercropping of legume cover crops in organic winter wheat: Effects on performance and resource availability. *Field Crops Research* 145: 78-87.

Andersen, M., Hauggaard-Nielsen, H., Ambus, P. and Jensen, E. (2005). Biomass production, symbiotic nitrogen fixation and inorganic N use in dual and tri-component annual intercrops. *Plant and Soil* 266(1): 273-287.

Andersen, M., Hauggaard-Nielsen, H., Høgh-Jensen, H. and Jensen, E. (2007). Competition for and utilisation of sulfur in sole and intercrops of pea and barley. *Nutrient Cycling in Agroecosystems* 77(2): 143-153.

Andersen, M. K., Hauggaard-Nielsen, H., Weiner, J., Jensen, E. S. (2007). Competitive dynamics in two- and three-component intercrops. *Journal of Applied Ecology* 44(3): 545-551.

Arslan, A. and Kurdali, F. (1996). Rainfed vetch-barley mixed cropping in the Syrian semi-arid conditions. *Plant and Soil* 183(1): 149-160.

Barberi, P. (2002). Weed management in organic agriculture: are we addressing the right issues? *Weed Research* 42(3): 177-193.

Bàrberi, P. (2013). Functional Agrobiodiversity: The Key to Sustainability? . *Agricultural Sustainability*. G. S. Bhullar and N. K. Bhullar. San Diego, CA, USA, Elsevier Academic Press: 3-20.

Bàrberi, P. and Mazzoncini, M. (2006). The MASCOT (Mediterranean Arable Systems Comparison Trial) long term experiment. *Long-term Field Experiments in Organic Farming*. Joakim Raupp, Pekrun, Carola, Oltmanns, Meike, Köpke, Ulrich (Eds.). Berlin, Germany, Verlag Dr. Köster. 1: 1-14.

Bartelheimer, M., Steinlein, T. and Beyschlag, W. (2006). Aggregative root placement: A feature during interspecific competition in inland sand-dune habitats. *Plant and Soil* 280(1-2): 101-114.

Bedoussac, L. and Justes, E. (2009). The efficiency of a durum wheat-winter pea intercrop to improve yield and wheat grain protein concentration depends on N availability during early growth. *Plant and Soil* 330(1): 19-35.

Bedoussac, L. and Justes, E. (2010). Dynamic analysis of competition and complementarity for light and N use to understand the yield and the protein content of a durum wheat–winter pea intercrop. *Plant and Soil* 330(1): 37-54.

Benincasa, P., Pace, R., Tosti, G. and Tei, F. (2012). Early interspecific interference in the wheat/faba bean (Triticum aestivum/ Vicia faba ssp. minor) and rapeseed/ squarrosum clover (Brassica napus var. oleifera/Trifolium squarrosum) intercrops. *Italian Journal of Agronomy* 7(2): 171-177.

Betencourt, E., Duputel, M., Colomb, B., Desclaux, D. and Hinsinger, P. (2012). Intercropping promotes the ability of durum wheat and chickpea to increase rhizosphere phosphorus availability in a low P soil. *Soil Biology and Biochemistry* 46: 181-190.

Blaser, B. C., Singer, J. W. and Gibson, L. R. (2011). Winter cereal canopy effect on cereal and interseeded legume productivity. *Agronomy Journal* 103(4): 1180-1185.

Bremner, J. M. and Mulvaney, C. S. (1982). Nitrogen total. *Methods of soil analysis. Part 2.* A. L. Page, X and Y. Madison, wisconsin (USA), ASA and SSSA: 595-624.

Brophy, L. S., Heichel, G. H. and Russelle, M. P. (1987). Nitrogen Transfer from Forage Legumes to Grass in a Systematic Planting Design. *Crop Science* 27(4): 753-758.

Bulson, H. A. J., Snaydon, R. W. and Stopes, C. E. (1997). Effects of plant density on intercropped wheat and field beans in an organic farming system. *The Journal of Agricultural Science* 128(01): 59-71.

Campo, R. J., Araujo, R. S. and Hungria, M. (2009). Molybdenum-enriched soybean seeds enhance N accumulation, seed yield, and seed protein content in Brazil. *Field Crops Research* 110(3): 219-224.

Caporali, F., Paolini, R. and Campiglia, E. (1987). La consociazione fra piante erbacee. Nota I. Basi biologiche e vantaggi produttivi. *Rivista di Agronomia*(21): 3-17.

Carpi, G., Antichi, D., Mazzoncini, M., Carlesi, S. and Barberi, P. (2009). Consociazione temporanea: uno strumento per migliorare le produzioni del frumento duro. IV Workshop GRAB-IT "Agricoltura Biologica: sistemi produttivi e modelli di commercializzazione e di consumo". M. Crescimanno and G. Schifani. Palermo: 379-381.

Carter, M. and Ambus, P. (2006). Biologically Fixed N2 as a Source for N2O Production in a Grass–clover Mixture, Measured by 15N2. *Nutrient Cycling in Agroecosystems* 74(1): 13-26.

Chalk, P. M. (1998). Dynamics of biologically fixed N in legume-cereal rotations: A review. *Australian Journal of Agricultural Research* 49: 303.

Cherr, C. M., Scholberg, J. M. S. and McSorley, R. (2006). Green manure approaches to crop production: A synthesis. *Agronomy Journal* 98(2): 302-319.

Chu, G. X., Shen, Q. R. and Cao, J. L. (2004). Nitrogen fixation and N transfer from peanut to rice cultivated in aerobic soil in an intercropping system and its effect on soil N fertility. *Plant and Soil* 263(1): 17-27.

CoHort-Software (2007). Costat. Monterey, California (USA). www.cohort.com

Corre-Hellou, G., Brisson, N., Launay, M., Fustec, J. and Crozat, Y. (2007). Effect of root depth penetration on soil nitrogen competitive interactions and dry matter production in pea-barley intercrops given different soil nitrogen supplies. *Field Crops Research* 103(1): 76-85.

Corre-Hellou, G., Dibet, A., Hauggaard-Nielsen, H., Crozat, Y., Gooding, M., Ambus, P., Dahlmann, C., von Fragstein, P., Pristeri, A., Monti, M. and Jensen, E. S. (2011). The competitive ability of pea-barley intercrops against weeds and the interactions with crop productivity and soil N availability. *Field Crops Research* 122(3): 264-272.

Corre-Hellou, G., Fustec, J. and Crozat, Y. (2006). Interspecific Competition for Soil N and its Interaction with N2 Fixation, Leaf Expansion and Crop Growth in Pea–Barley Intercrops. *Plant and Soil* 282(1): 195-208.

Crews, T. E. and Peoples, M. B. (2004). Legume versus fertilizer sources of nitrogen: ecological tradeoffs and human needs. *Agriculture, Ecosystems & Environment* 102(3): 279-297.

Darnhofer, I., Lindenthal, T., Bartel-Kratochvil, R. and Zollitsch, W. (2010). Conventionalisation of organic farming practices: from structural criteria towards an assessment based on organic principles. A review. *Agronomy for Sustainable Development* 30(1): 67-81.

Dhima, K. V., Lithourgidis, A. S., Vasilakoglou, I. B. and Dordas, C. A. (2007). Competition indices of common vetch and cereal intercrops in two seeding ratio. *Field Crops Research* 100(2-3): 249-256.

Di Miceli, G., Giambalvo, D., Ruisi, P., Amato, G. and Frenda, A. S. (2009). Effetti della consociazione temporanea con favino sulla produttività e qualità del frumento duro in sistemi biologici. IV Workshop GRAB-IT "Agricoltura Biologica: sistemi produttivi e modelli di commercializzazione e di consumo". M. Crescimanno and G. Schifani. Palermo: 237-243.

Eaton, A. D., Clesceri, L. S. and Greenberg, A. E. (1995). Determination of anions by ion chromatography, part 4000 Inorganic nonmetallic constituents 4-1 4-8. *Standard Methods for the examination of water and wastewater*. AWWA APHA, WEF.

Erisman, J. W., Sutton, M. A., Galloway, J., Klimont, Z. and Winiwarter, W. (2008). How a century of ammonia synthesis changed the world. *Nature Geosci* 1(10): 636-639.

Ervin, D. E., Glenna, L. L. and Jussaume, R. A. (2010). Are biotechnology and sustainable agriculture compatible? *Renewable Agriculture and Food Systems* 25(2): 143-157.

Eskandari, H. (2011). Intercropping of wheat (Triticum aestivum) and bean (Vicia faba): Effects of complementarity and competition of intercrop components in resource consumption on dry matter production and weed growth. *African Journal of Biotechnology* 10(77): 17755-17762.

(2012). http://epp.eurostat.ec.europa.eu/portal/page/portal/eurostat/home.

Fagnano, M., Fiorentino, N., D'Egidio, M. G., Quaranta, F., Ritieni, A., Ferracane, R. and Raimondi, G. (2012). Durum Wheat in Conventional and Organic Farming: Yield Amount and Pasta Quality in Southern Italy. *The Scientific World Journal* 2012: 9.

Fan, F., Zhang, F., Song, Y., Sun, J., Bao, X., Guo, T. and Li, L. (2006). Nitrogen Fixation of Faba Bean ( Vicia faba L.) Interacting with a Non-legume in Two Contrasting Intercropping Systems. *Plant and Soil* 283(1): 275-286.

FAO (2009). FAO profile for climate change. Rome, FOOD AND AGRICULTURE ORGANIZATION OF THE UNITED NATIONS. Available for download at http://www.fao.org/docrep/012/i1323e/i1323e00.htm

Fernàndez-Aparicio, M., Sillero, J. C. and Rubiales, D. (2007). Intercropping with cereals reduces infection by Orobanche crenata in legumes. *Crop Protection* 26 (8): 1166-1172.

Finckh, M. R., Gacek, E. S., Goyeau, H., Lannou, C., Merz, U., Mundt, C. C., Munk, L., Nadziak, J., Newton, A. C., De Vallavielle-Pope, C. and Wolfe, M. S. (2000). Cereal variety and species mixtures in practice, with emphasis on disease resistance. *Agronomie* 20(7): 813-837.

Francis, C. A. (1986). *Distribution and importance of multiple cropping systems*. New York, Macmillan.

French, S., Levy-Booth, D., Samarajeewa, A., Shannon, K. E., Smith, J. and Trevors, J. T. (2009). Elevated temperatures and carbon dioxide concentrations: effects on selected microbial activities in temperate agricultural soils. *World Journal of Microbiology and Biotechnology* 25: 1887-1900.

Frye, W. W., Blevins, R. L., Smith, M. S., Corak, S. J., Varco, J. J. (1988). Role of annual legume cover crops in efficient use of water and nitrogen. *Cropping Strategies for Efficient Use of Water and Nitrogen, Special Pubblication* 51: 129-154.

Fujita, K., Ofosu-Budu, K. G. and Ogata, S. (1992). Biological nitrogen fixation in mixed legume-cereal cropping systems. *Plant and Soil* 141(1): 155-175.

Fukai, S. (1993). Intercropping -- bases of productivity. *Field Crops Research* 34(3-4): 239-245.

Fukai, S. and Trenbath, B. R. (1993). Processes determining intercrop productivity and yields of component crops. *Field Crops Research* 34(3-4): 247-271.

Garnett, T., Appleby, M. C., Balmford, A., Bateman, I. J., Benton, T. G., Bloomer, P., Burlingame, B., Dawkins, M., Dolan, L., Fraser, D., Herrero, M., Hoffmann, I., Smith, P., Thornton, P. K., Toulmin, C., Vermeulen, S. J. and Godfray, H. C. J. (2013). Sustainable intensification in agriculture: Premises and policies. *Science* 341(6141): 33-34.

Garrido-Lestache, E., López-Bellido, R. J. and López-Bellido, L. (2005). Durum wheat quality under Mediterranean conditions as affected by N rate, timing and splitting, N form and S fertilization. *European Journal of Agronomy* 23(3): 265-278.

Gee, G. W. and Bauder, J. W. (1986). Particle-size analysis. *Methods of soil analysis. Part 1. Physical and mineralogical methods*. A. Klute. Madison, Wisconsin (USA), American Society of Agronomy, Inc.: 383-411.

Ghaley, B., Hauggaard-Nielsen, H., Høgh-Jensen, H. and Jensen, E. (2005). Intercropping of Wheat and Pea as Influenced by Nitrogen Fertilization. *Nutrient Cycling in Agroecosystems* 73(2): 201-212.

Ghanbari-Bonjar, A., Lee, H., nbsp and C (2002). Intercropped field beans (Vicia faba) and wheat (Triticum aestivum) for whole crop forage: effect of nitrogen on forage yield and quality. *The Journal of Agricultural Science* 138(03): 311-315.

Ghosh, P. K., Tripathi, A. K., Bandyopadhyay, K. K. and Manna, M. C. (2009). Assessment of nutrient competition and nutrient requirement in soybean/sorghum intercropping system. *European Journal of Agronomy* 31(1): 43-50.

Giuliani, M. M., Giuzio, L., de Caro, A. and Flagella, Z. (2011). Relationships between nitrogen utilization and grain technological quality in durum wheat: II. Grain yield and quality. *Agronomy Journal* 103(6): 1668-1675.

Godfray, H. C. J., Beddington, J. R., Crute, I. R., Haddad, L., Lawrence, D., Muir, J. F., Pretty, J., Robinson, S., Thomas, S. M. and Toulmin, C. (2010). Food security: The challenge of feeding 9 billion people. *Science* 327(5967): 812-818.

Gomez, K. A. and Gomez, A. A. (1984). *Statistical Procedures for Agricultural Research*, John Wiley & Sons.

Gooding, M. J., Kasyanova, E., Ruske, R., Hauggaard-Nielsen, H., Jensen, E. S., Dahlmann, C., Von Fragstein, P., Dibet, A., Corre-Hellou, G., Crozat, Y., Pristeri, A., Romeo, M., Monti, M. and Launay, M. (2007). Intercropping with pulses to concentrate nitrogen and sulphur in wheat. *The Journal of Agricultural Science* 145(05): 469-479.

Gunes, A., Inal, A., Adak, M., Alpaslan, M., Bagci, E., Erol, T. and Pilbeam, D. (2007). Mineral nutrition of wheat, chickpea and lentil as affected by mixed cropping and soil moisture. *Nutrient Cycling in Agroecosystems* 78(1): 83-96.

Hansen, B., Kristensen, E. S., Grant, R., Høgh-Jensen, H., Simmelsgaard, S. E. and Olesen, J. E. (2000). Nitrogen leaching from conventional versus organic farming systems - A systems modelling approach. *European Journal of Agronomy* 13(1): 65-82.

Hanson, J. D., Liebig, M. A., Merrill, S. D., Tanaka, D. L., Krupinsky, J. M. and Stott, D. E. (2007). Dynamic Cropping Systems: Increasing Adaptability Amid an Uncertain Future. *Agronomy Journal* 99(4): 939-943.

Hardarson, G., Zapata, F. and Danso, S. K. A. (1988). Dinitrogen Fixation Measurements in Alfalfa-Ryegrass Swards Using Nitrogen-15 and Influence of the Reference Crop. *Crop Science* 28(1): 101-105.

Harper, J. L. (1977). *Population biology of plants*, Academic Press.

Hauggaard-Nielsen, H., Ambus, P. and Jensen, E. S. (2001a). Interspecific competition, N use and interference with weeds in pea-barley intercropping. *Field Crops Research* 70(2): 101-109.

Hauggaard-Nielsen, H., Ambus, P. and Jensen, E. S. (2001b). Temporal and spatial distribution of roots and competition for nitrogen in pea-barley intercrops – a field study employing 32P technique. *Plant and Soil* 236(1): 63-74.

Hauggaard-Nielsen, H., Ambus, P. and Jensen, E. S. (2003). The comparison of nitrogen use and leaching in sole cropped versus intercropped pea and barley. *Nutrient Cycling in Agroecosystems* 65(3): 289-300.

Hauggaard-Nielsen, H., Andersen, M. K., Jornsgaard, B. and Jensen, E. S. (2006). Density and relative frequency effects on competitive interactions and resource use in pea-barley intercrops. *Field Crops Research* 95(2): 256-267.

Hauggaard-Nielsen, H., Gooding, M., Ambus, P., Corre-Hellou, G., Crozat, Y., Dahlmann, C., Dibet, A., von Fragstein, P., Pristeri, A. and Monti, M. (2009a). Pea-barley intercropping for efficient symbiotic N2-fixation, soil N acquisition and use of other nutrients in European. *Field Crops Research* 113(1): 64-71.

Hauggaard-Nielsen, H., Gooding, M., Ambus, P., Corre-Hellou, G., Crozat, Y., Dahlmann, C., Dibet, A., von Fragstein, P., Pristeri, A., Monti, M. and Jensen, E. (2009b). Pea–barley intercropping and short-term subsequent crop effects across European organic cropping conditions. *Nutrient Cycling in Agroecosystems* 85(2): 141-155.

Hauggaard-Nielsen, H., J?rnsgaard, B., Kinane, J. and Jensen, E. S. (2008). Grain legume-cereal intercropping: The practical application of diversity, competition and facilitation in arable and organic cropping systems. *Renewable Agriculture and Food Systems* 23(Special Issue 01): 3-12.

Hauggaard-Nielsen, H. and Jensen, E. (2005). Facilitative Root Interactions in Intercrops. *Plant and Soil* 274(1): 237-250.

Haymes, R. and Lee, H. C. (1999). Competition between autumn and spring planted grain intercrops of wheat (Triticum aestivum) and field bean (Vicia faba). *Field Crops Research* 62(2-3): 167-176.

Herridge, D., Roughley, R. and Brockwell, J. (1984). Effect of rhizobia and soil nitrate on the establishment and functioning of the soybean symbiosis in the field. *Australian Journal of Agricultural Research* 35(2): 149-161.

Hill, E. C., Ngouajio, M. and Nair, M. G. (2007). Allelopathic potential of hairy vetch (Vicia villosa) and cowpea (Vigna unguiculata) methanol and ethyl acetate extracts on weeds and vegetables. *Weed Technology* 21(2): 437-444.

Hiltbrunner, J., Liedgens, M., Stamp, P. and Streit, B. (2005). Effects of row spacing and liquid manure on directly drilled winter wheat in organic farming. *European Journal of Agronomy* 22(4): 441-447.

Hinsinger, P., Betencourt, E., Bernard, L., Brauman, A., Plassard, C., Shen, J., Tang, X. and Zhang, F. (2011). P for Two, Sharing a Scarce Resource: Soil Phosphorus Acquisition in the Rhizosphere of Intercropped Species. *Plant Physiology* 156 (3): 1078-1086.

Högberg, P. (1997). Tansley Review No. 95. 15N Natural Abundance in Soil-Plant Systems. *New Phytologist* 137(2): 179-203.

Hooks, C. and Johnson, M. (2006). Population densities of herbivorous lepidopterans in diverse cruciferous cropping habitats: Effects of mixed cropping and using a living mulch. *BioControl* 51(4): 485-506.

Horwith, B. (1985). A Role for Intercropping in Modern Agriculture. *BioScience* 35(5): 286-291.

Houlton, B. Z., Wang, Y.-P., Vitousek, P. M. and Field, C. B. (2008). A unifying framework for dinitrogen fixation in the terrestrial biosphere. *Nature* 454(7202): 327-330.

IFOAM (2012). 2011 IFOAM Consolidated Annual Report. Bonn.

IPCC (2007). Climate change 2007: Synthesis Report. Contribution of Working Groups I, II and III to the Fourth Assessment Report of the Intergovernmental Panel on Climate Change. Geneva, Switzerland, IPCC.

(2012). http://www.istat.it/it/.

Jensen, E. S. (1996). Grain yield, symbiotic N2 fixation and interspecific competition for inorganic N in pea-barley intercrops. *Plant and Soil* 182(1): 25-38.

Jensen, E. S. and Hauggaard-Nielsen, H. (2003). How can increased use of biological N2 fixation in agriculture benefit the environment? *Plant and Soil* 252(1): 177-186.

Keating, B. A. and Carberry, P. S. (1993). Resource capture and use in intercropping: solar radiation. *Field Crops Research* 34(3-4): 273-301.

Kirkegaard, J., Christen, O., Krupinsky, J. and Layzell, D. (2008). Break crop benefits in temperate wheat production. *Field Crops Research* 107(3): 185-195.

Kopke, U. and Nemecek, T. (2010). Ecological services of faba bean. *Field Crops Research* 115(3): 217-233.

Kurdali, F., Sharabi, N. and Arslan, A. (1996). Rainfed vetch-barley mixed cropping in the Syrian semi-arid conditions. *Plant and Soil* 183(1): 137-148.

Lauk, R. and Lauk, E. (2008). Pea-oat intercrops are superior to pea-wheat and pea-barley intercrops. *Acta Agriculturae Scandinavica Section B: Soil and Plant Science* 58(2): 139-144.

Lauk, R., Lauk, E., Lauringson, E. and Talgre, L. (2007). Vetch-wheat crops are superior to vetch-oat crops in terms of protein yield. *Acta Agriculturae Scandinavica Section B: Soil and Plant Science* 57(2): 116-121.

Launay, M., Brisson, N., Satger, S., Hauggaard-Nielsen, H., Corre-Hellou, G., Kasynova, E., Ruske, R., Jensen, E. S. and Gooding, M. J. (2009). Exploring options for managing strategies for pea-barley intercropping using a modeling approach. *European Journal of Agronomy* 31(2): 85-98.

Li Destri Nicosia, O., Paoletta, G. and Codianni, P. (2005). Valutazione di tecniche a basso impatto ambientale per il controllo delle infestanti e il miglioramento qualitativo della produzione in frumento duro in coltura biologica. Atti del XXXV Convegno della Società Italiana di Agronomia. M. M. Giuliani and G. Gatta. Foggia**:** 53-54.

Li, L., Sun, J., Zhang, F., Guo, T., Bao, X., Smith, F. and Smith, S. (2006). Root distribution and interactions between intercropped species. *Oecologia* 147(2): 280-290.

Li, L., Tang, C., Rengel, Z. and Zhang, F. (2003). Chickpea facilitates phosphorus uptake by intercropped wheat from an organic phosphorus source. *Plant and Soil* 248(1-2): 297-303.

Li, L., Tang, C., Rengel, Z. and Zhang, F. S. (2004). Calcium, magnesium and microelement uptake as affected by phosphorus sources and interspecific root interactions between wheat and chickpea. *Plant and Soil* 261(1): 29-37.

Li, Y., Ran, W., Zhang, R., Sun, S. and Xu, G. (2009). Facilitated legume nodulation, phosphate uptake and nitrogen transfer by arbuscular inoculation in an upland rice and mung bean intercropping system. *Plant and Soil* 315(1): 285-296.

Liebman, M. and Dyck, E. (1993). Crop rotation and intercropping strategies for weed management. *Ecological Applications* 3(1): 92-122.

Lithourgidis, A. S., Dhima, K. V., Vasilakoglou, I. B., Dordas, C. A. and Yiakoulaki, M. D. (2007). Sustainable production of barley and wheat by intercropping common vetch. *Agronomy for Sustainable Development* 27(2): 95-99.

Lithourgidis, A. S., Vlachostergios, D. N., Dordas, C. A. and Damalas, C. A. (2011). Dry matter yield, nitrogen content, and competition in pea-cereal intercropping systems. *European Journal of Agronomy* 34(4): 287-294.

Malézieux, E., Crozat, Y., Dupraz, C., Laurans, M., Makowski, D., Ozier-Lafontaine, H., Rapidel, B., Tourdonnet, S. and Valantin-Morison, M. (2009). Mixing plant species in cropping systems: concepts, tools and models. A review. *Agronomy for Sustainable Development* 29(1): 43-62.

Mariotti, M., Masoni, A., Ercoli, L. and Arduini, I. (2009). Above- and below-ground competition between barley, wheat, lupin and vetch in a cereal and legume intercropping system. *Grass and Forage Science* 64(4): 401-412.

Mariotti, M., Masoni, A., Ercoli, L. and Arduini, I. (2012). Optimizing forage yield of durum wheat/field bean intercropping through N fertilization and row ratio. *Grass and Forage Science* 67(2): 243-254.

Markham, J. H. and Chanway, C. P. (1996). Measuring Plant Neighbour Effects. *Functional Ecology* 10(4): 548-549.

Martens, J. R. T., Hoeppner, J. W. and Entz, M. H. (2001). Legume cover crops with winter cereals in southern Manitoba: Establishment, productivity, and microclimate effects. *Agronomy Journal* 93(5): 1086-1096.

(2005). *Fertilizzazine azotata dei cereali autunno-vernini*. Venturina, Italy http://www.irri.it/fert_cer/start_fertcer.asp.

McGilchrist, C. A. (1965). Analysis of competition experiments. *Biometrics* 21: 975-985.

McLean, E. O. (1982). Soil pH and lime requirement. *Methods of soil analysis, Part 2*. A. L. Page, X and Y. Madison, Wisconsin (USA), ASA and SSSA**:** 199-224.

Mehlich, A. (1984). Mehlich 3 soil test extractant: A modification of the Mehlich 2 extractant. *Communications in Soil Science and Plant Analysis* 15: 1409-1416.

Midmore, D. J. (1993). Agronomic modification of resource use and intercrop productivity. *Field Crops Research* 34(3-4): 357-380.

Moonen, A. C. and Barberi, P. (2004). Size and composition of the weed seedbank after 7 years of different cover-crop-maize management systems. *Weed Research* 44(3): 163-177.

Morris, R. A. and Garrity, D. P. (1993a). Resource capture and utilization in intercropping; non-nitrogen nutrients. *Field Crops Research* 34(3-4): 319-334.

Morris, R. A. and Garrity, D. P. (1993b). Resource capture and utilization in intercropping: water. *Field Crops Research* 34(3-4): 303-317.

Naudin, C., Corre-Hellou, G., Pineau, S., Crozat, Y. and Jeuffroy, M.-H. (2010). The effect of various dynamics of N availability on winter pea-wheat intercrops: Crop growth, N partitioning and symbiotic N2 fixation. *Field Crops Research* 119(1): 2-11.

Nelson, D. W. (1982). Carbonate and gypsum. *Methods of soil analysis, Part 2.* A. L. Page, X and Y. Madison, Wisconsin (USA), ASA and SSSA. 9**:** 182-197.

Nelson, D. W. and Sommers, L. E. (1982). Total carbon, organic carbon and organic matter. *Methods of soil analysis, Part 2.* A. L. Page, X and Y. Madison, Wisconsin (USA), ASA and SSSA. 9**:** 539-579.

Ofori, F. and Stern, W. R. (1987). Cereal–Legume Intercropping Systems. *Advances in Agronomy*. N. C. Brady, Academic Press. Volume 41**:** 41-90.

Olsen, S. R. and Sommers, L. E. (1982). Phosphorus. *Methods of soil analysis, Part 2*. A. L. Page, X and Y. Madison, Wisconsin (USA), ASA and SSSA**:** 403-430.

Palmer, R., Perez, P., Ortiz-Perez, E., Maalouf, F. and Suso, M. (2009). The role of crop-pollinator relationships in breeding for pollinator-friendly legumes: from a breeding perspective. *Euphytica* 170(1): 35-52.

Pappa, V. A., Rees, R. M., Walker, R. L., Baddeley, J. A. and Watson, C. A. (2011). Nitrous oxide emissions and nitrate leaching in an arable rotation resulting from the presence of an intercrop. *Agriculture, Ecosystems & Environment* 141(1-2): 153-161.

Peigné, J., Ball, B. C., Roger-Estrade, J. and David, C. (2007). Is conservation tillage suitable for organic farming? A review. *Soil Use and Management* 23(2): 129-144.

Pelzer, E., Bazot, M., Makowski, D., Corre-Hellou, G., Naudin, C., Al Rifaï, M., Baranger, E., Bedoussac, L., Biarnès, V., Boucheny, P., Carrouée, B., Dorvillez, D., Foissy, D., Gaillard, B., Guichard, L., Mansard, M. C., Omon, B., Prieur, L., Yvergniaux, M., Justes, E. and Jeuffroy, M. H. (2012). Pea-wheat intercrops in low-input conditions combine high economic performances and low environmental impacts. *European Journal of Agronomy* 40: 39-53.

Peoples, M. B., Hauggaard-Nielsen, H. and Jensen, E. S. (2009a). The potential environmental benefits and risks derived from legumes in rotations. *Nitrogen fixation in crop production*. D. W. and Krishnan Emerich, H. B. 677 South Segoe Road, Madison, WI 53711-1086 USA, American Society of Agronomy, Inc. Crop Science Society of America, Inc. Soil Science Society of America, Inc.**:** 349-385.

Peoples, M. B., Hauggaard-Nielsen, H. and Jensen, E. S. (2009a). The potential environmental benefits and risks derived from legumes in rotations. *Nitrogen fixation in crop production*. D. W. and Krishnan Emerich, H. B. 677 South Segoe Road, Madison, WI 53711-1086 USA, American Society of Agronomy, Inc. Crop Science Society of America, Inc. Soil Science Society of America, Inc.**:** 349-385.

Peoples, M. B., Herridge, D. F. and Ladha, J. K. (1995a). Biological nitrogen fixation: An efficient source of nitrogen for sustainable agricultural production? *Plant and Soil* 174(1): 3-28.

Peoples, M. B., Ladha, J. K. and Herridge, D. F. (1995b). Enhancing legume N2 fixation through plant and soil management. *Plant and Soil* 174(1): 83-101.

Peoples, M. B., Turner, G. L., Shah, Z., Shah, S. H., Aslam, M., Ali, S., Maskey, S. L., Bhattarai, S., Afandi, F., Schwenke, G. D. and Herridge, D. F. (1997). Evaluation of the 15N natural abundance technique to measure N2 fixation in experimental plots and farmers' fields. International workshop on managing legume nitrogen fixation in the cropping systems of Asia, Andhra Pradesh, India, International Crops Research Institute for the Semi-Arid Tropics.

Peoples, M. B., Unkovich, M. J. and Herridge, D. F. (2009b). Measuring symbiotic nitrogen fixation by legumes. *Nitrogen fixation in crop production*. D. W. and Krishnan Emerich, H. B. 677 South Segoe Road, Madison, WI 53711-1086 USA, American Society of Agronomy, Inc. Crop Science Society of America, Inc. Soil Science Society of America, Inc.**:** 125-170.

Peoples, M. B., Unkovich, M. J. and Herridge, D. F. (2009b). Measuring symbiotic nitrogen fixation by legumes. *Nitrogen fixation in crop production*. D. W. and Krishnan Emerich, H. B. 677 South Segoe Road, Madison, WI 53711-1086 USA, American Society of Agronomy, Inc. Crop Science Society of America, Inc. Soil Science Society of America, Inc.**:** 125-170.

Perrin, R. and Phillips, M. (1978). Some effects of mixed cropping on the population dynamics of insect pests. *Entomologia Experimentalis et Applicata* 24(3): 585-593.

Pimentel, D., Hepperly, P., Hanson, J., Douds, D. and Seidel, R. (2005). Environmental, Energetic, and Economic Comparisons of Organic and Conventional Farming Systems. *BioScience* 55(7): 573-582.

Poggio, S. L. (2005). Structure of weed communities occurring in monoculture and intercropping of field pea and barley. *Agriculture, Ecosystems & Environment* 109(1-2): 48-58.

Preston, K. R., March, P. R. and Tipples, K. H. (1982). An assessment of the sds-sedimentation test for the prediction of canadian bread wheat quality. *Canadian Journal of Plant Science* 62(3): 545-553.

Pridham, J. C. and Entz, M. H. (2008). Intercropping spring wheat with cereal grains, legumes, and oilseeds fails to improve productivity under organic management. *Agronomy Journal* 100(5): 1436-1442.

Quaranta, F., Amoriello, T., Aureli, G., Belocchi, A., D'Egidio, M. G., Fornara, M., Melloni, S. and Desiderio, E. (2010). Grain yield, quality and deoxynivalenol (DON) contamination of durum wheat (Triticum durum desf.): Results of national networks in organic and conventional cropping systems. *Italian Journal of Agronomy* 5(4): 353-366.

Rhoades, D. W. (1982). Soluble salts. *Methods of soil analysis, Part 2.* A. L. Page, X and Y. Madison, Wisconsin (USA), ASA and SSSA. 9**:** 167-179.

Rochester, I. J., Peoples, M. B., Hulugalle, N. R., Gault, R. R. and Constable, G. A. (2001). Using legumes to enhance nitrogen fertility and improve soil condition in cotton cropping systems. *Field Crops Research* 70(1): 27-41.

Rosset, P. M. and Altieri, M. A. (1997). Agroecology versus input substitution: A fundamental contradiction of sustainable agriculture. *Society and Natural Resources* 10(3): 283-295.

Saggar, S. (2010). Estimation of nitrous oxide emission from ecosystems and its mitigation technologies. *Agriculture, Ecosystems & Environment* 136(3-4): 189-191.

Sainju, U. M., Schomberg, H. H., Singh, B. P., Whitehead, W. F., Tillman, P. G. and Lachnicht-Weyers, S. L. (2007). Cover crop effect on soil carbon fractions under conservation tillage cotton. *Soil & Tillage Research* 96(1-2): 205-218.

San-nai, J. and Ming-pu, Z. (2000). Nitrogen transfer between N2-fixing plant and non-N2-fixing plant. *Journal of Forestry Research* 11(2): 75-80.

Schmidt, O. and Curry, J. (1999). Effects of earthworms on biomass production, nitrogen allocation and nitrogen transfer in wheat-clover intercropping model systems. *Plant and Soil* 214(1): 187-198.

Shearin, A. F., Chris Reberg-Horton, S. and Gallandt, E. R. (2009). Cover Crop Effects on the Activity-Density of the Weed Seed Predator Harpalus rufipes (Coleoptera: Carabidae). *Weed Science* 56(3): 442-450.

(2012). *Bio in cifre 2012: i dati del biologico italiano*. http://www.sinab.it/share/img_lib_files/2290_bio_in_cifre_2012_anticipazioni.pdf.

Smith, P. (2013). Delivering food security without increasing pressure on land. *Global Food Security* 2(1): 18-23.

Smith, S. R. and Hadley, P. (1990). Carbon and nitrogen mineralization characteristics of organic nitrogen fertilizers in a soil-less incubation system. *Fertilizer Research* 23(2): 97-103.

Stauss, R., Ciba-Geigy and Forstwirtschaft, B. B. f. L.-u. (1994). *Compendium of Growth Stage Identification Keys for Mono- and Dicotyledoneus Plants: Extended BBCH Scale*, Ciba-Geigy.

Stehfest, E. and Bouwman, L. (2006). N2O and NO emission from agricultural fields and soils under natural vegetation: summarizing available measurement data and modeling of global annual emissions. *Nutrient Cycling in Agroecosystems* 74(3): 207-228.

Stehfest, E., Bouwman, L., Vuuren, D. P. v., Elzen, M. G. J. d., Eickhout, B. and Kabat, P. (2009). Climate benefits of changing diet. *Climatic Change* 95: 83-102.

Stern, W. R. (1993). Nitrogen fixation and transfer in intercrop systems. *Field Crops Research* 34(3-4): 335-356.

Stinner, W., Möller, K. and Leithold, G. (2008). Effects of biogas digestion of clover/ grass-leys, cover crops and crop residues on nitrogen cycle and crop yield in organic stockless farming systems. *European Journal of Agronomy* 29(2-3): 125-134.

Szumigalski, A. R. and Van Acker, R. C. (2006). Nitrogen yield and land use efficiency in annual sole crops and intercrops. *Agronomy Journal* 98(4): 1030-1040.

Szumigalski, A. R. and Van Acker, R. C. (2008). Land Equivalent Ratios, Light Interception, and Water Use in Annual Intercrops in the Presence or Absence of In-Crop Herbicides. *Agron J* 100(4): 1145-1154.

Thiessen Martens, J. R., Hoeppner, J. W. and Entz, M. H. (2001). Legume cover crops with winter cereals in southern manitoba: Establishment, productivity, and microclimate effects. *Agronomy Journal* 93(5): 1086-1096.

Thorsted, M. D., Olesen, J. E. and Weiner, J. (2006). Width of clover strips and wheat rows influence grain yield in winter wheat/white clover intercropping. *Field Crops Research* 95(2-3): 280-290.

Tosti, G., Benincasa, P. and Guiducci, M. (2010). Competition and facilitation in hairy vetch-barley intercrops. *Italian Journal of Agronomy* 5(3): 239-247.

Tosti, G. and Guiducci, M. (2010). Durum wheat-faba bean temporary intercropping: Effects on nitrogen supply and wheat quality. *European Journal of Agronomy* 33 (3): 157-165.

Trenbath, B. R. (1993). Intercropping for the management of pests and diseases. *Field Crops Research* 34(3-4): 381-405.

Trydeman Knudsen, M., Hauggaard-Nielsen, H., Oslash, Rnsg, Aring, Rd, B. and Steen Jensen, E. (2004). Comparison of interspecific competition and N use in pea-barley and lupin-barley intercrops grown at two temperate locations. *The Journal of Agricultural Science* 142(06): 617-627.

Unkovich, M. J., Herridge, D. F., Peoples, M. B., Boddey, R. M., Cadisch, G., Giller, K. E., Alves, B. and Chalk, P. (2008). Measuring plant-associated nitrogen fixation in agricultural systems. *ACIAR Monography* 136.

Unkovich, M. J., Pate, J. P., Sanford, P. and Armstrong, E. L. (1994). Potential precision of the $\delta^{15}N$ natural abundance method in field estimates of nitrogen fixation by crop and pasture legumes in south-west Australia. *Australian Journal of Agricultural Research* 45: 119-132.

Unkovich, M. J. and Pate, J. S. (2000). An appraisal of recent field measurements of symbiotic N2 fixation by annual legumes. *Field Crops Research* 65(2-3): 211-228.

Urbatzka, P., Graß, R., Haase, T., Schüler, C. and Heß, J. (2009). Fate of legume-derived nitrogen in monocultures and mixtures with cereals. Agriculture, Ecosystems and Environment. **132:** 116-125.

Vandermeer, J. (1989). *The ecology of intercropping*, Cambridge University Press.

Vandermeer, J. (1992). *The ecology of intercropping*, Cambridge University Press.

Varvel, G. E. and Peterson, T. A. (1990). Nitrogen Fertilizer Recovery by Corn in Monoculture and Rotation Systems. *Agron. J.* 82(5): 935-938.

Violante, P. and Adamo, P. (2000). Determinazione della conduttività elettrica. *Metodi di analisi chimica del suolo. Parte IV*. Milano, Franco Angeli Editore.

Wagger, M. G. (1989). Cover Crop Management and Nitrogen Rate in Relation to Growth and Yield of No-Till Corn. *Agron. J.* 81(3): 533-538.

Willey, R. W. (1979). Intercropping - Its importance and research needs. *Field Crop Abstracts* 32: 1-10.

Willey, R. W. and Rao, M. R. (1980). A Competitive Ratio for Quantifying Competition Between Intercrops. *Experimental Agriculture* 16(02): 117-125.

Wojtkowski, P. (2006). *Introduction to Agroecology*. Birmingham, NY, Hawort Press Inc.

Zhang, L., Wang, G., Zhang, E., Zhang, B., He, C., Wang, Q., Qiang, S. and Huang, G. (2011). Effect of phosphorus application and stripintercropping on yield and some wheat-grain components in a wheat/maize/potato intercropping system. *African Journal of Agricultural Research* 6(27): 5860-5869.

Zuo, Y. and Zhang, F. (2009). Iron and zinc biofortification strategies in dicot plants by intercropping with gramineous species. A review. *Agronomy for Sustainable Development* 29(1): 63-71.

# Appendix 1 - Omitted results

*Table A - Concentration of P and accumulation of $P_2O_5$ in the different plant portions (grain, chaff, straw, residues, total biomass) of wheat sampled at maturity in 2009/10 . Data expressed as means ± SD (n = 3). Within each column, data followed by different letters are significantly different*

| **Treatment** | **P concentration (mg $g^{-1}$)** | | | **$P_2O_5$ accumulation (g $m^{-2}$)** | | | | |
|---|---|---|---|---|---|---|---|---|
| | **Chaff** | **Straw** | **Grain** | **Chaff** | **Straw** | **Residues** | **Grain** | **Total** |
| N0 | 1.70 cdef | 0.80 def | 3.27 cd | 0.18 | 0.37 | 0.55 | 0.85 | 1.40 |
| N40 | 1.03 f | 0.70 ef | 3.20 cd | 0.18 | 0.56 | 0.74 | 1.46 | 2.20 |
| N80 | 0.97 f | 0.60 f | 3.50 abc | 0.25 | 0.55 | 0.80 | 2.02 | 2.82 |
| N120 | 1.47 def | 0.60 f | 3.40 abc | 0.32 | 0.61 | 0.93 | 2.19 | 3.12 |
| N160 | 1.07 ef | 0.60 f | 3.40 abc | 0.29 | 0.64 | 0.93 | 2.44 | 3.37 |
| PB-DW TIC | 2.37 bcd | 1.00 cd | 3.33 bc | 0.40 | 0.59 | 0.99 | 1.57 | 2.56 |
| FP-DW TIC | 2.07 bcde | 0.67 ef | 2.97 d | 0.33 | 0.37 | 0.70 | 1.15 | 1.85 |
| HV-DW TIC | 2.97 ab | 0.90 de | 3.30 bc | 0.45 | 0.56 | 1.01 | 1.62 | 2.63 |
| PB-DW PIC | 3.83 a | 2.20 a | 3.60 ab | 0.02 | 0.43 | 0.45 | 0.03 | 0.48 |
| FP-DW PIC | 2.63 bc | 1.27 b | 3.47 abc | 0.31 | 0.65 | 0.96 | 1.00 | 1.96 |
| HV-DW PIC | 2.60 bc | 1.17 bc | 3.70 a | 0.28 | 0.62 | 0.90 | 0.85 | 1.75 |
| Significance[1] | ** | ** | ** | ** | ns | ns | ** | ** |
| *LSD* | *1.02* | *0.26* | *0.32* | *1.11* | *2.62* | *3.62* | *5.41* | *8.70* |
| *CV (%)[2]* | *29.1* | *15.8* | *5.5* | *24.5* | *28.0* | *26.1* | *22.9* | *23.2* |

*[1] **, *, ns are, respectively, significant for p≤.01, significant for p≤.05 and not significant (Fisher's Protected LSD test)*

*[2] Coefficient of variation*

*Table B - Concentration of P and accumulation of $P_2O_5$ in the different plant portions (grain, chaff, straw, residues, total biomass) of wheat sampled at maturity in 2010/11 . Data expressed as means ± SD (n = 3). Within each column, data followed by different letters are significantly different*

| **Treatment** | **P concentration (mg $g^{-1}$)** | | | **$P_2O_5$ accumulation (g $m^{-2}$)** | | | | |
|---|---|---|---|---|---|---|---|---|
| | **Chaff** | **Straw** | **Grain** | **Chaff** | **Straw** | **Residues** | **Grain** | **Total** |
| N0 | 1.47 cd | 0.93 bc | 3.27 | 0.24 | 0.27 | 0.51 | 1.33 | 1.84 |
| N40 | 0.80 e | 0.30 d | 3.07 | 0.18 | 0.12 | 0.30 | 1.80 | 2.10 |
| N80 | 1.07 de | 0.60 cd | 3.17 | 0.25 | 0.26 | 0.51 | 1.92 | 2.43 |
| N120 | 0.93 de | 0.33 d | 3.17 | 0.21 | 0.14 | 0.35 | 2.06 | 2.41 |
| N160 | 0.63 e | 0.23 d | 2.97 | 0.17 | 0.11 | 0.28 | 2.27 | 2.55 |
| PB-DW TIC | 2.37 ab | 2.23 a | 3.80 | 0.40 | 0.60 | 1.00 | 1.63 | 2.63 |
| FP-DW TIC | 2.40 ab | 2.30 a | 3.50 | 0.41 | 0.73 | 1.14 | 1.64 | 2.78 |
| HV-DW TIC | 2.27 ab | 1.93 a | 3.33 | 0.44 | 0.63 | 1.07 | 1.71 | 2.78 |
| PB-DW PIC | 2.47 a | 1.20 b | 3.43 | 0.24 | 0.24 | 0.48 | 0.83 | 1.31 |
| FP-DW PIC | 1.73 bc | 1.27 b | 3.40 | 0.25 | 0.35 | 0.60 | 1.28 | 1.88 |
| HV-DW PIC | 1.93 abc | 1.27 b | 3.30 | 0.26 | 0.32 | 0.58 | 1.07 | 1.65 |
| *Significance[1]* | ** | ** | *ns* | ** | ** | ** | ** | ** |
| *LSD* | *0.64* | *0.46* | *0.45* | *1.07* | *1.77* | *2.64* | *3.69* | *5.34* |
| *CV (%)[2]* | *22.7* | *23.7* | *8.0* | *22.8* | *30.0* | *24.9* | *13.6* | *14.1* |

*[1] **, *, ns are, respectively, significant for p≤.01, significant for p≤.05 and not significant (Fisher's Protected LSD test)*

*[2] Coefficient of variation*

*Table C - P concentration (mg g$^{-1}$) in aboveground dry matter of wheat under sole- (N0, N40, N80, N120, N160) and inter-cropping (PB-DW PIC, FP-DW PIC, HV-DW PIC, PB-DW TIC, FP-DW TIC, HV-DW TIC) in 2009/10*

| **Treatment** | **BBCH 30** | **BBCH 59** | | **BBCH 69** | | **BBCH 75** | | **BBCH 89** | | |
|---|---|---|---|---|---|---|---|---|---|---|
| | **Straw** | **Straw** | **Ears** | **Straw** | **Ears** | **Straw** | **Ears** | **Straw** | **Ears** | **Grain** |
| N0 | 1.50 | 1.70 abc | 3.00 bc | 1.20 cde | 2.50 bc | 0.90 cde | 2.80 bc | 1.70 cdef | 0.80 def | 3.27 cd |
| N40 | 1.50 | 1.20 d | 2.50 c | 1.00 e | 2.30 de | 0.50 e | 2.40 c | 1.03 f | 0.70 ef | 3.20 cd |
| N80 | 1.50 | 1.70 abc | 3.00 bc | 1.40 bcd | 2.40 bcd | 0.50 e | 2.60 bc | 0.97 f | 0.60 f | 3.50 abc |
| N120 | 1.40 | 1.40 cd | 2.80 bc | 1.10 de | 2.50 bc | 0.60 de | 2.80 bc | 1.47 def | 0.60 f | 3.40 abc |
| N160 | 1.60 | 1.50 bcd | 3.20 b | 1.40 bcd | 2.50 bc | 0.80 cde | 2.70 bc | 1.07 ef | 0.60 f | 3.40 abc |
| PB-DW TIC | 1.70 | 1.80 abc | 2.90 bc | 1.60 b | 2.50 bc | 1.10 bcd | 2.90 bc | 2.37 bcd | 1.00 cd | 3.33 bc |
| FP-DW TIC | 1.60 | 1.80 abc | 2.70 c | 1.40 bcd | 2.40 bcd | 0.60 de | 2.70 bc | 2.07 bcde | 0.67 ef | 2.97 d |
| HV-DW TIC | 1.70 | 1.90 ab | 2.90 bc | 1.50 bc | 2.20 e | 0.90 cde | 2.80 bc | 2.97 ab | 0.90 de | 3.30 bc |
| PB-DW PIC | 1.70 | 2.20 a | 3.70 a | 2.30 a | 3.80 a | 2.20 a | 4.60 a | 3.83 a | 2.20 a | 3.60 ab |
| FP-DW PIC | 1.60 | 1.80 abc | 2.80 bc | 1.40 bcd | 2.30 de | 1.20 bc | 2.60 bc | 2.63 bc | 1.27 b | 3.47 abc |
| HV-DW PIC | 1.70 | 2.00 ab | 2.90 bc | 1.60 b | 2.40 bcd | 1.40 b | 3.10 b | 2.60 bc | 1.17 bc | 3.70 a |
| *Significance*[1] | *ns* | * | * | ** | ** | ** | ** | ** | ** | ** |
| *LSD* | *0.57* | *0.48* | *0.49* | *0.34* | *0.24* | *0.52* | *0.53* | *1.02* | *0.26* | *0.32* |
| *CV (%)*[2] | *21.3* | *16.4* | *9.7* | *13.7* | *4.6* | *31.3* | *10.8* | *29.1* | *15.8* | *5.5* |

[1] ***, *, ns are, respectively, significant for p≤.01, significant for p≤.05 and not significant (LSD test)*

[2] *Coefficient of variation*

*Table D - $P_2O_5$ accumulation (g $m^{-2}$) in aboveground dry matter of wheat under sole- (N0, N40, N80, N120, N160) and inter-cropping (PB-DW PIC, FP-DW PIC, HV-DW PIC, PB-DW TIC, FP-DW TIC, HV-DW TIC) in 2009/10*

| Treatment | BBCH 30 | | BBCH 59 | | | BBCH 69 | | | BBCH 75 | | | BBCH 89 | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | **Straw** | **Total** | **Straw** | **Ears** | **Total** | **Straw** | **Ears** | **Total** | **Straw** | **Ears** | **Total** | **Straw** | **Ears** | **Grain** | **Total** |
| N0 | 0.32 c | 0.32 c | 0.70 cd | 0.18 cd | 0.88 de | 0.62 cd | 0.27 cd | 0.89 de | 0.43 | 0.86 d | 1.29 d | 0.37 | 0.18 c | 0.85 e | 1.40 e |
| N40 | 0.60 ab | 0.60 ab | 0.80 cd | 0.30 bc | 1.10 cde | 1.08 abc | 0.46 bc | 1.54 bcd | 0.41 | 1.30 cd | 1.71 cd | 0.56 | 0.18 c | 1.46 cd | 2.20 bcde |
| N80 | 0.66 a | 0.66 a | 1.36 ab | 0.44 ab | 1.80 ab | 1.67 a | 0.69 ab | 2.36 ab | 0.46 | 1.90 ab | 2.36 abc | 0.55 | 0.25 bc | 2.02 ab | 2.82 abc |
| N120 | 0.64 a | 0.64 a | 1.57 a | 0.55 a | 2.12 a | 1.35 ab | 0.82 a | 2.17 abc | 0.62 | 2.14 ab | 2.76 ab | 0.61 | 0.32 ab | 2.19 a | 3.12 ab |
| N160 | 0.76 a | 0.76 a | 1.28 abc | 0.55 a | 1.83 abc | 1.81 a | 0.95 a | 2.76 a | 0.90 | 2.23 a | 3.13 a | 0.64 | 0.29 bc | 2.44 a | 3.37 a |
| PB-DW TIC | 0.34 bc | 0.34 bc | 1.15 abc | 0.37 abc | 1.52 abcd | 1.01 bc | 0.51 bc | 1.52 cd | 0.68 | 1.62 bc | 2.30 abc | 0.59 | 0.40 a | 1.57 bc | 2.56 abcd |
| FP-DW TIC | 0.44 abc | 0.44 abc | 0.95 bcd | 0.28 abc | 1.23 bcd | 0.91 bcd | 0.34 cd | 1.25 d | 0.35 | 1.36 cd | 1.71 cd | 0.37 | 0.33 bc | 1.15 cde | 1.85 de |
| HV-DW TIC | 0.57 abc | 0.57 abc | 1.01 abc | 0.31 bc | 1.32 bcd | 0.98 bcd | 0.47 bc | 1.45 cd | 0.57 | 1.59 bc | 2.16 bcd | 0.56 | 0.45 a | 1.62 bc | 2.63 abcd |
| PB-DW PIC | 0.30 c | 0.30 c | 0.40 d | 0.02 d | 0.42 e | 0.43 d | 0.02 d | 0.45 e | 0.41 | 0.04 e | 0.45 e | 0.43 | 0.02 d | 0.03 f | 0.48 f |
| FP-DW PIC | 0.45 abc | 0.45 abc | 0.93 bcd | 0.23 c | 1.16 bcd | 0.93 bcd | 0.27 cd | 1.20 de | 0.67 | 0.84 d | 1.51 cd | 0.65 | 0.31 ab | 1.00 de | 1.96 cde |
| HV-DW PIC | 0.54 abc | 0.54 abc | 1.13 abc | 0.19 cd | 1.32 bcd | 1.05 bc | 0.33 bc | 1.38 cd | 0.78 | 0.85 d | 1.63 cd | 0.62 | 0.28 bc | 0.85 e | 1.75 de |
| *Significance[1]* | * | * | * | ** | ** | ** | ** | ** | *ns* | ** | ** | *ns* | ** | ** | ** |
| *LSD* | *0.28* | *0.28* | *0.57* | *0.19* | *0.72* | *0.59* | *0.34* | *0.78* | *0.42* | *0.53* | *0.86* | *2.62* | *1.11* | *5.41* | *8.70* |
| *CV (%)[2]* | *32.1* | *32.1* | *32.3* | *36.2* | *31.5* | *31.8* | *34.2* | *29.5* | *41.6* | *23.0* | *25.9* | *28.0* | *24.5* | *22.9* | *23.2* |

[1] ***, *, ns are, respectively, significant for $p \leq .01$, significant for $p \leq .05$ and not significant (LSD test)*

[2] *Coefficient of variation*

Table E - P concentration (mg $g^{-1}$) in aboveground dry matter of wheat under sole- (N0, N40, N80, N120, N160) and inter-cropping (PB-DW PIC, FP-DW PIC, HV-DW PIC, PB-DW TIC, FP-DW TIC, HV-DW TIC) in 2010/11

| Treatment | BBCH 30† | BBCH 59‡ | | BBCH 69‡ | | BBCH 75‡ | | BBCH 89‡ | | |
|---|---|---|---|---|---|---|---|---|---|---|
| | Straw | Straw | Ears | Straw | Ears | Straw | Ears | Straw | Ears | Grain |
| N0 | 2.87 | 2.40 abc | 2.37 | 2.57 abc | 2.47 | 1.57 b | 2.80 ab | 0.93 bc | 1.47 cd | 3.27 |
| N40 | 3.03 | 1.63 f | 2.30 | 1.90 cde | 2.13 | 1.10 bc | 2.37 d | 0.30 d | 0.80 e | 3.07 |
| N80 | 3.07 | 1.73 ef | 2.43 | 2.10 cde | 2.37 | 1.07 bc | 2.57 cd | 0.60 cd | 1.07 de | 3.17 |
| N120 | 3.00 | 1.80 def | 2.40 | 1.77 de | 2.30 | 0.90 c | 2.53 cd | 0.33 d | 0.93 de | 3.17 |
| N160 | 2.87 | 1.77 ef | 2.33 | 1.67 e | 2.30 | 0.80 c | 2.47 cd | 0.23 d | 0.63 e | 2.97 |
| PB-DW TIC | 3.37 | 2.13 bcde | 2.50 | 2.93 a | 2.50 | 2.47 a | 2.90 a | 2.23 a | 2.37 ab | 3.80 |
| FP-DW TIC | 2.63 | 2.43 abc | 2.53 | 2.80 ab | 2.60 | 2.23 a | 2.97 a | 2.30 a | 2.40 ab | 3.50 |
| HV-DW TIC | 2.70 | 2.27 abcd | 2.40 | 3.00 a | 2.37 | 2.40 a | 2.80 ab | 1.93 a | 2.27 ab | 3.33 |
| PB-DW PIC | 3.27 | 2.73 a | 2.33 | 2.40 abcd | 2.33 | 1.53 b | 2.80 ab | 1.20 b | 2.47 a | 3.43 |
| FP-DW PIC | 2.50 | 2.00 cdef | 2.27 | 2.13 bcde | 2.47 | 1.27 bc | 2.67 bc | 1.27 b | 1.73 bc | 3.40 |
| HV-DW PIC | 2.70 | 2.53 ab | 2.37 | 2.23 bcde | 2.50 | 1.53 b | 2.90 a | 1.27 b | 1.93 abc | 3.30 |
| *Significance[1]* | *ns* | ** | *ns* | ** | *ns* | ** | ** | ** | ** | *ns* |
| *LSD* | *0.62* | *0.50* | *0.25* | *0.67* | *0.47* | *0.30* | *0.22* | *0.46* | *0.64* | *0.45* |
| *CV (%)[2]* | *12.6* | *13.7* | *6.2* | *16.9* | *11.5* | *31.9* | *4.9* | *23.7* | *22.7* | *8.0* |

[1] **, *, ns are, respectively, significant for p≤.01, significant for p≤.05 and not significant (LSD test)

[2] Coefficient of variation

*Table F - $P_2O_5$ accumulation (g $m^{-2}$) in aboveground dry matter of wheat under sole- (N0, N40, N80, N120, N160) and inter-cropping (PB-DW PIC, FP-DW PIC, HV-DW PIC, PB-DW TIC, FP-DW TIC, HV-DW TIC) in 2010/11*

| **Treatment** | **BBCH 30** | | **BBCH 59** | | | **BBCH 69** | | | **BBCH 75** | | | **BBCH 89** | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | **Straw** | **Total** | **Straw** | **Ears** | **Total** | **Straw** | **Ears** | **Total** | **Straw** | **Ears** | **Total** | **Straw** | **Ears** | **Grain** | **Total** |
| N0 | 0.31 | 0.31 | 0.84 | 0.27 cd | 1.11 | 1.00 | 0.36 cde | 1.36 | 0.51 bcd | 1.13 def | 1.64 bc | 0.27 bc | 0.24 b | 1.33 de | 1.84 d |
| N40 | 0.41 | 0.41 | 0.76 | 0.34 abc | 1.10 | 1.20 | 0.49 abcd | 1.69 | 0.52 bcd | 1.55 abc | 2.07 ab | 0.12 c | 0.18 b | 1.80 bc | 2.10 bcd |
| N80 | 0.40 | 0.40 | 0.91 | 0.43 a | 1.34 | 1.27 | 0.54 abc | 1.81 | 0.55 cd | 1.72 ab | 2.27 ab | 0.26 bc | 0.25 b | 1.92 abc | 2.43 ab |
| N120 | 0.38 | 0.38 | 1.03 | 0.46 a | 1.49 | 1.14 | 0.58 a | 1.72 | 0.48 cd | 1.71 ab | 2.19 ab | 0.14 c | 0.21 b | 2.06 ab | 2.41 abc |
| N160 | 0.42 | 0.42 | 0.88 | 0.42 ab | 1.30 | 0.95 | 0.55 ab | 1.5 | 0.43 d | 1.96 a | 2.39 a | 0.11 c | 0.17 b | 2.27 a | 2.55 ab |
| PB-DW TIC | 0.29 | 0.29 | 0.70 | 0.25 cd | 0.95 | 1.03 | 0.31 de | 1.34 | 0.74 abc | 1.38 bcd | 2.12 ab | 0.60 a | 0.40 a | 1.63 cd | 2.63 ab |
| FP-DW TIC | 0.35 | 0.35 | 0.85 | 0.29 bcd | 1.14 | 1.18 | 0.40 bcde | 1.58 | 0.80 ab | 1.54 abcd | 2.34 a | 0.73 a | 0.41 a | 1.64 cd | 2.78 a |
| HV-DW TIC | 0.26 | 0.26 | 0.74 | 0.25 cd | 0.99 | 1.14 | 0.38 bcde | 1.52 | 0.86 a | 1.25 cde | 2.11 ab | 0.63 a | 0.44 a | 1.71 bc | 2.78 a |
| PB-DW PIC | 0.29 | 0.29 | 0.68 | 0.17 d | 0.85 | 0.80 | 0.27 e | 1.07 | 0.37 d | 0.80 f | 1.17 c | 0.24 bc | 0.24 b | 0.83 f | 1.31 e |
| FP-DW PIC | 0.28 | 0.28 | 0.54 | 0.19 d | 0.73 | 0.74 | 0.29 e | 1.03 | 0.34 d | 0.91 ef | 1.25 c | 0.35 b | 0.25 b | 1.28 e | 1.88 cd |
| HV-DW PIC | 0.30 | 0.30 | 0.64 | 0.20 d | 0.84 | 0.78 | 0.28 e | 1.06 | 0.47 bcd | 0.85 ef | 1.32 c | 0.32 b | 0.26 b | 1.07 ef | 1.65 de |
| Significance[1] | *ns* | *ns* | *ns* | **** | *ns* | *ns* | **** | *ns* | *** | *** | **** | **** | **** | **** | **** |
| *LSD* | *0.15* | *0.15* | *0.40* | *0.13* | *0.50* | *0.60* | *0.19* | *0.75* | *0.30* | *0.44* | *0.68* | *0.18* | *0.11* | *0.37* | *0.53* |
| *CV (%)*[2] | *26.6* | *26.6* | *29.6* | *24.7* | *27.2* | *34.0* | *27.4* | *30.6* | *31.9* | *19.1* | *21.0* | *30.0* | *22.8* | *13.6* | *14.1* |

[1] ***, *, ns are, respectively, significant for p≤.01, significant for p≤.05 and not significant (LSD test)*

[2] *Coefficient of variation*

*Table G - P concentration (mg g$^{-1}$) in aboveground dry matter of legumes under sole- (PB, FP, HV) and inter-cropping (PB-DW PIC, FP-DW PIC, HV-DW PIC, PB-DW TIC, FP-DW TIC, HV-DW TIC) in 2009/10*

| **Treatment** | **BBCH 30†** | **BBCH 59‡** | | **BBCH 69‡** | | **BBCH 75‡** | | **BBCH 89‡** | | |
|---|---|---|---|---|---|---|---|---|---|---|
| | **Straw** | **Straw** | **Pods** | **Straw** | **Pods** | **Straw** | **Pods** | **Straw** | **Pods** | **Grain** |
| PB | 1.77 | 1.83 | 5.00 | 1.70 | 3.93 | 0.63 | 3.83 | 0.67 | 0.57 a | 5.63 |
| PB-DW PIC | 1.70 | 1.97 | 4.80 | 1.53 | 4.13 | 0.90 | 3.27 | 0.70 | 0.47 b | 4.97 |
| PB-DW-TIC | 1.57 | - | - | - | - | - | - | - | - | - |
| *$P_{.05}$* | *0.80* | *0.72* | *0.72* | *0.65* | *0.84* | *0.43* | *0.22* | *0.81* | *0.00* | *0.31* |
| FP | 1.90 | 1.27 | 2.43 | 1.27 | 2.30 | 0.80 b | 1.90 | 0.73 | 0.87 | 3.07 |
| FP-DW PIC | 1.70 | 1.03 | 2.37 | 0.93 | 2.33 | 1.03 a | 2.53 | 0.90 | 1.10 | 3.43 |
| FP-DW-TIC | 1.77 | - | - | - | - | - | - | - | - | - |
| *$P_{.05}$* | *0.46* | *0.32* | *0.80* | *0.20* | *0.53* | *0.02* | *0.06* | *0.50* | *0.46* | *0.56* |
| HV | 2.00 | 2.57 | - | 2.37 | - | 1.67 | 4.30 | 1.97 | 1.33 | 5.33 |
| HV-DW PIC | 2.50 | 2.67 | - | 2.33 | - | 1.67 | 3.83 | 1.40 | 1.73 | 4.80 |
| HV-DW-TIC | 2.37 | - | - | - | - | - | - | - | - | - |
| *$P_{.05}$* | *0.24* | *0.73* | *-* | *0.87* | *-* | *1.43* | *0.18* | *0.14* | *0.38* | *0.13* |

*† Treatments analyzed under one-way ANOVA (Fisher's protected LSD test)*

*‡ Paired comparison t-test performed with treatments concerning the same legume species (i.e. PB vs PB-DW PIC; FP vs FP-DW PIC, HV vs HV-DW PIC)*

*Table H - $P_2O_5$ accumulation (g $m^{-2}$) in aboveground dry matter of legumes under sole- (PB, FP, HV) and inter-cropping (PB-DW PIC, FP-DW PIC, HV-DW PIC, PB-DW TIC, FP-DW TIC, HV-DW TIC) in 2009/10*

| **Treatment** | **BBCH 30** | | **BBCH 59** | | | **BBCH 69** | | | **BBCH 75** | | | **BBCH 89** | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | **Straw** | **Total** | **Straw** | **Pods** | **Total** | **Straw** | **Pods** | **Total** | **Straw** | **Pods** | **Total** | **Straw** | **Pods** | **Grain** | **Total** |
| PB | 0.92 | 0.92 | 1.91 | 0.23 | 2.14 a | 1.93 | 1.30 a | 3.23 | 0.78 | 4.90 a | 5.68 | 0.85 | 0.23 | 5.69 | 6.77 a |
| PB-DW PIC | 0.53 | 0.53 | 1.42 | 0.22 | 1.64 b | 1.35 | 0.65 b | 2.00 | 0.85 | 2.77 b | 3.62 | 0.68 | 0.14 | 4.63 | 5.45 b |
| PB-DW-TIC | 0.49 | 0.49 | - | - | - | - | - | - | - | - | - | - | - | - | - |
| *$P_{.05}$* | *0.18* | *0.18* | *0.13* | *0.78* | *0.03* | *0.32* | *0.01* | *0.12* | *0.96* | *0.06* | *0.10* | *0.36* | *0.05* | *0.06* | *0.02* |
| FP | 0.30 | 0.30 | 0.31 | 0.57 | 0.88 | 0.36 | 1.17 | 1.53 | 0.30 | 1.27 | 1.57 | 0.30 | 0.39 | 1.02 | 1.71 |
| FP-DW PIC | 0.08 | 0.08 | 0.08 | 0.26 | 0.34 | 0.08 | 0.43 | 0.51 | 0.12 | 0.55 | 0.67 | 0.13 | 0.05 | 0.82 | 1.00 |
| FP-DW-TIC | 0.16 | 0.16 | - | - | - | - | - | - | - | - | - | - | - | - | - |
| *$P_{.05}$* | *0.18* | *0.18* | *0.19* | *0.30* | *0.24* | *0.21* | *0.29* | *0.27* | *0.26* | *0.30* | *0.29* | *0.35* | *0.27* | *0.47* | *0.36* |
| HV | 0.72 | 0.72 | 1.52 | - | 1.52 | 1.58 | - | 1.58 | 1.18 | 0.44 a | 1.62 | 1.48 | 0.07 | 0.29 | 1.84 |
| HV-DW PIC | 0.50 | 0.50 | 0.75 | - | 0.75 | 0.80 | - | 0.80 | 0.66 | 0.14 b | 0.80 | 0.69 | 0.05 | 0.16 | 0.90 |
| HV-DW-TIC | 0.43 | 0.43 | - | - | - | - | - | - | - | - | - | - | - | - | - |
| *$P_{.05}$* | *0.30* | *0.30* | *0.20* | *-* | *0.20* | *0.09* | *-* | *0.09* | *0.28* | *0.10* | *0.21* | *0.15* | *0.76* | *0.25* | *0.18* |

*† Treatments analyzed under one-way ANOVA (Fisher's protected LSD test)*

*‡ Paired comparison t-test performed with treatments concerning the same legume species (i.e. PB vs PB-DW PIC; FP vs FP-DW PIC, HV vs HV-DW PIC)*

*Table I - P concentration (g kg$^{-1}$) in aboveground dry matter of legumes under sole- (PB, FP, HV) and inter-cropping (PB-DW PIC, FP-DW PIC, HV-DW PIC, PB-DW TIC, FP-DW TIC, HV-DW TIC) in 2010/11*

| **Treatment** | **BBCH 30** | **BBCH 59** | | **BBCH 69** | | **BBCH 75** | | **BBCH 89** | | |
|---|---|---|---|---|---|---|---|---|---|---|
| | **Straw** | **Straw** | **Pods** | **Straw** | **Pods** | **Straw** | **Pods** | **Straw** | **Pods** | **Grain** |
| PB | 2.17 | 1.53 | 3.53 | 1.47 | 3.50 | 1.30 | 3.67 | 0.80 | 0.63 | 4.93 b |
| PB-DW PIC | 1.87 | 1.33 | 3.47 | 1.40 | 3.57 | 1.20 | 3.30 | 0.73 | 1.07 | 5.27 a |
| PB-DW-TIC | 1.93 | - | - | - | - | - | - | - | - | - |
| *$P_{.05}$* | *0.76* | *0.18* | *0.74* | *0.82* | *0.81* | *0.42* | *0.19* | *.73* | *.27* | *.01* |
| FP | 1.73 | 0.70 | 2.70 | 0.73 | 2.67 | 0.47 | 2.43 | 0.60 | 1.00 | 3.50 b |
| FP-DW PIC | 1.90 | 0.73 | 2.43 | 0.60 | 2.70 | 0.50 | 2.23 | 0.73 | 0.70 | 4.40 a |
| FP-DW-TIC | 1.90 | - | - | - | - | - | - | - | - | - |
| *$P_{.05}$* | *0.26* | *0.83* | *0.27* | *0.42* | *0.94* | *0.87* | *0.44* | *.46* | *.32* | *.02* |
| HV | 2.43 | 1.67 | 4.00 | 1.43 | 2.63 b | 0.70 | 2.97 | 0.77 | 0.40 | 3.93 |
| HV-DW PIC | 2.37 | 1.53 | 3.80 | 1.23 | 2.90 a | 0.67 | 2.60 | 0.73 | 0.33 | 3.80 |
| HV-DW-TIC | 2.33 | - | - | - | - | - | - | - | - | - |
| *$P_{.05}$* | *0.79* | *0.18* | *0.63* | *0.07* | *0.02* | *0.74* | *0.09* | *.81* | *.67* | *.75* |

*† Treatments analyzed under one-way ANOVA (Fisher's protected LSD test)*

*‡ Paired comparison t-test performed with treatments concerning the same legume species (i.e. PB vs PB-DW PIC; FP vs FP-DW PIC, HV vs HV-DW PIC)*

*Table J - $P_2O_5$ accumulation (g $m^{-2}$) in aboveground dry matter of legumes under sole- (PB, FP, HV) and inter-cropping (PB-DW PIC, FP-DW PIC, HV-DW PIC, PB-DW TIC, FP-DW TIC, HV-DW TIC) in 2010/11*

| **Treatment** | **BBCH 30** | | **BBCH 59** | | | **BBCH 69** | | | **BBCH 75** | | | **BBCH 89** | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | **Straw** | **Total** | **Straw** | **Pods** | **Total** | **Straw** | **Pods** | **Total** | **Straw** | **Pods** | **Total** | **Straw** | **Pods** | **Grain** | **Total** |
| PB | 0.94 | 0.94 | 0.98 | 0.42 | 1.40 | 0.70 | 1.03 | 1.73 | 0.60 | 1.19 | 1.79 | 0.34 | 0.08 | 1.63 | 2.05 |
| PB-DW PIC | 0.77 | 0.77 | 0.83 | 0.47 | 1.30 | 0.79 | 0.77 | 1.56 | 0.60 | 1.01 | 1.61 | 0.34 | 0.14 | 1.20 | 1.68 |
| PB-DW-TIC | 0.81 | 0.81 | - | - | - | - | - | - | - | - | - | - | - | - | - |
| *$P_{.05}$* | *0.27* | *0.27* | *0.05* | *0.87* | *0.66* | *0.70* | *0.27* | *0.61* | *0.83* | *0.43* | *0.40* | *0.84* | *0.38* | *0.54* | *0.62* |
| FP | 0.37 | 0.37 | 0.18 | 0.34 | 0.52 | 0.17 | 0.47 | 0.64 | 0.09 | 0.60 | 0.69 | 0.10 | 0.16 | 0.50 | 0.76 |
| FP-DW PIC | 0.33 | 0.33 | 0.15 | 0.33 | 0.48 | 0.12 | 0.53 | 0.65 | 0.08 | 0.61 | 0.69 | 0.10 | 0.14 | 0.46 | 0.70 |
| FP-DW-TIC | 0.30 | 0.30 | - | - | - | - | - | - | - | - | - | - | - | - | - |
| *$P_{.05}$* | *0.80* | *0.80* | *0.82* | *0.97* | *0.92* | *0.40* | *0.83* | *0.99* | *0.49* | *0.97* | *0.92* | *0.74* | *0.07* | *0.90* | *0.80* |
| HV | 0.34 | 0.34 | 0.62 | 0.43 | 1.05 | 0.40 | 0.69 | 1.09 | 0.18 | 0.95 | 1.13 | 0.16 | 0.05 | 0.95 | 1.16 |
| HV-DW PIC | 0.29 | 0.29 | 0.44 | 0.21 | 0.65 | 0.25 | 0.52 | 0.77 | 0.11 | 0.68 | 0.79 | 0.11 | 0.03 | 0.73 | 0.87 |
| HV-DW-TIC | 0.28 | 0.28 | - | - | - | - | - | - | - | - | - | - | - | - | - |
| *$P_{.05}$* | *0.80* | *0.80* | *0.11* | *0.28* | *0.20* | *0.06* | *0.57* | *0.38* | *0.30* | *0.49* | *0.46* | *0.34* | *0.11* | *0.66* | *0.59* |

*† Treatments analyzed under one-way ANOVA (Fisher's protected LSD test)*

*‡ Paired comparison t-test performed with treatments concerning the same legume species (i.e. PB vs PB-DW PIC; FP vs FP-DW PIC, HV vs HV-DW PIC)*

*Table K - P concentration -Pconc- (g kg$^{-1}$) and $P_2O_5$ accumulation -$P_2O_5$acc- (g m$^{-2}$) in weed aboveground dry matter in 2009/10. Values are means ± SD (n=3). Within each column, data followed by different letters are significantly different*

| **Treatment** | **BBCH 30** | | **BBCH 59** | | **BBCH 69** | | **BBCH 75** | | **BBCH 89** | |
|---|---|---|---|---|---|---|---|---|---|---|
| | **Pconc** | **$P_2O_5$acc** | **Pconc** | **$P_2O_5$acc** | **Pconc** | **$P_2O_5$acc** | **Pconc** | **$P_2O_5$acc** | **Pconc** | **$P_2O_5$acc** |
| N0 | 1.87 | 0.06 cde | 2.67 | 0.30 bcd | 2.47 cd | 0.32 bcd | 4.27 | 1.13 a | 3.30 a | 1.71 a |
| N40 | 1.07 | 0.04 cde | 3.33 | 0.24 cd | 2.47 cd | 0.35 bcd | 2.53 | 0.79 ab | 2.80 abcde | 1.09 b |
| N80 | 1.67 | 0.10 bc | 2.70 | 0.46 abc | 2.27 d | 0.64 abc | 2.07 | 0.97 ab | 2.53 cdef | 1.26 a |
| N120 | 1.67 | 0.10 bc | 2.73 | 0.58 ab | 2.27 d | 0.89 a | 1.93 | 0.94 ab | 2.43 def | 1.32 a |
| N160 | 1.77 | 0.17 a | 2.87 | 0.65 a | 2.30 d | 0.70 ab | 2.17 | 1.21 a | 2.10 fg | 1.24 a |
| PB-DW TIC | 3.90 | 0.03 e | 3.70 | 0.01 d | 2.57 bcd | 0.14 cd | 2.90 | 0.36 c | 3.07 fg | 0.56 b |
| FP-DW TIC | 2.13 | 0.04 cde | 2.05 | 0.05 d | 3.00 ab | 0.07 d | 3.40 | 0.30 c | 2.87 abcd | 0.63 b |
| HV-DW TIC | 1.83 | 0.02 e | 4.05 | 0.04 d | 2.33 d | 0.14 cd | 2.80 | 0.49 bc | 3.10 abc | 0.98 b |
| PB-DW PIC | 2.50 | 0.07 cde | 3.17 | 0.10 d | 2.93 abc | 0.10 d | 3.07 | 0.12 c | 2.53 cdef | 0.12 c |
| FP-DW PIC | 2.27 | 0.05 cde | 2.85 | 0.10 d | 2.40 d | 0.17 cd | 3.17 | 0.33 c | 2.63 bcdef | 0.35 c |
| HV-DW PIC | 1.27 | 0.01 de | 2.70 | 0.04 d | 3.30 a | 0.07 d | 3.03 | 0.12 c | 2.10 fg | 0.35 c |
| PB | 2.00 | 0.04 cde | 3.05 | 0.07 d | 2.50 bcd | 0.07 cd | 3.33 | 0.11 c | 3.10 abc | 0.14 c |
| FP | 1.87 | 0.19 a | 3.37 | 0.59 ab | 2.53 bcd | 0.75 ab | 2.07 | 1.19 a | 2.30 ef | 1.35 a |
| HV | 1.93 | 0.16 ab | 2.90 | 0.33 bcd | 3.20 a | 0.38 bcd | 2.50 | 0.54 bc | 1.73 g | 0.53 c |
| *Significance[1]* | *ns* | **** | *ns* | **** | **** | *** | *ns* | **** | **** | **** |
| *LSD* | *2.41* | *0.67* | *1.63* | *0.51* | *0.60* | *0.64* | *1.54* | *0.59* | *0.54* | *0.55* |
| *CV (%)[2]* | *72.6* | *52.8* | *18.4* | *56.7* | *11.1* | *86.8* | *32.8* | *62.8* | *12.2* | *39.9* |

*[1] **, *, ns are, respectively, significant for $p \leq .01$, significant for $p \leq .05$ and not significant (LSD test)*

*[2] Coefficient of variation*

*Table L - P concentration -Pconc- (g kg$^{-1}$) and $P_2O_5$ accumulation -$P_2O_5$acc- (g m$^{-2}$) in weed aboveground dry matter in 2010/11. Values are means ± SD (n=3). Within each column, data followed by different letters are significantly different*

| **Treatment** | **BBCH 30** | | **BBCH 59** | | **BBCH 69** | | **BBCH 75** | | **BBCH 89** | |
|---|---|---|---|---|---|---|---|---|---|---|
| | **Pconc** | **$P_2O_5$acc** | **Pconc** | **$P_2O_5$acc** | **Pconc** | **$P_2O_5$acc** | **Pconc** | **$P_2O_5$acc** | **Pconc** | **$P_2O_5$acc** |
| N0 | 2.97 | 0.05 | 2.77 a | 0.24 | 3.10 a | 0.31 | 2.07 | 0.56 b | 1.97 ab | 0.56 bcd |
| N40 | 2.13 | 0.07 | 1.97 abc | 0.24 | 2.10 cd | 0.39 | 1.60 | 0.40 b | 1.47 cde | 0.53 bcd |
| N80 | 2.47 | 0.13 | 1.90 bc | 0.36 | 2.37 bcd | 0.55 | 2.03 | 0.88 ab | 2.17 ab | 1.37 a |
| N120 | 2.33 | 0.09 | 1.93 abc | 0.30 | 2.03 d | 0.57 | 1.67 | 0.69 ab | 1.43 de | 1.02 a |
| N160 | 2.40 | 0.10 | 1.87 c | 0.48 | 1.90 d | 0.53 | 1.83 | 0.60 ab | 1.33 e | 0.91 abc |
| PB-DW TIC | 2.70 | 0.04 | 2.80 a | 0.01 | - | - | - | - | 2.30 a | 0.04 e |
| FP-DW TIC | 2.47 | 0.03 | - | - | - | - | - | - | 1.85 abcd | 0.06 e |
| HV-DW TIC | 2.20 | 0.04 | - | - | - | - | - | - | 1.87 abc | 0.09 de |
| PB-DW PIC | 2.67 | 0.07 | 2.50 ab | 0.10 | 2.27 cd | 0.17 | 1.97 | 0.30 b | 1.93 ab | 0.32 de |
| FP-DW PIC | 2.27 | 0.03 | 2.30 abc | 0.16 | 2.67 abc | 0.25 | 1.87 | 0.32 b | 2.07 ab | 0.41 de |
| HV-DW PIC | 2.47 | 0.05 | 2.40 abc | 0.12 | 2.37 bcd | 0.26 | 1.77 | 0.27 b | 1.83 bcd | 0.30 de |
| PB | 2.57 | 0.11 | 2.77 a | 0.40 | 2.93 ab | 0.49 | 1.90 | 0.57 ab | 2.30 a | 1.06 a |
| FP | 2.47 | 0.10 | 2.13 abc | 0.46 | 2.67 abc | 0.71 | 2.07 | 0.97 ab | 2.17 ab | 1.08 a |
| HV | 2.43 | 0.10 | 2.50 ab | 0.40 | 2.93 ab | 0.69 | 2.13 | 1.04 a | 2.13 ab | 1.07 a |
| *Significance*[1] | *ns* | *ns* | * | *ns* | ** | *ns* | *ns* | ** | ** | ** |
| *LSD* | *0.83* | *0.10* | *1.07* | *0.52* | *0.61* | *0.45* | *0.47* | *0.47* | *0.51* | *0.47* |
| *CV (%)*[2] | *20.1* | *84.3* | *15.8* | *62.7* | *14.3* | *58.6* | *14.4* | *47.0* | *12.8* | *35.0* |

[1] ***, *, ns are, respectively, significant for p≤.01, significant for p≤.05 and not significant (LSD test)*

[2] *Coefficient of variation*

# Appendix 2 - Report

## Report: Substitution of mineral fertilizer N use with leguminous $N_2$-fixation in a climate change perspective

Daniele Antichi[1]
Henrik Hauggaard-Nielsen[2]

[1] Visiting PhD Student, Università di Pisa-Scuola Sant'Anna, Pisa (Italy)
[2] Senior Scientist, Risø National Lab for Sustainable Energy, Risø (Denmark)

**Summary**

*Together with intensive livestock management, nitrogen fertilizers are the main reason for agriculture's contribution to global warming, due to their strong reliance on fossil energy sources and even more because of the high emissions of $N_2O$, a greenhouse gas 300 times more potent than $CO_2$ which can arise from denitrification of nitrogen in the soil. A suitable option to reduce the negative impact of agriculture on the climate can be the substitution of mineral N fertilizers by cultivation of legume crops that fix significant amounts of atmospheric $N_2$ due to their symbiosis with Rhizobium spp. bacteria in the roots. Currently areas cultivated with legumes represent only 15% of globally cultivated areas, whilst in the past they were consistent components of crop rotations, being important both as fertility-building crops aimed at maintaining soil fertility and as feedstuff for animal production. The decline of legume cultivation in the last decades was due to increased use of mineral N fertilizers, which allowed farmers to allocate more land to cereal crops and shift livestock to intensive dairy farming. Attributing more importance to legumes in crop rotations and integrating old knowledge with modern technologies is recognized as extremely promising in terms of climate change mitigation both by IPCC and FAO, provided negative externalities of legumes (e.g. $N_2O$ emission during biological $N_2$ fixation and fossil fuels consumption for field operations) are kept under control. This can be realized by properly measuring the amount of $N_2$ fixed by Rhizobia and by reducing both N losses from legume cultivation and the intensiveness of legume management. The aim of this report is to collect updated knowledge about symbiotic $N_2$ fixation efficiency and measurement, and to define some advanced technical solutions to include more legumes in cropping systems and to enhance their potential to mitigate climate change.*

### 1. Introduction

In the last century, the so called "green revolution" enabled farmers around the world to replace fertility-building crops, such as legumes, with newly available nitrogen fertilizers, thus intensifying crop management (Crews and Peoples, 2004). The systematic application of mineral fertilizers and other chemical products, such as pesticides, produced a dramatic increase of crop and food production, so that today 48% of global population is estimated to be dependent on food produced by using N fertilizers (Erisman et al., 2008). However, a lot of negative impacts have been consistently related to these practices, e.g. numerous environmental hazards and high fossil energy consumption (Peoples et al., 1995; Pimentel, 1996; Smil, 2001; Hanson et al., 2007; Erisman et al., 2008). Recently, mineral fertilizers have been identified as one of the main sources for greenhouse gasses (GHG) emissions and consequently for the contribution of agriculture to global warming (IPCC, 2007a). Moreover, as the supply of not renewable energy (i.e. mineral oil) will become scarcer, the reliance of agriculture on N fertilizers could be unsuitable from an economical point of view due to the increasing price of oil.

Thus, designing sustainable cropping systems should also include a significant reduction in the use of mineral fertilizers, possibly by including legumes in crop rotations, which are extremely promising in terms of agronomical, economical and environmental efficiency and thereby provide a strong tool for mitigation of climate change in agriculture (Crews and Peoples, 2004).

## 1.1 Climate change perspective

Since 1990, when IPCC (Intergovernmental Panel on Climate Change of UN) published its First Assessment Report, climate change (CC) has become an important issue for international agreements and national governments regarding development policies. Climate change can produce heavy consequences not only on mechanisms involved in the evolution of environment, but also on lot of different aspects of human society, ranging from specific economic activities, constrained by climate conditions, to general mankind lifestyle, richness and demographic trend.

In its widest definition provided by IPCC (2007a), climate change has been defined as "*a change in the state of the climate that can be identified (e.g. using statistical tests) by changes in the mean and/or the variability of its properties, and that persists for an extended period, typically decades or longer. It refers to any change in climate over time, whether due to natural variability or as a result of human activity*".

Therefore, CC is a natural phenomenon that can be scientifically studied by measuring all its representative parameters over time. And data that IPCC scientists presented in the AR4 (Fourth Assessment Report) (IPCC, 2007a) showed a significant global warming during the last century, with a considerable acceleration from 1950s and onwards (Figure 1).

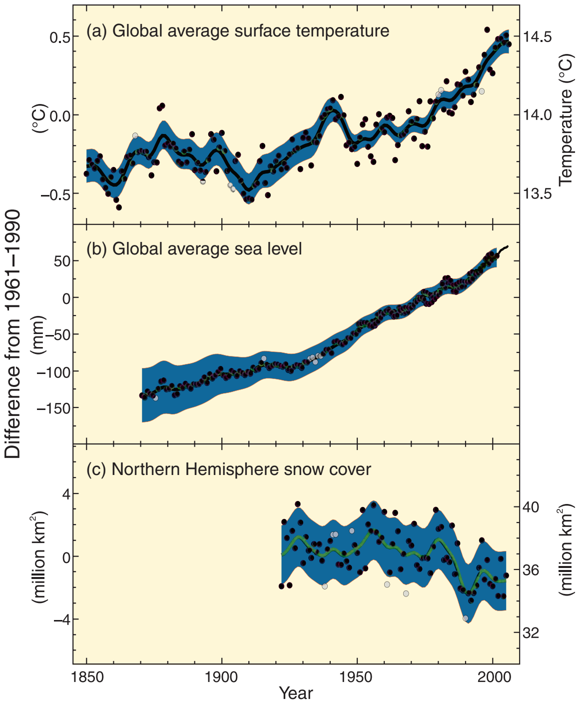


*Figure 1 - Observed changes in (a) global average surface temperature; (b) global average sea level from tide gauge (blue) and satellite (red) data; and (c) Northern Hemisphere snow cover for March-April (months in which snow cover starts to melt). All differences are relative to corresponding averages for the period 1961-1990 (IPCC, 2007)*

In Europe the warming is documented to be +0.90°C calculated from 1901 to 2005 being higher in central, north-eastern and mountainous regions, while lower trends are found in the Mediterranean area (IPCC, 2007b). Temperatures are increasing more in winter than in summer, and a significant increase of daily variability is also observed due to an increase in warm, rather than a decrease in cold, extremes. Precipitation trends are more fluctuating over time and more spatially variable, with mean winter values increasing in northern regions, whilst in the Mediterranean area yearly trends are negative in the east and non-significant in the west. An increase in intense rainfall frequency is observed in most parts of the European continent, even in some areas which are generally becoming drier.

Like for every foresight model, there are uncertainties due to the nature of prediction models and the amount of different factors included to estimate the dynamic climate processes in the future (Räisänen, 2007). Nonetheless, IPCC (2007a) hypothesized that the climate in the next 100 years will be characterized by even more rapidly increasing temperatures (with a mean rate of +0.2°C per decades), rising sea levels all over the world, by higher precipitations, more frequent extreme weather events at higher latitudes and drought at lower latitudes.

These changes will negatively affect living organisms with predictions of extinction of up to 30% of present animal and plant species. Obviously, human activities will be strongly affected as well. For instance, according to FAO agriculture in temperate northern european countries will experience productivity gains due to higher temperatures, longer vegetation periods and $CO_2$ enrichment, whilst Mediterranean, Tropical and Sub-tropical regions will face decreasing crop and animal productivity caused by temperature increases of 2-3°C and risk of drought, flooding, soil erosion, desertification and salinity (FAO, 2009).

The observed trend in CC, and especially the temperature increase, is due to an increase in greenhouse gasses (GHG) in the atmosphere. Human activities are regarded one of the main causes for this increase. For this reason, the six prediction scenarios for the future climate developed by IPCC (A1FI, A1T, A1B, A2, B1, B2) are based on different levels of GHG emissions, according to a function of human population and economical growth (IPCC, 2007a). Currently observed global GHG emissions match the most pessimistic scenarios (A1FI and A2). However, in the EU-27, GHG emissions are starting to decrease and in 2008 emissions were at the lowest level since 1990 (EEA, 2009). Nevertheless, in this report we assume that if we do not reduce our fossil energy use by general savings combined with alternative energy substitutions we can expect a significantly warmer climate, making the living conditions in several parts of the world more difficult.

### 1.2 Contribution of agriculture to climate change

Atmospheric carbon dioxide ($CO_2$) levels increase globally, primarily due to fossil fuel use. The agricultural sector plays a major role in this calculation accounting for about 13.5% and 9.2% of total global and European GHG emissions, respectively (Figure 2). This contribution can be even higher if also $CO_2$ emitted by soil respiration, mechanical field operations and manufacturing of agrochemicals is taken into account.

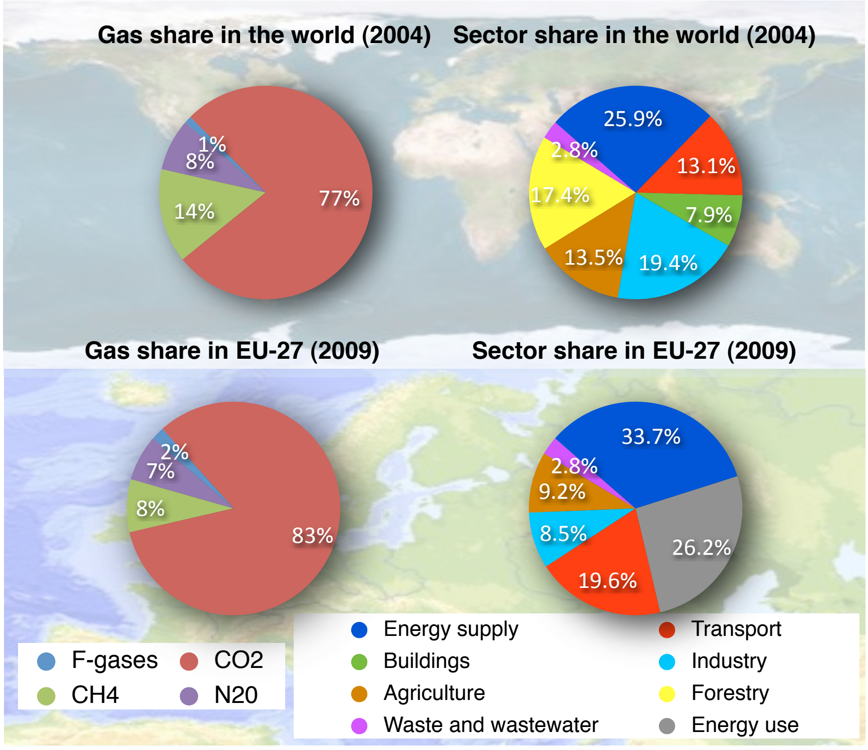


*Figure 2 - (a) Share of different anthropogenic GHGs in total emissions in the world (2004) and in Europe (2009) in terms of $CO_2$-eq. (b) Share of different sectors in total anthropogenic GHG emissions in the world(2004) and in Europe (2009). Modified from EPA, 2009 and IPCC, 2007a*

Obviously, the impact of agriculture on national GHG emissions varies according to its relative importance in local economy including land area used for agricultural production. In a country like Italy, where the main contributors to GHG emissions are energy production and use together with emissions from the transportation sector, the agricultural sector accounts only for 6.7%, whereas in Denmark it reaches 15% of the total national GHG emissions. Nevertheless, comparing the quantitative GHG contributions of the two countries Italian agriculture accounts for 36 Mt $CO_2$-eq. as compared to 9.5 Mt $CO_2$-eq. in Denmark possibly caused by differences in total area between the two countries (EEA, 2009).

The specific contribution of agricultural sector to global warming is caused mainly by emissions of methane ($CH_4$) from ruminants and nitrous oxide ($N_2O$) from N fertilization (Figure 2 and 3).

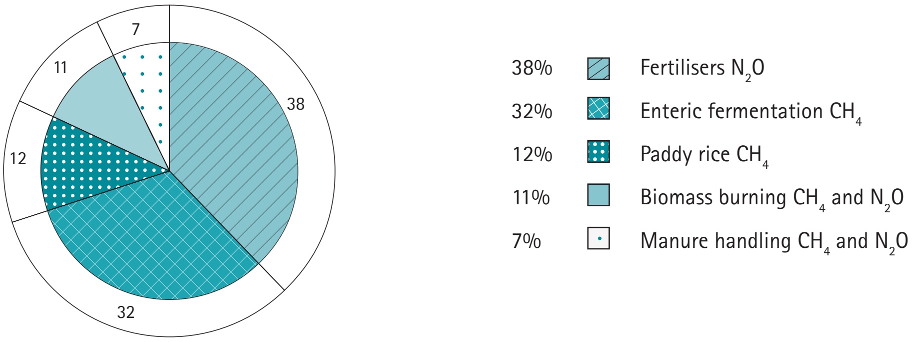


*Figure 3 - Share of different anthropogenic GHGs in total agriculture emissions in Europe (EEA, 2009)*

In this report most emphasis will be put on emissions of $N_2O$, as it can be reasonably considered the most important in terms of contribution to greenhouse effect and atmospheric ozone depletion. Despite its low concentration of 319±12 ppbv (IPCC, 2007a) and its radiative forcing potential substantially lower than $CO_2$, $N_2O$ has a 310 times higher global warming potential (GWP) than $CO_2$ due to its longer persistence in the atmosphere. In agricultural soils, $N_2O$ is an intermediate product of anaerobic denitrification, a bacteria-mediated process which transforms nitrates to gaseous $N_2$. Mineral N serving as substrate for denitrification originates from applied N fertilizer and/ or mineralization of N-rich organic matter in the soil. Ruminants can cause additional $N_2O$ emission from soils through excrements deposition during grazing and also as a consequence of manure application to the fields ((Saggar, 2010)).

### 1.3 Mitigation of climate change from agriculture

As agriculture is significantly contributing to global warming most stakeholders including FAO and IPCC (FAO, 2009; IPCC, 2007a) recognize it as a crucial factor with an unexploited potential to influence CC all over the world. This is especially true as agriculture is the most important human activity in terms of land use and to providing a wide range of different ecological services each with a relevant impact on GHG emission.

Within a novel development model based on environmental and energetic efficiency, agriculture is expected to change to achieve at least two goals: i) to face to the immediate risk of food insecurity, by providing new strategies and techniques for improving productivity in areas mostly impacted by CC (*adaptation*) and ii) to contrast effectively CC all over the world by reducing its own impact on climate (*mitigation*).

Whereas adaptation strategies (e.g. adjustment of planting time and crop species/ variety) have the potential to reduce the effects of CC on agriculture and thereby the short term global food supply (IPCC, 2007a), mitigation strategies aim at long-term CC mitigation by reduction of GHG emissions through a better management of natural resources and less reliance on non-renewable energy inputs.

IPCC (2007a) recommended four major actions for agriculture:
*a) crop rotations and farming systems design;*
*b) nutrient and manure management;*
*c) livestock management, pasture and fodder supply improvement;*
*d) fertile soil maintenance and restoration of degraded land.*

Niggli et al. (2009) explain in detail each of the four actions, examining the main practicable techniques, providing estimates of their mitigation potential and also an attempting balance between their costs and benefits.

Overall, these mitigating actions aim to reduce $CO_2$, $CH_4$ and $N_2O$ emission from soils or, even better, to create some carbon negative initiatives like for example systems integrating bioenergy crops production with a net C sequestration from the atmosphere, e.g. by returning significant amount of crop residues as stable biomass like biochar to the soil (Mathews, 2008). Conservation techniques, such as minimum or no tillage, can also contribute to reduce GWP of cropping systems. For instance, Lal (2004) estimated a potential mitigation of 23-72% of total GHG emission from agriculture simply by converting all cultivated areas to conservation techniques, in order to exploit the C sink capacity of the soil. However, this might be regarded as rather theoretical taking into account a rising world population and the need for appropriate food supply.

Thus, what is really needed now to combat CC effectively is to build a novel model of agriculture including all the most sustainable techniques adapted to each specific context, in order to reduce externalities, to enhance the efficiency of natural resource use and to slow down the reliance on external not-renewable inputs.

As pointed out by the IPCC and FAO recommendations listed above, a major role for this can be played by nutrient management. Under a CC perspective, fertilization, with a particular regard for nitrogen, should be considered as the crucial issue for agroecosystems management not only because it can be the major responsible for direct contribution of agriculture to environmental pollution and global warming (e.g. by GHG emission or fossil energy consumption), but especially because it influences the most important farmers' decisions including crop rotation design, species/variety choice and crop protection strategy (Crews and Peoples, 2004).

In this context, the most promising tool seems to be the replacement of mineral N fertilizers, reliant on fossil energy and responsible for most $N_2O$ emission from agriculture, with $N_2$-fixing legumes, which has the potential to strongly reduce the use of synthetically fixed N in cropping systems and to provide many other ecological services and mitigate CC (Crews and Peoples, 2004; Peoples et al., 2009a).

## 2. Symbiotic $N_2$-fixation and climate change

### 2.1 Synthetic and biological $N_2$-fixation: historical and current trend

N availability is one of the most limiting factors for both natural and agricultural ecosystems, being extremely important for the productivity of autotroph organisms, plants included. Man has been aware of the importance of N supply since ancient times, using the effect of natural $N_2$-fixing plants and manures on soil fertility. After the discovery of industrial $N_2$-fixation by Fritz Haber and Carl Bosch in the first decades of twentieth century, nitrogen became fully exploited for agricultural production, as farmers realized the benefits of mineral fertilizers rapidly.

Both Erisman et al. (2008) and Smil (2001) argued that the Haber-Bosch technology was the most important discovery in the twentieth century due to its large impact on crop and food production. Nevertheless, Erisman et al. (2008), Smil (2001) and also Hanson et al. (2007) pointed out equivalent negative impacts of N fertilizers dealing with the high fossil energy required for their industrial production, transport and application combined with a rather low crop fertilizer use efficiency of around 50% (Peoples et al., 1995), showing the potential of high atmospheric and aquatic emissions (Figure 4).

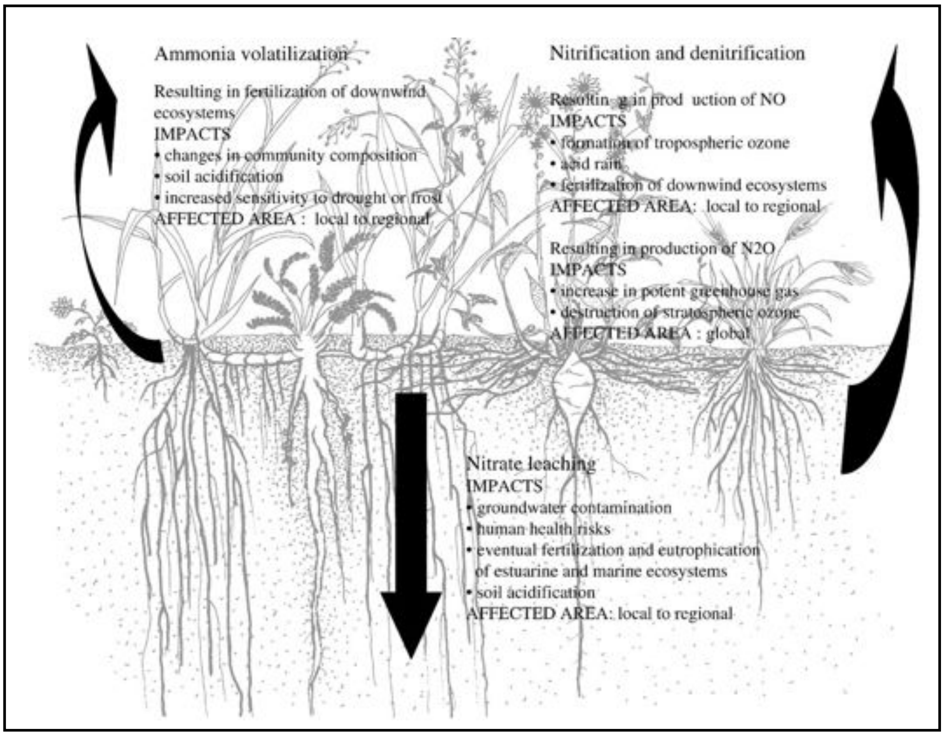


*Figure 4 - Major avenues of N loss from agroecosystems and associated environmental hazards (from Crews and Peoples, 1995)*

Historically, the only available alternative to N fertilizers and animal manures to supply cropping systems with N was biological atmospheric $N_2$-fixation from either the unique symbiotic nodulation of leguminous roots by *Rhizobium* spp. bacteria, or by non-symbiotic bacteria, like *Azotobacter* spp. and *Azospirillum* spp. However, the importance of symbiotic $N_2$-fixation in agroecosystems largely exceeds that of free living $N_2$-fixing organisms, as well pointed out by Peoples et al. (2009a), who estimated in the range of 33-46 million tons N year$^{-1}$ (if also below-ground N is considered) the global amount of nitrogen fixed by leguminous plants, a value that non-symbiotic bacteria do not even come close to.

Despite their important contribution to agroecosystems, in 2007, areas with legume-crop cultivation accounted only for approximately 15% of the total grown area worldwide (FAOSTAT, 2009). They are mostly concentrated in sparsely populated countries such as Australia and Canada, where still a large portion of total agricultural area is extensively managed (e.g. being dedicated to permanent pastures or perennial meadows, where legumes are components), and in areas where pulses still play a major role in the traditional diet such as Asia, Central and South America, and Africa (Crews and Peoples, 2004; FAOSTAT, 2009). Trends of total production are radically different comparing the yields of these areas with the highest yields in North, Central and South America and Europe. Africa is the only continent where yields are less than 1 t ha$^{-1}$ (Figure 5). The most important legume crop in the world is soybean, with a share of 48% of total legume-cultivated area. Field beans, groundnuts and field peas are equally represented (FAOSTAT, 2009).

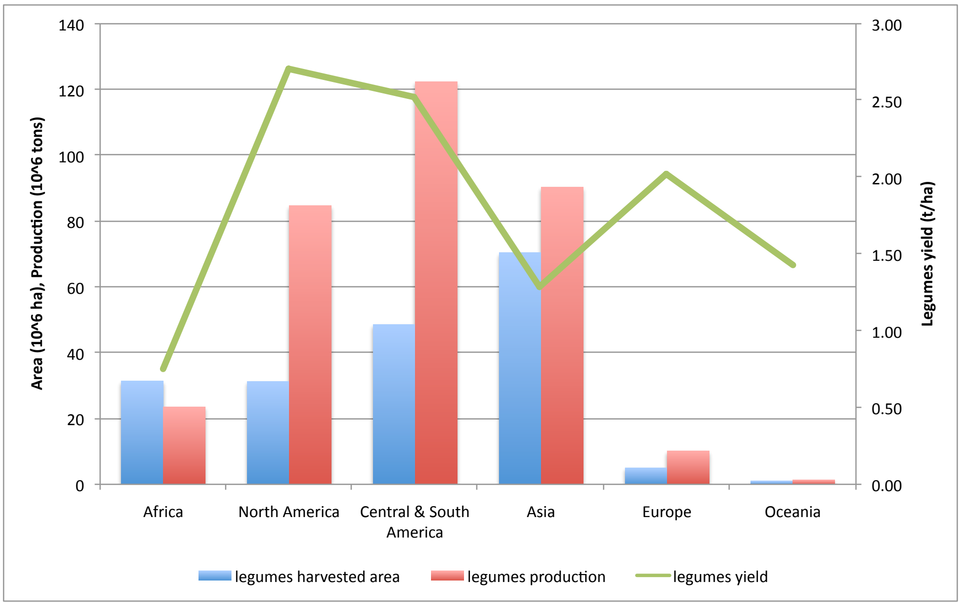


*Figure 5 - World grown area, total production and mean yield of legumes in 2007 (FAOSTAT, 2009)*

In Europe, legume fields represent only 3% of total harvested area and only 2% of total crop production (FAOSTAT, 2009), being mostly cultivated in Eastern and Southern European regions. For example, in Italy legumes are cultivated on about 260,000 ha (3.5% of total cultivated area), in contrast to Denmark, where only 8,000 ha or 0.5% of total cultivated area. The species of legume grown in the two countries are likewise different with 82% field pea and the rest as pulses in Denmark as compared to at least 10 different kinds of legumes recorded in Italy (Figure 7). The different importance of legume among countries is mainly driven by factors like climate (legumes are sensitive to frost and drought, for instance) and intensity of cropping systems, with more specialized dairy or industrial crop farms operating in Northern and Central European Countries.

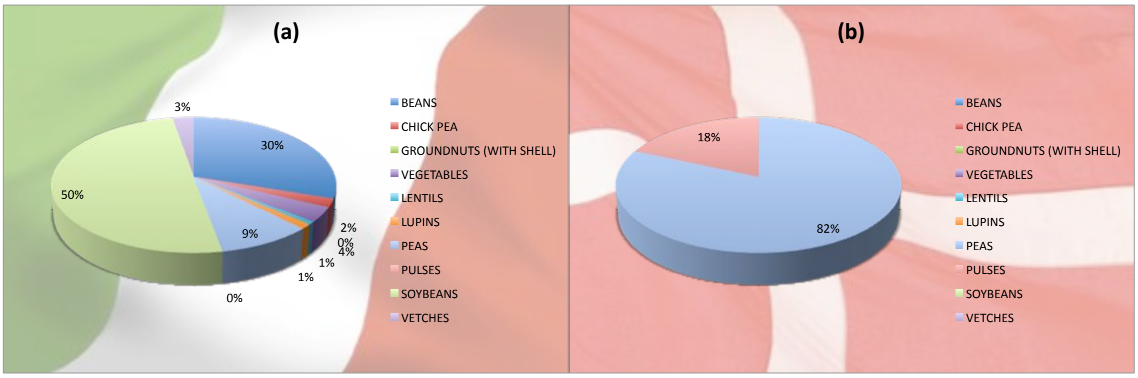


*Figure 6 - Share of total area grown with legumes in Italy (a) and Denmark (b) in 2007 (FAOSTAT, 2009)*

Although the area cultivated with legumes is absolutely increasing globally, this however is due to an increase in total land under cultivation in developing countries rather than a higher importance of legumes within crop rotations. Only soybean has been increasingly grown, whilst the more fertility-generating pulses (e.g. alfalfa, clovers, beans, peas and vetches) have been progressively reduced (Figure 7).

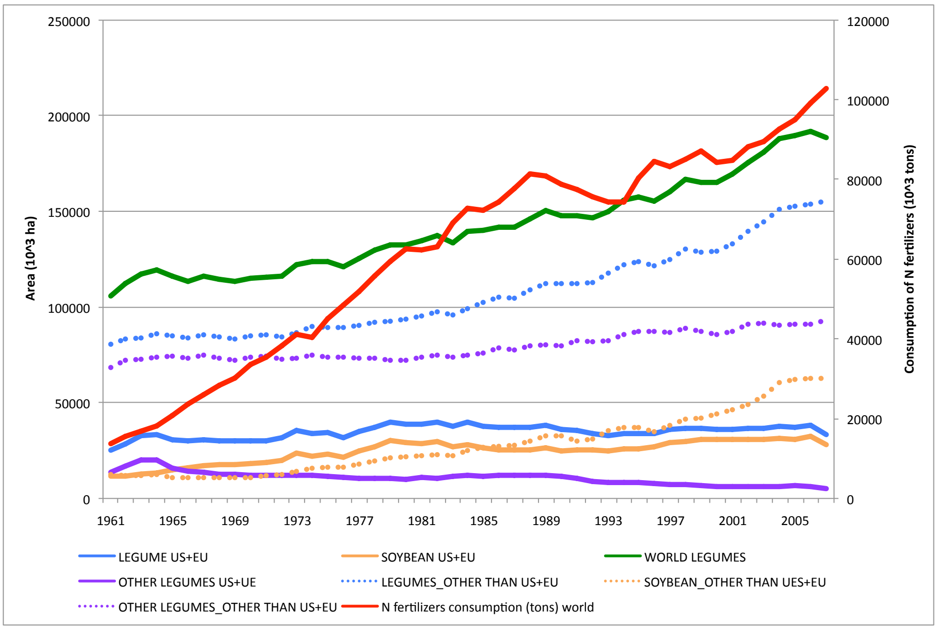


Figure 7 - Trend of world consumption of N fertilizers and extension of legume grown area in the world, USA+Europe and remaining countries (1961-2007) (FAOSTAT, 2009; IFA Statistics, 2009)

Crews and Peoples (2004) stated this trend was due to the progressively larger availability of cheap mineral N fertilizers, revealing a lower dependency of cropping systems on $N_2$-fixing crops. Smil (2001) estimated that while as much as 50% of all available N may have originated from biological $N_2$-fixation by leguminous crops in the 1950s, this value had dropped to around 20% by the mid-1990s. The rapid adoption of synthetic N, reflected in global fertilizer consumption which increased from about 10 Mt N per year in 1960 to approximately 100 Mt per year in 2007 (Figure 7), has not only brought a change in crop nutrient management, consisting of the shift from organic to mineral nutrient sources, but also a real revolution to the existing and traditional cropping systems all over the world with a related impact on the environment (Peoples et al., 1995; Smil, 2001; Peoples et al., 2009a).

In addition to increasing farmland productivity, the adoption of synthetic N fertilizers increased the overall farm production of food crops by allowing farmers to grow cereals or other crops on land that would have otherwise been dedicated to fertility-generating legume rotations including green manures, pastures, fodder, or grain pulses, also avoiding the sometimes high labour and time costs for these crops ((Crews and Peoples, 2004)). Excluding pulses and fallows from crop rotations has also led to shorten the time of the recurrence of main crops, mostly cereals, on the same field, giving rise to an increased risk of crop attack by specific weeds, pests and pathogens due to the more frequent occurrence of their plant hosts both in space and time. Thus, also a higher use of crop protection products and mechanical field operations had been made necessary, increasing the reliance of crop production on not-renewable external inputs ((Pimentel et al., 2005)).

As nitrogen ceased to be the most important limiting factor for crop production, a significant increase in land area under cultivation, P fertilization and water use for irrigation had also occurred ((Hanson et al., 2007)). Moreover, the shift from legumes to fertilizers caused spatial separation between crop and animal husbandry, with the latter pushed into specialized dairy farms, focusing on maximize milk and meat production in order to match the needs of the human diet shifting towards a preference of animal protein over plant protein.

The present use of N fertilizers can be evaluated from two perspectives. On one side, there is increasing public interest in food that is produced under various organic certifications, including considerations about transformation of global agricultural production towards more robust and resilient cropping strategies with a higher proportion of legumes. In contrast the food demands for current and future human populations may exceed the potential productivity of legume-based agriculture. Thus, some argues that the productivity of the best farmlands should be maximized with a more efficient use of mineral inputs rather than developing other systems to replace fertilizers (Smil, 2001).

Here a point-to-point comparison between legumes and mineral fertilizers under a CC perspective is provided.

### 2.2 $N_2$-fixing legumes and climate change

Legumes have three ecological functions to offer under a CC perspectives:

a. they can mitigate CC;
b. they can contribute to CC;
c. they can be affected by CC.

a. IPCC (2007a) and FAO (2009) recommended a wider use of legumes as one of the most effective tools for agriculture to CC mitigation.

Firstly, legumes can reduce or even replace the application of mineral N fertilizers, thereby reducing fossil energy consumption and $N_2O$ emissions, without reducing N supply to the cropping system nor yield or quality of high profitable crops (Crews and Peoples, 2004; Nemecek et al., 2008). These benefits have been attributed both to the addiction of N derived from fixation and to “N sparing” effect(Chalk, 1998). According to the estimations of Peoples et al. (2009a) legumes currently grown in the world account for the equivalent of 72-100 Mt of urea (corresponding to 33-46 Mt of N) on a yearly base when including also rhizodeposition. Thus, as experimental data report a global average of around 1% of $N_2O$ emission from N applied as fertilizer ((Stehfest and Bouwman, 2006)), an indirect reduction of 330-460 thousand t of $N_2O$ per year is already achieved by currently grown legumes, and a shift towards more legumes in crop rotations can have an even bigger impact on $N_2O$ emission.

Furthermore, legumes can help to reduce $CO_2$ emission from agriculture by positively affecting soil C sink capacity either by increasing cropping systems productivity and the addiction of organic C to the soil, or by reducing C losses from protecting soil in otherwise fallow periods, reducing erosion and avoiding high soil respiration rates (Lal, 2004). All these benefits are most valuable in degraded land, where legumes may strongly contribute to counteract desertification and to recover marginalized soils. Also C dynamics can be optimized by growing legumes, as they can decrease C:N ratio of plant materials incorporated into the soil stimulating N mineralization-immobilization processes and thereby microbial activity and diversity (Peoples et al., 2009a).

Overall, agroecosystems may benefit of a higher presence of legumes in crop rotations also in terms of resistance and resilience against adverse events (e.g. flooding, drought, fires), predicted to be come more frequent in the future. Water budget, for example, may be improved by legumes both in dry and wet areas, as they can reduce erosion, enhance water infiltration, and also obstruct evaporation and deep percolation of soil water ((Frye, 1988; Kirkegaard et al., 2008)).

Other important issues are also indirect mitigation effect coming from several ecological services provided by legumes, like the break crop effects in rotations dominated by cereals, indicating the great role of biodiversity in ecosystems endurance (Altieri, 1999). Including legumes in crop rotations would result in a higher diversification not only of crops, but also of weeds, animals and microorganisms as a whole (Table 1), potentially decreasing the risk of high incidence of pests and diseases. Thus, legumes can also indirectly reduce also the application of pesticides, further contributing to lower GHG emission from agriculture.

A shift towards more legume-based cropping systems may imply also a change in human diet composition, by replacing meat intake with legume proteins. Such a change would lead towards a lower density of livestocks possibly more linked to crops rather than concentrated feedstuff. A transition to less animal production would reduce $CH_4$ emissions from agriculture, and also it would free up to 2,700 Mha of pastures and 100 Mha of cropland, being newly available for vegetation and carbon storage ((Stehfest et al., 2009)).

Finally, Crews and Peoples (2004) state legume-based agriculture has the potential to feed current and future human populations. Nevertheless, as a reduction in global invested area and production of crops other than legumes is expected due to their reduced recurrence in crop rotations, they contend also important changes in both national and international food distribution and market strategies would be necessary to address the future food demand.

| | Effect of $N_2$-fixing legumes | Environmental benefit | Reference |
|---|---|---|---|
| Soil physical and chemical fertility | Organic matter supply | Improved soil structure, lower bulk density | (Rochester et al., 2001) |
| | Soil exploration by roots, hardpan breaking | Increased soil depth and porosity | (Martens et al., 2001) |
| | Less soil disturbance within conservative strategies | Increased porosity and improved structure | (Sainju et al., 2007) |
| | Symbiosis with AMF | Increased P availability | (Li et al., 2009) |
| | Soil acidification by root exudates | Increased P and other nutrient availability | (Kopke and Nemecek, 2010) |
| Soil & ecosystem biodiversity | Supply of organic matter with balanced C:N ratio | Higher microbial biomass and activity | (Peoples et al., 2009a) |
| | Increased microbial diversity in rhyzosphere | Lower soil pathogens and dangerous nematodes | (Jensen and Hauggaard-Nielsen, 2003; Kirkegaard et al., 2008) |
| | Released of $H_2$ from nodules | Stimulation of plant growth by $Hup^+$ (producing Hydrogenase Uptake enzymes) rhizobial strains | (Peoples et al., 2009a) |
| | Production of flowering, creeping biomass, mulch | Increased presence and diversity of pollinator, predator and antagonist insects | (Palmer et al., 2009; Shearin et al., 2009) |
| | More diversified crop rotations and tillage (time, technique) | Lower occurrence and higher diversity of weeds (seedlings and seed-bank) | (Moonen and Barberi, 2004) |

Table 1 - Main benefits of legumes on soil fertility and biodiversity

A last point to be addressed is the application of legumes in the no-food sector, especially in bioenergy production, where legumes they can be part of the process either as substitute of mineral fertilizers, or as biomass itself to be converted into energy (by direct burning or by high quality oil production) ((Peoples et al., 2009a)). In any case, legumes may have the potential to improve the environmental and energetic efficiency of bioenergy crops.

b. On the other hand, leguminous $N_2$-fixation is to some extent responsible for $CO_2$ and $N_2O$ emission.

Addressing especially the potent GHG $N_2O$ emissions, several studies in the past have investigated whether the process of $N_2$-fixation itself may give rise to $N_2O$ emission, as some *Rhizobium* spp. can denitrify nitrate in root nodules. But recent data has lowered the importance of this, putting more emphasis towards accumulation of mineral fixed N in the soil due to asynchronism between N release from root exudates or residues mineralization and N uptake by plants ((Carter and Ambus, 2006; Peoples et al., 2009a)).

Rochette and Janzen (2005), for instance, suggest to lower the legume-related $N_2O$ emission IPCC coefficient to better match actual documented field measurements. It has been clearly demonstrated that, despite the low C:N ratio, legume biomass is not immediately mineralized after soil incorporation. Firstly, it is rapidly assimilated by soil microbial population proportionally to its requirement of C. That evidence applies particularly to the legumes rich in lignin like some herbaceous tropical species and shrubs or woody plants (Peoples et al., 2009a).

However, it's important to address potential $N_2O$ emissions from legume cropping when evaluating legumes in a CC perspective, especially for green manures or forages when high amounts of N rich biomass are incorporated followed by fast N mineralization (Peoples et al., 2009a). For example, Robertson et al. (2000) have stressed the importance of $N_2O$ emission when comparing the GWP of cropping systems differently reliant on legumes and mineral fertilizers. In some cases, the benefits of a net C sequestration in legume-based systems were made fruitless by significant $N_2O$ emission.

Resource use efficiency is regarded as an other key factor in order also to achieve a more sustainable agriculture. Rather poor N economy and efficiency can be observed when growing legumes, as it has been estimated that less than 30% of total legume N is commonly made available for the subsequent crop, while the remaining part is mineralized at a quite slower rate (< 5-10% on a yearly base). Overall, the highest impact of legumes on $N_2O$ emission seems to be the need to match subsequent crops N demand with soil mineral N levels, which is one of the main advantages attributed to mineral fertilizers.

Anyway, Peoples et al. (2009a) report a substantial similarity between synthetic and biological N sources in the efficiency of N uptake by crops with a decreasing efficiency increasing the amounts of N applied, and with slight predominance of fertilizer in dry environment and of legumes in wetlands. Nevertheless, Peoples et al. (2009) also argued that all the reviewed studies had some constraints which resulted in an underestimation of N recovery either of fertilizer (e.g. the variable adoption of “best management practices” for fertilizer application) and, even more, of legume (e.g. the omission of below-ground biomass nitrogen, the exclusion of biologically fixed N firstly immobilized in microbial pool, and the short duration of the studies, ineffective to reveal the real impact of legumes on N availability for crop rotations).

Likewise leguminous $N_2$-fixation is responsible for some $CO_2$ emissions. Atkins (1984) has estimated that between 0.5 and 2.7 $CO_2$-C is respired in the legume nodule for every kg of N fixed and even if a 30-60% of this amount can be captured by plants and soil microorganisms, after the decomposition and mineralization of their biomass a positive net increase in $CO_2$ emission from legumes can be expected ((Peoples et al., 2009a)).

Peoples et al. (2009a) estimated a $CO_2$ emission from fertilizers of 1.7÷6 tons $CO_2$-eq. per ton of $NH_4^+$ produced, which means a global contribution of 400÷1600 Mtons $CO_2$-eq., stated a consumption of 100 Mtons of N fertilizers in 2007. Legumes may account only for 23÷127 Mtons $CO_2$-eq but, if also mineralization of residues and nitrous oxide losses from excess of mineralized N would be considered, it may even exceed the contribution from fertilizers. However, in this context it is important to stress that this $CO_2$ arises from renewable source and not from fossil fuel, as in the case of fertilizers.

This difference between the nature of energy source of legumes and fertilizers will be even more crucial in the future, when a higher food demand and an increased fertilizer consumption are expected following increasing cost per produce due to lower global availability of fossil oil and higher prices. Mineral fertilizers are indeed the most oil-dependent agricultural commodity, and historically a strong increase in oil price has always been linked to a proportionally higher increase in fertilizer price (Baffes, 2007). Moreover, fossil energy sources are not equally distributed worldwide, so there are lot of concerns about future energy supplying and fertilizers accessibility of many countries (Peoples et al., 2009a).

The energetic costs of $N_2$-fixation reduce legume plant growth and production due to transportation of photosynthates to the symbiotic bacteria. Thus leguminous species could be regarded as less relevant when taking into account that their land use efficiency becomes lower than other crops (Brehmer et al., 2008).

Even though it might directly be linked to this rather low yield potential for many species, the increase in soybean cultivation currently going on (Figure 7) especially in South America results in a high proportion of natural and semi-natural habitats being converted to arable land. This is causing a comprehensive decrease in soil carbon storage and typically increasing use of pesticides, related to the fact that a large fraction of soybean is the genetically modified types resistant to herbicides (Nemecek et al., 2008).

Another issue is that many legume species require an often intensive mechanical seedbed preparation. Furthermore, a sufficient amount of P is necessary especially for initial growth, consequently increasing fossil fuel consumption and $CO_2$ emission for fertilizer manufacturing, transport and application. For legumes grown as cover crops, an additional emission may arise from their devitalization before the establishment of the main crop. This can be performed before legume maturity either mechanically, by ploughing under or cutting and chopping green manures, or chemically, by spraying herbicide on pulses devoted to provide a dead mulch (Dabney et al., 2001).

c. Legumes can also be affected by changes of growing conditions in future climates.

Lack of water, which is one of the three main traits of CC, has to be considered as a limiting factor for photosynthesis and plant growth, as many legume species are well-known to be extremely sensitive to drought (McDonald and Paulsen, 1997).

Nevertheless, legumes might take advantage from higher $CO_2$ and temperature, as they significantly increase their photosynthetic activity and efficiency (a lower stomatal conductance is required), thereby enhancing their biomass production (Ainsworth et al., 2003). While other plants cannot sustain this increase for long, as other nutrients than C (e.g. N) would become limiting factors, legumes might be able to exploit this potential further being provided with N and P by symbiotic microorganisms (i.e. *Rhizobium* spp. and AMF), even though such symbiosis requires a carbohydrate prize. However interactions and relationship with the soil microbial biomass and CC is a complex matter to address (Figure 8).

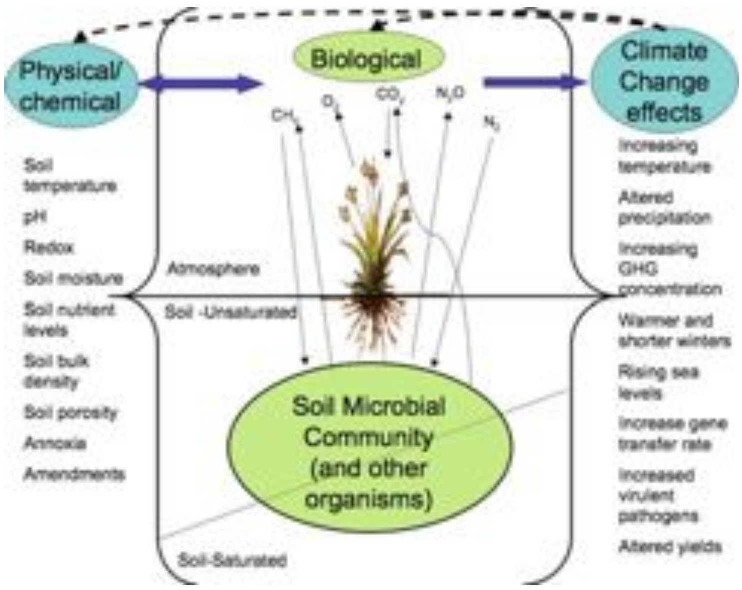


*Figure 8 - Some complex interactions between climate change and soil-plant-microorganism communities (from (French et al., 2009))*

For example, regarding increase in temperature, general findings suggested an increase of soil respiration and soil microbial activity but with some uncertainty in the long-term due to the dynamic changes occurring in microbial population composition ((French et al., 2009)). Houlton et al. (2008), producing a synthesis of the relationship between temperature and nitrogenase activity, spanning diverse species, strains, latitudes and environments, demonstrated a strong convergent effect of temperature on biochemical $N_2$ fixation, with nitrogenase activity reaching a maximum at 26°C and decreasing at higher temperatures, probably in response to depletion of C supplies (Figure 9).

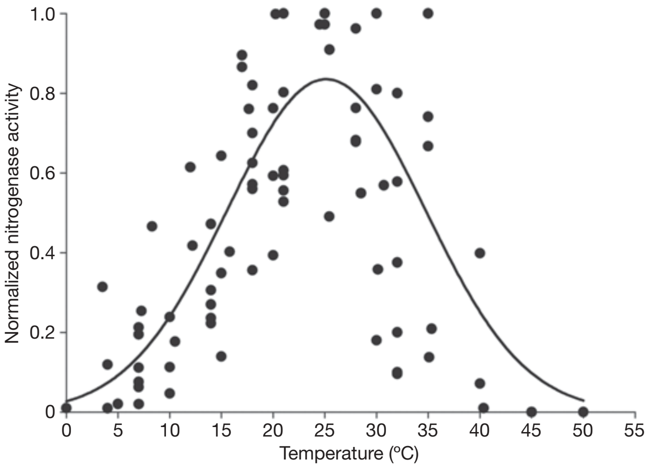


*Figure 9 - Relationship between nitrogenase activity and temperature ((Houlton et al., 2008))*

On the other hand, an enrichment in $CO_2$ is generally related to a proportional enhancement in soil respiration and microbial biomass, maybe due to the higher plant production and the consequent higher organic matter availability in the soil.

Both these responses seemed to be quicker in rhizosphere communities, and especially for arbuscular mycorrhizal fungi and $N_2$-fixing bacteria an increase in biomass at high $CO_2$ and T has been demonstrated. But simultaneous interactions between temperature, $CO_2$ and water are still unclear. Garten et al. (2008), for example, studied the effect of all these climate factors on the $N_2$-fixation capacity and reported no net change in symbiotically fixed $N_2$ due to environmental manipulated factors, whilst a stronger effect came from legume species and temporal changes of their activity. French et al. (2009) collected results coming from studies carried out either in the field or under controlled growing conditions in growth chambers, demonstrating an evident increase in symbiotic $N_2$-fixation in legumes grown in warmer and $CO_2$ enriched air.

Therefore, trying to summarize all these evidences, CC is expected to negatively affect those legume species being more exposed to drought, whilst for annual winter grown pulses (e.g. winter cover crops and green manures) an increase of biomass production and nitrogen fixation is likely to occur. For perennials, there are no clear trends, so different behaviors can be expected depending on the species and the environment.

Assuming continuation of past practices, another possible effect of CC on legume growth and $N_2$-fixation may come from the predicted increase of N fertilizers application to cropping systems in order to face the higher food demand of a rising human population ((Erisman et al., 2008)). Soil nitrate and $N_2$ fixation are complementary in meeting the N requirements for growth by a legume, and the inhibitory effect of nitrate on legume root nodulation by *Rhizobium* spp is well documented ((Herridge et al., 1984; Peoples et al., 1995b)).

### 2.3 Substitution of N fertilizers with legume N-fixation: technical improvements

It is clear that legumes have promising potential dealing with CC mitigation.

Crews and Peoples (2004) contend an increased importance of legumes as N source is possible and it could be achieved by either: i) increasing the amounts of $N_2$ fixed where legumes are already included in cropping systems; ii) reducing the amount of N lost from legume-based cropping systems; iii) increasing the amount of land planted under legumes.

Peoples et al. (2002) argue that the most promising approach to increase the contribution of fixed N to agriculture in the short-medium term is in the local fine-tuning and implementation of already existing agronomic knowledge. Specifically, $N_2$ fixation and biomass production by legumes could be increased by fertilizing with deficient phosphorus, making sure the crop legumes are inoculated with effective and efficient rhizobia, and addressing other agronomic limitations (e.g. soil acidity, water stress, and high N carryover from previous crops). Thus, such simple technical improvements may lead to include more legumes not only in organically managed cropping systems, but also in integrated agriculture, where they can substantially reduce the application rate of N fertilizers.

Despite the currently low interest of breeders in developing genetically improved legumes, we can imagine that in the future the higher importance of these crops could stimulate the production of new varieties featuring high resistance to specific environmental and biological stresses (e.g. high temperatures, drought, pests and

diseases). The efficiency of $N_2$-fixation may be enhanced not only through plant but also *Rhizobium* strains improvement, for example by amplifying their cross-inoculation traits, by increasing the resistance of nitrogenase to high temperatures and by reducing energy cost of $N_2$-fixation process through an enhanced metabolism of carbohydrates.
Synchrony between legume N supply and N demand by subsequent crops may be improved for instance by modulating N mineralization of legume residues through addition of nitrification inhibitors prior to their incorporation into the soil. Other approaches with a higher environmental worth may be based on incorporating legume biomass into the soil not immediately at the end of their cycle, but only after composting or biodigestion in plants dedicated to biogas production. For this latter solution, the application of N rich stable effluents can be performed directly to the target main crop, for example a cereal crop, simultaneously with the most N demanding stage ((Stinner et al., 2008)).
Likewise, reliance of legumes on P fertilizers can be lowered through inoculation of AMF if doesn't occur naturally and microcapsulation of seeds with P and/or other nutrients, like molybdenum, ((Campo et al., 2009)).

Overall, increasing and monitoring N-fixation efficiency of legumes for matching the actual needs of the crop rotation is the major point for exploiting all the legume potentialities to reduce $N_2O$ emission from agriculture and to mitigate CC. So, a proper measurement of actual $N_2$-fixation, being performable also in farmers' fields, is necessary.

## 3. N-fixation measurements: methods, preciseness and uncertainty

Correct fixation estimation is regarded as extremely important for improving the knowledge about the agro-ecosystem effects of these species and making the use as efficient as possible when adopting legumes to mitigate CC.
The use of $^{15}N$ isotope techniques gives precise and reliable values for $N_2$-fixation and can be used as the benchmark for calibrating all other methods available (Peoples et al., 2009b). $^{15}N$ isotope techniques are indeed the only one able to directly detect the fraction of all the N accumulated in plant tissues that comes from fixation, whilst other methods are either simple estimations based on apparent N balance (e.g. N balance and N difference methods), or analytical measurements of products indirectly related to $N_2$-fixation process (e.g. acetylene-reduction, hydrogen-evolution).
This report mainly focus on $^{15}N$ techniques, as they are demonstrated to be the most versatile and reliable one for both experimental and more commercial purposes.

### 3.1 General principles behind $^{15}N$ techniques

Two main stable isotopes of N naturally occur with the lighter one ($^{14}N$) being more abundant than the heavier ($^{15}N$). Isotope abundance is generally expressed as a percentage of total N atoms. In the atmosphere $^{15}N$ has a constant abundance of 0.3663 atoms% ((Högberg, 1997)), whilst in the soil it is 0.001-0.007% more enriched. The difference between air and soil isotope abundance occurs because N in the soil can be involved in many different transformations, mainly driven by microorganisms, which can favor one isotope rather than the other. For instance, gaseous losses of N (e.g. denitrification) favor the lighter $^{14}N$ isotope, but also fertilization, fire, water-logging, organic manure application are reported to influence isotope abundance, whilst crop rotation has no strong effect (Peoples et al., 2009b).
The principle behind all $^{15}N$ techniques is that when $^{15}N$ concentration in the atmosphere significantly differs from that in the soil it is possible to calculate $N_2$ fixation on the basis of isotopes concentration in the tissues of a legume and a non-$N_2$ fixing reference plant. A well nodulated legume grown in a medium free of mineral N and

thereby totally depending on $N_2$-fixation will obtain an isotopic composition similar to that of the atmosphere (0.3663 atoms% $^{15}N$), while a non-$N_2$ fixing reference species, which can use only mineral N, will have a concentration of the $^{15}N$ very close to that of the soil. Thus, when a legume is assimilating N from both the atmosphere and from the soil intermediate $^{15}N/^{14}N$ abundance will be found, which is termed "isotope dilution".

One main assumption when using $^{15}N$-techniques is that any variability in $^{15}N$ abundance in the soil and in the air is small if compared to their overall difference (Peoples et al., 2009b). Thus $^{15}N$ natural abundance techniques can provide precise estimations of legume $N_2$ fixation in many different environments and cropping systems. Nevertheless, when soil N content is very poor (e.g. in sandy or flooded soils), this difference may be only little which will result in rather small differences between the $^{15}N$ abundance of $N_2$-fixing and non-fixing plants ((Unkovich and Pate, 2000)). When this is the case $^{15}N$ enrichment techniques can be recommended.

## 3.2 Natural abundance and N enrichment

### 3.2.1 $^{15}N$ enrichment technique

Before the development of high-precision mass spectrometers (±0.3‰ $\delta^{15}N$; ±0.0001 atom %$^{15}N$), $^{15}N$ enrichment was the only available technique for $N_2$-fixation estimates. The basis of the method is to widen at least an order of magnitude (with 10-fold being the optimum) higher than naturally the difference between $^{15}N$ abundance in atmosphere and soil. This is performed by applying $^{15}N$-enriched fertilizers at the same rate to legume and non-fixing reference plants ((Chalk, 1998)). As the cost of labeled material is extremely high, it should be applied only to a few small areas (1-2 $m^2$) which might be isolated from the rest of the field by the installation of barriers (e.g. steel boxes placed into the soil) aimed at reducing runoff and scavenging of $^{15}N$ by plants from outside the labeled area.

After the enrichment, the soil $^{15}N$ pool is increased giving a larger difference between $N_2$-fixing and non-fixing species. The dilution of plant tissue $^{15}N$ with $^{14}N$ from $N_2$-fixation will be easy to estimate assuming that both species have access to the same pool of soil mineral N in the root zone (Figure 10). Thus, the legume and non-legume plants have to be grown very close each other in order to obtain the same soil N uptake stated an identical N availability, a similar extension of roots and an equal growth period.

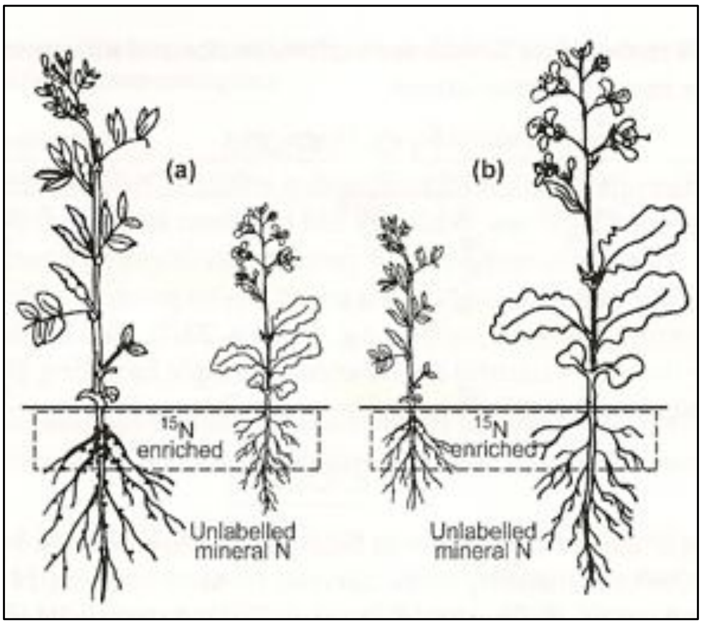


Figure 10 - N pools available to a fixing (a) and non $N_2$-fixing reference plant (b) following addition of $^{15}N$ labeled materials to soil (modified from Peoples et al., 2009b)

The principle is that the larger $^{15}N$ enrichment of the material, the greater is the potential accuracy of the method ((Hardarson et al., 1988)). In N-poor soils, such as sandy ones, a lower enrichment should be acceptable for achieving a good-quality analysis. As for natural abundance, it is extremely important to measure isotope abundance also in a non-$N_2$ fixing crop (*reference crop*), generally a grass weed or a cereal crop (or even better both), in order to estimate the fraction of total plant N taken up from the labeled and unlabeled soil N pools.

The value of $^{15}N$ enrichment of a plant sample is usually expressed as:

$$\text{atom\% } ^{15}N\,excess = [(atom\%\ ^{15}N_{sample} - 0.3663)/0.3663] \quad [1]$$

with 0.3663 being atom% $^{15}N$ of atmospheric $N_2$.

Atom% $^{15}N$ excess of reference plant can be assumed as an accurate reflection of the $^{15}N$ enrichment of soil N taken up by the legume, then the total uptake of soil N (*Ndfs, Nitrogen derived from soil*) by the legume can be quantified and N derived from symbiotic $N_2$-fixation (*Ndfa, Nitrogen derived from atmosphere)* calculated by difference:

$$\%Ndfa = 100 \times [(\text{atom\% } ^{15}N\ excess_{reference} - \text{atom\% } ^{15}N\ excess_{legume})/\text{atom\% } ^{15}N\ excess_{reference}] \quad [2]$$

$^{15}N$ enrichment technique allows to trace the fate of legumes within cropping systems, as it is possible to relate N content also of subsequent crop to labelled or unlabelled pool revealing the importance of N supplied by legumes in soil N pool. In legume-cereal intercropping, $^{15}N$ enrichment technique allows also to quantify N transfer from legume to companion crop ((Stern, 1993)).
Due to the high spatial variability of soil $^{15}N$-enriched pool (e.g. due to soil depth and variability of plant root systems), the choice as reference crop of several non-$N_2$ fixing species with different root pattern is recommended. Concerning variability over time (e.g. an asynchronism between enriched N availability and plant needs may occur), suitable options could be to split fertilizer application over time or to apply fertilizers with slow N release (e.g. organic or pelletized products) (Peoples et al., 2009b).

### 3.2.2 $^{15}N$ natural abundance

Since the high-precision mass spectrometers have been available on the market the $^{15}N$ enrichment technique has been replaced in several research studies by $^{15}N$ natural abundance, applicable to every situation in which legumes and non-legumes coexist without the need to apply highly expensive labeled material. The two techniques are based upon the same principles but for natural abundance the legume isotope dilution as compared to non-$N_2$ fixing species is used directly (Figure 11).

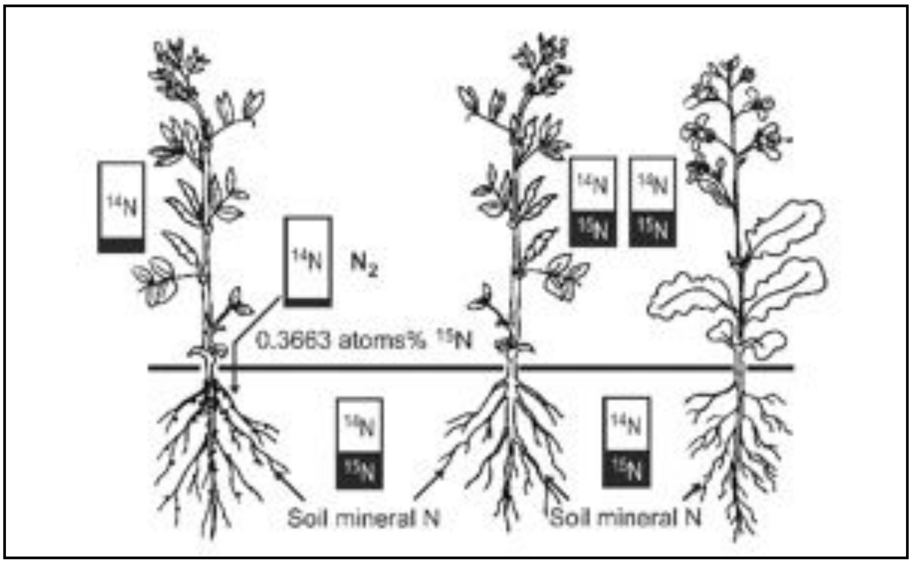


*Figure 11 - Diagrammatic representation of the $^{15}N$ composition (indicated by the size of the black bar at the bottom of the histogram) of N accumulated by a nodulated legume using both soil mineral N and atmospheric $N_2$ for growth. Also shown are the $^{15}N$ compositions of two non $N_2$–fixing plants, a non-nodulating legume and a non-legume, using only soil mineral N for growth (from (Peoples et al., 2009b))*

$^{15}N$ abundance in both legume and non-fixing plants is expressed in comparison to that of atmosphere:

$$\delta^{15}N_{sample} = 1000 \times [(atom\% \ ^{15}N_{sample} - 0.3663)/0.3663] \quad [3]$$

The *Ndfa* of legume is calculated by comparing $^{15}N$ enrichment of legumes with that of a reference plant, again to be chosen among grasses living in strict contact to legumes:

$$\% \ Ndfa_{legume} = [(\delta^{15}N_{reference} - \delta^{15}N_{legume})/(\delta^{15}N_{reference} - B)]*100 \quad [4]$$

where B is the $\delta^{15}N$ of legume grown in a N free medium (N is the only limiting factor) ((Peoples et al., 2009b)).

Overall, spatial variability of $^{15}N$ natural abundance represents one of the most important limitations of this technique and occurs due to landscape attributes, soil type and management.

Generally, in soil there are $\delta^{15}N$ values for plant available N between +3 and +8‰ (in Europe a mean value is 4.3 in arable crops, 3.4 for pastures). In soil with low organic matter content and under rain-fed conditions, very often there are values <2‰ . In these cases $^{15}N$ natural abundance can be implemented only if legumes have negative $\delta^{15}N$ values and a significant difference from reference plant occurs (Peoples et al., 2009b).

Hauck et al. (1972) and Bedard-Haughn et al. (2003) reported also predictable patterns of soil $^{15}N$ variability within different landscapes, mainly driven by topography, which can influence hydraulic and biological processes involved in N transformation in the soil. Hauggaard-Nielsen et al. (2010) did not confirm this evidence for pea plants and *Ndfa*, while they found landscape position to have affected isotope abundance in reference plants. They also argued $N_2$-fixation can evade these patterns, as it can quickly respond to particularly local, small-range environmental variability. Concerning management, although recent cropping and tillage history seems to not have a significant effect on isotope composition, a high variability is expected for soil added with animal manures and for grazed pastures, where the proportion of urines and feces may alter $^{15}N$ content (urines have less $^{15}N$ than feces) (Peoples et al., 2009b).

Natural abundance is regarded as the most feasible $^{15}N$ technique, and it seems to have the highest suitability also under a CC perspective, as it can provide precise estimations of biological $N_2$ fixation in worldwide agro-ecosystems and thereby helping farmers, researchers and all the stakeholders to rate either already implemented or still potential CC mitigation actions. On the other hand, the sensitive nature of the technique implies the crucial importance of taking into account the possible sources of uncertainty and error that can affect the reliability of the results. A synoptic comparison of the main pros and cons of both $^{15}N$ techniques is reported in table 2.

In this report we will mainly focus on $^{15}N$ natural abundance technique, providing detailed guidelines for a proper implementation of each step of the whole analytical process, as well as some pictures and data from our practical experience provided in the Appendix.

| | **$^{15}N$ enrichment** | **$^{15}N$ natural abundance** |
|---|---|---|
| Suitability for field-grown legumes | + | + |
| Suitability for on-farm measurements and for fields not intended for experimental purposes | - | + |
| Costliness and technical skills requirement | ++ | + |
| Importance of reference plants choice | + | ++ |
| Suitability for N poor soils | ++ | = |
| High numerousness of sampling areas with the same investment | - | + |
| Sensitivity to spatial variability | + | ++ |
| Sensitivity to temporal variability | + | + |
| Suitability for natural areas and organic farms | - | + |
| Cruciality of correct identification of samples | + | + |
| Cruciality of samples contamination prevention | + | ++ |
| Suitability for tracing legume fate within cropping systems | ++ | + |
| Representativeness of natural processes involving N | + | ++ |

Table 2 - Comparison of main issues of $^{15}N$ enrichment and $^{15}N$ natural abundance techniques
"++" is extremely true, "+" is true, "=" is conditionally true, "-" is not true

### 3.3 Suggested guidelines for appropriate estimations in $^{15}N$ natural abundance technique

*A. Plot size, numerousness and size of samples*

As estimates of *Ndfa* depend on values of plant biomass, the correct choice of sampling area extension and number of replications is crucial (Peoples et al., 2009b). For annual legumes, ideally, at least 0.5 $m^2$ should be harvested from each plot, with a number of replicates ranging from 4 for largest sampling areas (1 $m^2$) to 10 for smaller ones. Number of samples has to be increased if high weed presence and heterogeneity are assessed. If the legume is grown in intercropping with a different crop, also the variability in composition of the mixture should be considered. For perennial systems, the number of samples should reflect the spatial variability of plant growth, weed density and composition, grazing patterns and, if legumes are grown in mixture, also their proportion. If repetitive samplings are planned, plot size should be set in order to provide researchers with the opportunity to choose where sampling should be performed in each date.

*B. Plant sampling*

All the components of aboveground biomass should be sampled, as isotope abundance is reported to even strongly vary among individual plant tissues, with differences due to species and environments ((Peoples et al., 1997; Peoples et al., 2009b)). Provided that the amount of root system that can be physically recovered from the soil is small and root separation is unfeasible for trees, only above-ground biomass is generally sampled, and final estimations can be corrected with specific multiplication factor (see below).

In agroforestry, a further complication arises from legume tree sampling, which should be seasonally performed taking into account all the plant components, and at the same time without compromising plant life. For these purposes, non-destructive methods are available to estimate biomass from trunk diameter, for example ((Peoples et al., 2009b)). Regarding N composition, perennial species are reported to have a lower variability among organs than annual ones, so very often sampling only new leaves, litter fall, pruned branches and some sprouts at the main growing stages can be enough.

*C. Sampling time*

For annual species, and especially for grain legumes, to reduce investments of time, labour and money, it may be planned even only one sampling at crop maturity, when most N is concentrated in shoots and seeds. Anyway, Hauggaard-Nielsen et al. (2010) demonstrated differences in *%Ndfa* of pea when measured at flowering stage and at maturity, with a decreasing importance of $N_2$-fixation at the end of crop cycle. They also reported a different performance of straw when sampled at flowering respect to crop maturity, as a possible result of N remobilization during grain filling.

The number of samplings per season will also depend on the type of agronomical management. For instance, for intercropped legumes at least an additional sampling should be scheduled at flowering stage in order to assess competitive interactions for soil N at this key growth phase. For green manures plowed down before maturity, the sampling should be very close to soil incorporation. For perennial crops more than one sampling is recommended in order to monitor the changes in $N_2$-fixation throughout the year. In agroforestry there may be at least two important seasons to consider, the one of vegetative growth and that of overwintering.

When repetitive samplings are done, a problem may occur due to spatial soil $^{15}N$ variability, with collected plants in a plot/field at a determined time which may have a completely different $\delta^{15}N$ as compared to previously sampled material. But as long as the $N_2$-fixing crop and the reference crop are grown in close proximity no major error will occur.

*D. Choice of reference plant*

The choice of reference plant is crucial for the success of the $N_2$-fixation estimation. As pointed out by Unkovich et al. (1994), a minimum difference of 2‰ in δ values of reference and legume plants is necessary to detect a difference of 10% in %*Ndfa.*

Therefore non-legume plants grown as close as possible to legumes are the most correct, as they share the same environmental conditions than legumes. While this option is usually available for legumes grown in mixed systems, like in intercropping with cereals, a problem may occur when a legume is grown as a sole crop and no potential reference plants are available. In this case, collecting non-$N_2$ fixing weeds or sowing non-fixing crops on small areas close to legume plots may solve the problem.

Nevertheless, even though a suitable reference crop exists, it would be better to collect samples of many different species (e.g. cereal crop and some weeds) in order to cover all the potential types of root systems and to reduce the effect of variability in space and/or time in soil plant available N (Peoples et al., 2009b). In this case, it is recommended to measure $\delta^{15}N$ separately for each species following calculations of weighted mean.

Another important point about reference crop size and growth stage is that it should be as similar as possible to those of the legume plants, as for lot of species huge differences in $^{15}N$ abundance over growth cycle are demonstrated ((Peoples et al., 2009b)).

*E. Sampling procedure*

For all the analytical chain, a clear identification of samples is crucial. To ensure a successful analysis, it is extremely important also to avoid any potential contamination between legume and reference samples, starting already at the initial field activities. According to $^{15}N$ enrichment signal legumes are collected first (lowest $^{15}N$ level) followed by the reference plants. In addition different harvest tools for each species are recommended, as well as to separate samples in different and new paper bags. Paper bags should be kept closed and separated as much as possible from sampling onwards, in order to prevent contamination between reference and $N_2$-fixing species. When different plant tissues are collected, their separation should be done already when sampling in the field.

For completely avoiding contaminations, if natural abundance and N-enrichment samplings simultaneously occur, it would be necessary to handle first natural abundance samples and thereafter the other ones. A recommended practice is also to oven-dry legumes samples either before non-legume plants or even in a different oven.

*F. Grinding of samples*

All harvested plant samples have to be oven-dried until constant weight for 24-48 hours at 60°C (recommended temperature to avoid proteins alteration) following a two-steps grinding procedure. The first step consisting in a course grinding, and the second one in a fine grinding. The fine grinding is performed using a sample mill with a 500 µm mesh. For both course and fine grinding particular care has to be taken during cleaning procedures for the respective mills, as this is a step where potential heavy contaminations can occur.

If working with both enrichment and natural abundance techniques in the same lab two different mills in two separated rooms should be used to grind samples (see Figure 1 in the Appendix). It's also important to be sure than nobody else is using the same mill for grinding other samples. Before starting up on a batch of samples an accurate cleaning with alcohol is recommended.

During the grinding procedure legumes expected to have lower $^{15}N$ content than the reference crops should be initiated first. Furthermore, the operator should proceed with the different replicates of the same treatment (e.g. intercropped legume). Thus, the right sequence for an intercropping experiment should be:

*intercropped legume (all the replicates)→monocropped legume (all the replicates) →intercropped reference crop (all the replicates)→monocropped reference crop (all the replicates)*

Before proceeding with a new sample, the grinding equipment should be cleaned very carefully, using both compressed air, vacuum cleaner and brush in order to remove any dust from rotary blade, filters and the body of the machinery.
When starting to grind a sample, the best practice should be to throw away the first two-three spoons of biomass. In this way, a complete removal of potential residues of previous sample can be ensured.

Stated the importance of avoiding any contaminations, the two steps of grinding are very time-consuming, as for each about 6 samples per hour can be completed.

*G.Precision weighting*

For being pushed into the analyzer, approximately 5 mg (4.5÷5.4) of each ground samples (weighted on a precision scale) must be put into tin combustion cups for elemental analysis (e.g. 5 mm diameter x 8 mm height). For plant analysis, acetanilide (0.9÷1.2 mg), as a N-reference compound, and certified plant materials (e.g. 3÷4 mg of peach leaves) standards must be prepared, in a proportion of 1 each 10 samples for acetanilide and 1 each 6 samples for plant material. Also empty cups (1 each 25 samples) as blanks should be included.
The exact weight of each sample and standard is saved in a worksheet file in a PC. For ensuring good quality results, precision of weighting should be absolutely verified, for example by cleaning scale plate as even a minimum amount of dust falls down and by recalibrating the scale when requested. This is another high time-consuming step of the process, as all the tin cups should be firmly closed around the weighted samples and standards, and then shaped spherically, all by mean of two tweezers. These two operations are extremely important in order to avoid stops and problems when the samples are put into the combustion chamber of elemental analyzer (EA).
Once they are sealed and shaped, tin cups are put into a standard micro titration plates in an exact position (e.g. row A, column 5), reported in the worksheet (see Figure 2 in the Appendix).
To perform this step, approximately 6 minutes (samples), 5 minutes (plant material standards) and 8 minutes (acetanilide standards) per sample are required including frequent weighting errors, recalibration of scale and damage on tin cups occur.

*H. Laboratory analysis*

The analysis of isotope abundance is performed by coupling a carbon-hydrogen-nitrogen elemental analyzer (EA) with a gas mass-spectrometer (MS) (see Figure 3 in the Appendix). Samples are continuously loaded into the EA, where a carrier gas (Helium) first pushes them in the so called “flash dynamic combustion chamber”, in which they are burned at 1700°C in presence of pure $O_2$ and a catalyzer (see Figure 4 in the Appendix). Then the resulting combustion gases are swept through a reduction furnace and onto a series of column where C and H gases are separated from N ones, which go through the GC column and then into a Continuous-Flow (CON-FLOW) allowing operator to select the gas to be introduced into the MS ($N_2$ from sample combustion, reference $N_2$ or pure helium).

A $N_2$ international standard gas with a fixed isotope ratio is introduced before the sample $N_2$ gas into the MS, where a ionizer transforms gasses into ions. Then, an electromagnet properly set generates a magnetic field deviating ions with an angle increasing with their electric charge and mass. According to these angles, the spectrometer is able to discriminate the portion of each isotope within the sample gas. $N_2$ is normally made from both $^{14}N$ and $^{15}N$ isotopes, therefore it can be described as a mixture of $^{28}N_2$ ($^{14}N+^{14}N$), $^{29}N_2$ ($^{14}N+^{15}N$) and $^{30}N_2$ ($^{15}N+^{15}N$) being in a steady state with $^{28}N_2 > {}^{29}N_2 >> {}^{30}N_2$. The MS gives back the $^{15}N$ abundance of the sample expressed as δ value with reference to the standard which has the same isotope composition of the air (δ=0) (see Figure 5 in the Appendix).

*I. Calculations*

The MS gives back the δ values of each analyzed samples. First, they should be checked in order to detect any potential errors occurred during the whole process (from the field to the lab). This should not be done simply by looking at absolute value of δ, because the most important factor affecting results is the difference between legume and reference crop values. If a difference of at least 2‰ in δ values is not detected, there's no significant evidence of $N_2$-fixation ((Unkovich et al., 1994)) and this is most often due to some contamination between samples or inappropriate choice or sampling of reference crop(s).

In intercropping experiment or where different treatments including legumes are studied the comparison has to be made with respect to the same reference crop for all the legumes included. For example, while comparing $N_2$-fixation in a sole-cropping legume and the same legume intercropped with a cereal, for both the reference should be the sole-cropped cereal. Some arguments have been raised for that, as sole-cropped reference does not necessarily share the same soil N pool. But also interactions between $N_2$-fixing and non-fixing plants cannot be excluded for intercropped reference crop, as also some potential N can be taken up from legume root exudates.

The second step is to calculate the percentage of nitrogen derived from fixation (*%Ndfa*). If δ value of total above-ground biomass are available, *%Ndfa* can be calculated using equation [4]. When different plant tissues like e.g. seeds and straw are included, the calculation can be slightly more complicated. In this case, a total biomass δ should be calculated by calculating the weighted mean of each contribution both for legume and reference crops. For example, if both grain and straw samples are available:

$$\delta^{15}N_{\text{total biomass}} = \frac{[(\delta^{15}N_{\text{straw}} \text{ x straw N accumulation}) + (\delta^{15}N_{\text{grain}} \text{ x grain N accumulation})]}{\text{total N accumulation}} \quad [5]$$

Then, the *%Ndfa* in legumes can be calculated as shown in Equation [4] by using total δ values for each crop.

Once *%Ndfa* is obtained, it will be possible to calculate the mass units of N derived from symbiotic fixation, simply by multiplying *%Ndfa* per total N accumulation of each crop, and consequently also legume N uptake from the soil (*Ndfs)* can be easily calculated by doing the difference between total N accumulation and *Ndfa* (g N $m^{-2}$). Obviously, for reference crop *Ndfs* and total N accumulation will coincide.

Some times calculated *%Ndfa* can be less than 0, thereby indicating negative amount of *Ndfa* (g N $m^{-2}$)! Obviously, that doesn't make sense. In this case, even though a negative value occurs only for one plant portion (e.g. grain), all the isotope-related values of the sample, namely δ values, *Ndfa%*, *Ndfa* and *Ndfs* should be excluded from the statistical analysis.

In intercropping experiment or when legumes and non-$N_2$ fixing species are grown very close to each other (e.g. pastures), this method can provide information on potential N transfer from legumes to non-legumes, simply by comparing δ values of intercropped and mono-cropped reference plants. If a significant difference is detected between these values a potential transfer might have been occurred ((Jensen, 1996)). For this purpose, N-enrichment seems to be more precise than natural abundance ((Chalk, 1998)) because for natural abundance uptake of soil N from a different pool cannot be absolutely excluded, whilst in N-enriched experiment we can be sure that all soil N is easily recognizable being labeled. However, some uncertainty still remains as we don't know for sure whether transfer takes place at the same rate as N taken up from the soil and $N_2$-fixation or how these two pools are differently involved ((Stern, 1993)). Moreover, transfer of fixed N seems not to be a very rapid phenomenon, because it is most often mediated by soil microbial pool ((Stern, 1993)).

In addiction to $^{15}N$ data, for a complete exploitation of competition for soil N between companion crops, a competition index, such as LER (*Land Equivalent Ratio,* (Fukai, 1993)) or CR (*Competitive Ratio,* (Willey and Rao, 1980)) can be calculated and results shown within an apposite column in the same table of N concentration and content (see Table 1 and 2 in the Appendix).

### *J.Choice of B-value*

The B-value (see equation 4) is necessary to correct observed $\delta^{15}N$ with respect to maximum $N_2$-fixation conditions, such as when a legume is grown in a medium free of N.

Peoples et al. (2009b) summarized the findings of several reviews on the importance of B-value on final estimation of $N_2$ fixation. They concluded its importance was high only when *%Ndfa* was low, whilst it decreased when ≥85%. This evidence was confirmed also in our personal experience. For example, *Ndfa* of 24 samples of hairy vetch aboveground biomass varied only at most of 9.4% when calculated using two B values suggested by literature examination differing for about 60% (i.e. -0.76 vs -0.47).

The B-value should be different if only above-ground or also below-ground N is considered, as $^{15}N$ is distributed differently in roots and shoots. Usually $\delta^{15}N$ values are >0 in roots and <0 in shoots ((Peoples et al., 2009b)). Unkovich et al. (2008) provided a comprehensive list of legume shoot B-values, which can be used by researchers all over the world, as this estimation is demonstrated to not change among different cultivars and different lab typologies. They also provided some useful suggestions for dealing with B-value under a range of environmental conditions.

When a preliminary study is carried out, a particular care should be taken in the detection of the *Rhizobium* sp. strains looking at root nodulations. The best recommended practice in laboratory assays should be the inoculation of a mixture of different strains instead of only one ((Peoples et al., 2009b)), as rhizobial strain can be extremely relevant for *Ndfa* estimation. Anyway, overall the most important advice is to determine B-value only for mature well-grown plants, as pointed out by Peoples et al. (1997).

### *K. Data corrections: outliers, contribution of N from seeds and from below-ground biomass*

As reported by Hauggaard-Nielsen et al. (2010), high variability in δ values may occur also among plant collected very close to each other, due to the strong effect on soil N uptake of many micro-scale phenomena. Therefore, even though some δ values could be identified as outliers, they should not be removed from datasets, as they can lead to a better explanation of spatial variability, especially if they can be correlated to some soil parameters.

Nitrogen content in seeds can lead to an overestimation of $N_2$ fixation, as some of total N accumulated by crop in produced biomass may be that initially content in the seeds. Jensen et al. (1985) reported that N content in seed is distributed equally to shoots and roots, so they suggested to correct δ values of both legume and reference crop as follow:

$$\delta^{15}\text{N corrected} = (\text{total N accumulation x } \delta^{15}\text{N})/(\text{total N accumulation - N seeds}) \quad [6]$$

Obviously, when legume and/or reference plants are not seeded, it would be impossible to correct δ values for N arising from seeds. In our practical experience the difference between original and corrected *Ndfa* of hairy vetch grown as sole crop or intercropped with rye was negligible, as shown by the very little value of the intercept, and it did not differ among treatments (see Figure 6 in the Appendix).

If there is evidence for an underestimation of $N_2$ fixation an attempt can be done to correct *Ndfa* with below-ground N before going back to look at the whole process and repeat sampling and/or part of the analysis. As above mentioned, nowadays below-ground is estimated to be of great importance in legume N budget, accounting for up to 50% of total N accumulation. Stated the difficulties in root sampling, Peoples et al. (2009b) suggest a practical data correction, i.e. multiplying the estimated amount of fixed N with a factor of 2 for fodder/pasture legumes and chick-pea, 1.5 for soybean and 1.4 for all the remaining species. Anyway, as discussed above, a great uncertainty is undertaken with estimation of N dynamics in the rhizosphere, therefore this approach should be followed only to provide more information on the behavior of legumes within cropping systems, and not to build new levels of potential $N_2$-fixation. For instance, from our experience a correction factor of 1.4 produced an overestimation of $N_2$ fixation of hairy vetch resulting in a *Ndfa%* bigger than 100% of total N accumulated by the legume.

### L. *Data presentation*

In general terms, it must be avoided to present only final *Ndfa* estimations in order to give the reader the opportunity to go through the data and to have a more precise overview of range of variability and measures of error.
The best way to do this can be to present in a unique table δ values both for reference and legume crops, and for these latter also the computed %*Ndfa* should be shown. For intercropping data, in this way also a comparison between δ values of intercropped and sole cropped reference can be performable, leading the reader to make some hypotheses about a potential N transfer and the level of competition for soil N in intercropping plots (see Table 3 in the Appendix).

A diagram can be an alternative to present data of accumulated N share in terms of mass units of N per area units (e.g. g N $m^{-2}$), derived from atmosphere and soil, respectively (see Figures 7-8 in the Appendix). In this way, a comparison between total N accumulation by plants and the importance of different N sources can be done simultaneously.

As total N accumulation may be strongly affected by sampled biomass and N concentration, also a table with those original data is recommended, in order to exclude any potential sampling/analysis errors (see Table 4 in the Appendix).

*M. Integration of results with additional data*

Because natural abundance technique only provides estimations of $N_2$-fixation including a number of assumptions, $^{15}N$ data should be integrated with some additional information. Peoples et al. (2009b) suggest to consider the growing conditions of plants, pest/disease/weed occurrence and seriousness, weather conditions, cropping history, and, above all, the status of legume root nodulation, rooting patterns and soil N content and dynamics. By integrating all these information it would be possible to have all the necessary tools to identify possible real outliers within the dataset, leading to a comprehensive verification of the estimated levels of biological $N_2$ fixation and a correct interpretation of their effects on the cropping system.

## 4. Legume-based cropping systems for CC mitigation

In the long-term, when fossil oil is predicted to be scarcely available, the price and the availability of mineral fertilizers are expected to be, respectively, higher and lower than currently. Therefore cropping systems will have to be designed emphasizing nutrient conservation and self-sufficiency. Obviously, biological $N_2$ fixation will be extremely relevant for nitrogen management. At that time, a simple implementation of what we will already know would be insufficient, thus the next approach to increasing biological $N_2$-fixation will be research and implementation of alternative tools. As these solutions will be differently available in time, the full replacement of fertilizers with $N_2$-fixing legumes should be considered as a progressive action, where also research and technology development will play an important role ((Crews and Peoples, 2004)). Here we will try to define some advanced technical solutions for including more efficiently legumes in crop rotations based on currently available technologies and knowledge.

What we believe is in the long run a reductionist approach based on the adoption of novel technologies like those described within paragraph 2.3 will be extremely constrained to face a climate and a world which are simultaneously changing so fast. Diversification of cropping systems will be the real key factor for making consistent advances in growing legumes and providing endurability to such new model of agriculture. Highly diversified cropping systems, where as many as possible different crop species and varieties are included, can make agroecosystems very resistant against environmental stresses (e.g. a warmer and drier climate) and offer a sort of insurance to farmers with regard to fluctuating weather events, market dynamics and pest/disease occurrence, that are all expected to increase in the future ((Altieri, 1999)).

Diversification can be differently defined depending on: i) time, ii) space and iii) function.

### 4.1 Temporal diversification of legume-based cropping systems

Nowadays only few countries have crop rotations with an average cereals:legumes ratio lower than 10:1 ((Crews and Peoples, 2004)). Therefore legume portion in crop sequences should be increased to build cropping systems more diversified in time. Nevertheless, this option has to take into account an increasing food demand which is currently mainly based on cereal products. Therefore, an increase in legume cultivated area should not be performed to a significant detriment of cereal production, and this can be possible by increasing the cultivation of pulses not only as main crops but also as cover crops to be grown between two other crops or mixed with them.

Soil cover that legumes can produce in even short periods, such as from autumn to spring, consequently provides many environmental and agronomical benefits which are well documented to positively affect the productivity of the subsequent crops and the environmental quality of cropping systems ((Cherr et al., 2006)). On the other side, legume cover crops has some major constraints in the high requirement of fossil energy for their devitalization, the often relevant N losses after their incorporation into the soil and their low implementation during summer in low latitudes, due to the scarce availability of drought-resistant varieties.

As well as from genetical improvement, important solutions to these limitations may come from the adoption of particular conservative techniques aiming at combining advantages of no tillage (e.g. water retention, strong reduction of $CO_2$ emission from soil respiration) with a reduction in N losses and energetic/economical costs due to cover crop management. For instance, a promising technique seems to be the simultaneous devitalization of a legume cover crop at the beginning of flowering stage, performed by mean of a crimper roller and a reduced/null dose of herbicide, and the sod-seeding of a spring main crop, such as sunflower (Figure 12). With some important exceptions for weakly structured soils and less diversified crop rotations, such conservative strategy could be implemented in the future also in organic farming if non-chemical effective tools to reduce weed pressure have been made available ((Peigné et al., 2007)).

*Figure 12 - Sod-seeding of sunflower (Helianthus annuus L.) combined with a mechanical devitalization of a hairy vetch (Vicia villosa Roth) cover crop*
*(Pictures from an on-farm experiment in Lorenzana, Pisa)*

For legumes grown as food crops (e.g. grain crops), diversification may lead to an increased cultivation at different times of the year, for instance alternating winter grown (e.g. faba bean) with summer grown species (e.g. soybean), thus modifying usual dynamics of soil N pool, improving the N-budget of more than one subsequent crop and producing a break crop effect on weeds, pests and diseases.

With the increasing duration of the vegetative season likely to occur in future climate at many different latitudes, also a double cropping of legumes can be performed by rotating more than one legume species/variety in the same season, thus emphasizing natural resource use efficiency of legume cultivation ((Francis, 1986)).

### 4.2 Spatial diversification of legume-based cropping systems

Intercropping is the traditional technique adopted by farmers to increase the spatial diversification of cropping systems through the cultivation on the same field of different species, e.g. a cereal and a legume (*interspecific intercropping*), or different varieties of the same crop (*mixed populations*) ((Willey, 1979)). Both strategies of intercropping have the potential to achieve many different goals of crop production, ranging from an increased land use efficiency ((Fukai and Trenbath, 1993)) to an improved quality of crop products ((Ghaley et al., 2005)) and a reduction of crop damages caused by biological agents ((Trenbath, 1993)). Moreover, intercropping is one of the most dynamic tools for farmers for including legumes into cropping systems, as they can differently set the occurrence and the portion of legumes within the mixtures in function of the climate conditions they have to face year by year.

Interspecific intercropping may play a major role in the future for integration of legumes in crop rotations, allowing farmers to grow more crops per area unit and to shorten the time required for cultivation of crops without direct economical income, such as fertility-building legumes. A special kind of intercropping is when a legume is included in an intercropping design with the unique aim to provide benefits to the companion crop, namely the main crop (*facilitative intercropping*). Generally this is performed by devitalizing the legume at an intermediate growing stage either plowing it under the soil (e.g. for supplying more N to the companion crop) or leaving it on the surface as a dead mulch (e.g. for contrasting weeds). Both strategies are defined as *temporary intercropping*.

If contemporary sowing of companion crops can be seen as easier to perform by farmers and less time-consuming, the alternative of under-sowing legumes in an already established main crop seems to be globally more sustainable under a CC perspective, since it can reduce the fallow period preceding the plantation date of the subsequent crop, thus increasing C sequestration and N retention in the soil, whilst reducing soil respiration and $N_2O$ emission. Within this strategy, biennial legume species, such as for instance red clover (*Trifolium pratense* L.), can be extremely effective, as they can enlarge at most soil cover. We can provide two different examples, the first one suitable for a Northern European country like Denmark, where spring-sown cereals and legumes are available options due to the humid condition of summer, and the second one more useful for a Mediterranean country like Italy.

At higher latitude an exemplar solution may be the one starting from the under-sowing of an annual legume in a winter-sown cereal at the beginning of the stem elongation stage. After the harvest of the cereal, the legume can be left growing until the end of summer, when it can be plowed down as a green manure for the subsequent catch crop (e.g. an other winter cereal or a *Brassica* spp.) aimed at sequestering nitrates exceeding in the soil after legume cultivation. In the following spring a biennial legume could be under-seeded within the catch crop and left growing for one year before to be plowed under or cut and left on soil surface as dead mulch in favor of the subsequent summer crop.

Alternatively, instead to letting the catch crop grow until harvest time in the second year, an ulterior option could be to incorporate it into the soil as a green manure before the contemporary seeding of a grain legume/spring cereal intercropping which will be harvested at the end of summer.

In Italy integration between legume cultivation and crop rotations may pass through designing of cropping systems mainly based on winter grown crops, due to the dry conditions of summer which do not allow farmers to grow many spring-sown crops. For instance, a N-rich legume (e.g. hairy vetch) may be seeded in autumn following the harvest of a summer crop like sunflower and then incorporated as a green manure in spring in favor of a summer crop with high N demand like maize. After the harvest of maize a winter cereal could be sown in order to fully exploit all the residual N coming from the mineralization of hairy vetch biomass. At the beginning of its stem elongation stage an intercropping may be established by under-sowing a biennial legume to the cereal and leaving it growing after cereal had been harvested until the seeding time of the subsequent summer crop. If incorporation into the soil has not been performed, the red clover biomass would alternatively provide an effective dead mulch where to sodseed the summer crop preventing high weed pressure and losses of C and N from an intensive mineralization which would otherwise occur after a conventional tillage (i.e. deep plowing) in such conditions.

Moreover, all the potential benefits coming from intercropping could be enhanced by combining interspecific intercropping with mixed populations (i.e. different species or varieties) either of legumes and/or their companion crops, making cropping systems more stable towards environmental stresses, with particular emphasis on resistance to pests, diseases and weather extremes. But in this case a major restriction may come from market acceptance of the final product, made from a mixture of different crop products, whilst for standard intercropping such as the one between one cereal and one legume, a simple post-harvest sieving of grain differing in size can solve the problem.

An ulterior constraint of intercropping, especially for temporary intercropping, is the practicability of mechanical field operations requiring more specific machines, higher practical skill by farmers, and being higher time-consuming than sole cropping. Addressing also this need, an advanced option recently included in agronomical research is the so called "*strip-intercropping*" consisting in the cultivation of intercropping in small strips repeated within a field and separated from each other from strips of the sole cropped main crop (Figure 13).

*Figure 13 - A rye-hairy vetch intercropping strip alternated with a sole cropped white clover*
*(Pictures from Bioconcens trial at Risø DTU, Denmark)*

Strips are generally as wide as the required machines for their management (e.g. harrows, harvesting machines) are. Such technique is aimed at minimizing labour and time cost required by intercropping whilst saving its benefits in terms of diversification of cropping systems, mostly concentrated in the interface between intercropped and sole cropped strips.

### 4.3 Functional diversification of legume-based cropping systems

As well pointed out by Altieri (1999), biodiversity does not consist only of genetical but also of functional diversity, that is the different ecological function provided by the living organisms within the ecosystem.

Above we have discussed some examples of inclusion of legumes within crop rotations. Furthermore it has to be noticed that what really can make a difference is the number of different services provided by the legumes and not the legume species themselves. For instance, the great role of biennial pulses like red clover is mainly due to their multiple functions coming in series over time (i.e. soil cover, green manure, dead mulch) and not simply to their persistence on the field for a variably large period. In the same way the final destination of legumes (food/cover/fodder/energy), strongly influences their effect on the global diversity of cropping systems by differently affecting N benefits, weed suppression and carbon return to the soil. For instance, a legume grown for grain production can have only a limited effect on N budget of cropping systems as most N is harvested with seeds. On the other side, a legume grown for biogas production through biodigestion of residues can be very useful in terms of N supply if the by-products of the processes are returned into the soil as stable organic matter rich in N (i.e. as digested slurries), whilst they can provide only a weak benefit for soil C storage.

Besides crop rotation, diversification can be achieved also using legumes for populating some ecological infrastructures, such as for instance buffer strips, hedgerows and flower strips, which can be grown around the fields or even in the middle of them according to the aim of their establishment, possibly ranging from creating a corridor of vegetation between a natural space and cultivated fields, to placing some trap crops for protecting main crop by the attack of pests, insects and diseases or attracting some beneficial organisms close to the fields ((Altieri, 1999)).

Legumes may contribute also to increase biodiversity in extremely specialized cropping systems like tree plantations (e.g. vineyards, olive-yards), where they can be part of agroforestry complex systems, for instance as component of stripped cover crops alternated to tree rows. Such technique is not really so advanced, as it was traditionally performed by farmers until some decades ago and now it has become to be again considered for organic and integrated production systems. The main benefits such legume strips can provide are the release into the soil of biologically fixed N, soil cover and protection from erosion, weed suppression, attraction of beneficials and field practicability for machines.

### 4.4 Current restrictions for legume-based cropping systems

In Europe, a revolution towards an agriculture based on renewable sources of nitrogen is currently constrained mainly by economical limitations. First, current market trends favor cereal productions in spite of very low market prices due to the high demand for cereals by food and feed-stuff industry, whilst legume products usually are imported from North and South America, where cultivated land is larger and market prices are lower than in Europe. Among legumes only few species, especially soybean, are requested by the market, whilst minor species have only a low demand by feed-stuff industry due to the lower investments in breeding and crop protection, thereby reducing the profitability for farmers operating in areas unsuitable for soybean. A reduction in animal production would reduce the importance of cereals and enhance that of local protein crops expected to replace part of meat intake in human diet.

Furthermore, the current price system is based only on economical issues of agricultural facilities and it does not take into account their contribution to environmental pollution and climate change. In such context mineral fertilizers are still considered to be cheaper than legumes. If also the global warming potential is included in the price of fertilizers as an additional cost for famers, their profitability would become dramatically lower than for legumes. Obviously, this action is a recommended task for policy makers.

From a technical point of view, a significant increase of legume inclusion within cropping systems needs strong efforts by advisors in training and education of farmers currently only skilled in cereal production. A special care should be also paid to monitoring the actual effects of legume-based systems on the environment and their contribution to climate change mitigation. Subsidies should be granted by governments to help farmers to pay advisors and, on the other side, to allow advisors themselves to update their knowledge with novel findings provided by agronomical research.

## 5. Conclusions

In this report we have stated in the long term a new model of agriculture including more legumes can be possible from a technical point of view and extremely successful in terms of CC mitigation. The restrictions and constraints currently shown by legume-based cropping systems can be progressively overcome simply by exploiting all the novel technologies and the newest information coming from agronomical and biological research. Efficiency will be the key word for agriculture in the future, covering either the fine-tuning of farm practices and the monitoring of their effects on the environment.

Nevertheless, farmers are by definition a link between the human society and ecosystems. Therefore, the practices they implement can only be effective in terms of environmental sustainability and CC mitigation only if also the industry based on agricultural products and the behavior of end-users of food and no-food commodities is efficient as well. Likewise, a deeper comprehension of natural processes involved in agricultural production should be achieved for making this system approach feasible.

## References

Altieri, M. A. (1999). The ecological role of biodiversity in agroecosystems. Agriculture, Ecosystems & Environment, 74(1-3): 19-31.

Atkins, C. (1984). Efficiencies and inefficiencies in the legume/Rhizobium symbiosis—A review. Plant and Soil, 82(3): 273-284.

Baffes, J. (2007). Oil spills on other commodities. Resources Policy, 32(3): 126-134.

Bedard-Haughn, A., J. W. van Groenigen and C. van Kessel (2003). Tracing 15N through landscapes: potential uses and precautions. Journal of Hydrology, 272(1-4): 175-190.

Campo, R. J., R. S. Araujo and M. Hungria (2009). Molybdenum-enriched soybean seeds enhance N accumulation, seed yield, and seed protein content in Brazil. Field Crops Research, 110(3): 219-224.

Carter, M. and P. Ambus (2006). Biologically Fixed N2 as a Source for N2O Production in a Grass–clover Mixture, Measured by 15N2. Nutrient Cycling in Agroecosystems, 74(1): 13-26.

Chalk, P. M. (1998). Dynamics of biologically fixed N in legume-cereal rotations: A review. Australian Journal of Agricultural Research, 49: 303.

Cherr, C. M., J. M. S. Scholberg and R. McSorley (2006). Green manure approaches to crop production: A synthesis. Agronomy Journal, 98(2): 302-319.

Crews, T. E. and M. B. Peoples (2004). Legume versus fertilizer sources of nitrogen: ecological tradeoffs and human needs. Agriculture, Ecosystems & Environment, 102(3): 279-297.

EEA (2009). Greenhouse gas emission trends and projections in Europe 2009. Tracking progress towards Kyoto targets. EUROPEAN ENVIRONMENTAL AGENCY Report 09/2009. Copenhagen, 2009. Available for download at http://www.eea.europa.eu/publications/eea_report_2009_9.

Erisman, J. W., M. A. Sutton, J. Galloway, Z. Klimont and W. Winiwarter (2008). How a century of ammonia synthesis changed the world. Nature Geosci, 1(10): 636-639.

FAO (2009). FAO profile for climate change. FOOD AND AGRICULTURE ORGANIZATION OF THE UNITED NATIONS, Rome, 2009. Available for download at http://www.fao.org/docrep/012/i1323e/i1323e00.htm.

FAOSTAT (2009). FAO Statistical Databases and Datasets. Available at: http://faostat.fao.org/site/291/default.aspx.

Francis, C. A. (1986). Distribution and importance of multiple cropping systems. New York, Macmillan.

French, S., D. Levy-Booth, A. Samarajeewa, K. E. Shannon, J. Smith and J. T. Trevors (2009). Elevated temperatures and carbon dioxide concentrations: effects on selected microbial activities in temperate agricultural soils. World Journal of Microbiology and Biotechnology, 25: 1887-1900.

Frye, W. W., Blevins, R. L., Smith, M. S., Corak, S. J., Varco, J. J. (1988). Role of annual legume cover crops in efficient use of water and nitrogen. Cropping Strategies for Efficient Use of Water and Nitrogen, Special Pubblication, 51: 129-154.

Fukai, S. (1993). Intercropping -- bases of productivity. Field Crops Research, 34(3-4): 239-245.

Fukai, S. and B. R. Trenbath (1993). Processes determining intercrop productivity and yields of component crops. Field Crops Research, 34(3-4): 247-271.

Garten, C. T., A. T. Classen, R. J. Norby, D. J. Brice, J. F. Weltzin and L. Souza (2008). Role of N-2-fixation in constructed old-field communities under different regimes of [CO2], temperature, and water availability. Ecosystems, 11(1): 125-137.

Ghaley, B., H. Hauggaard-Nielsen, H. Høgh-Jensen and E. Jensen (2005). Intercropping of Wheat and Pea as Influenced by Nitrogen Fertilization. Nutrient Cycling in Agroecosystems, 73(2): 201-212.

Hanson, J. D., M. A. Liebig, S. D. Merrill, D. L. Tanaka, J. M. Krupinsky and D. E. Stott (2007). Dynamic Cropping Systems: Increasing Adaptability Amid an Uncertain Future. Agronomy Journal, 99(4): 939-943.

Hardarson, G., F. Zapata and S. K. A. Danso (1988). Dinitrogen Fixation Measurements in Alfalfa-Ryegrass Swards Using Nitrogen-15 and Influence of the Reference Crop. Crop Science, 28(1): 101-105.

Hauck, R. D., W. V. Bartholomew, J. M. Bremner, F. E. Broadbent, H. H. Cheng, A. P. Edwards, D. R. Keeney, J. O. Legg, S. R. Olsen, L. K. Porter, D. H. Kohl, G. B. Shearer and B. Commoner (1972). Use of Variations in Natural Nitrogen Isotope Abundance for Environmental Studies: A Questionable Approach. Science, 177(4047): 453-456.

Hauggaard-Nielsen, H., L. Holdensen, D. Wulfsohn and E. Jensen (2010). Spatial variation of N2-fixation in field pea (Pisum sativum L.) at the field scale determined by the 15N natural abundance method. Plant and Soil, 327(1): 167-184.

Herridge, D., R. Roughley and J. Brockwell (1984). Effect of rhizobia and soil nitrate on the establishment and functioning of the soybean symbiosis in the field. Australian Journal of Agricultural Research, 35(2): 149-161.

Högberg, P. (1997). Tansley Review No. 95. 15N Natural Abundance in Soil-Plant Systems. New Phytologist, 137(2): 179-203.

Houlton, B. Z., Y.-P. Wang, P. M. Vitousek and C. B. Field (2008). A unifying framework for dinitrogen fixation in the terrestrial biosphere. Nature, 454(7202): 327-330.

IFA Statistics (2009). International Fertilizer Industry Association Statistics. Available at: http://www.fertilizer.org/ifa/Home-Page/STATISTICS.

IPCC (2007a). Climate change 2007: Synthesis Report. Contribution of Working Groups I, II and III to the Fourth Assessment Report of the Intergovernmental Panel on Climate Change. Core Writing Team, Pachauri, R.K. and Reisinger, A. (Eds.) IPCC, Geneva, Switzerland. pp. 104.

IPCC (2007b). Climate Change 2007: Impacts, Adaptation and Vulnerability. Contribution of Working Group II to the Fourth Assessment Report of the Intergovernmental Panel on Climate Change. M.L. Parry, O.F. Canziani, J.P. Palutikof, P.J. van der Linden and C.E. Hanson, Eds. Cambridge University Press, Cambridge, UK, 976 pp.

IPCC (2007c). Climate Change 2007: Mitigation of Climate Change. Contribution of Working Group III to the Fourth Assessment Report of the Intergovernmental Panel on Climate Change, 2007. B. Metz, O.R. Davidson, P.R. Bosch, R. Dave, L.A. Meyer (eds) Cambridge University Press, Cambridge, United Kingdom and New York, NY, USA.

Jensen, E. S. (1996). Grain yield, symbiotic N2 fixation and interspecific competition for inorganic N in pea-barley intercrops. Plant and Soil, 182(1): 25-38.

Jensen, E. S. and H. Hauggaard-Nielsen (2003). How can increased use of biological N2 fixation in agriculture benefit the environment? Plant and Soil, 252(1): 177-186.

Jensen, E. S., A. J. Andersen and J. D. Thomsen (1985). The influence of seed-borne nitrogen in nitrogen-15 isotope dilution studies with legumes. Acta Agriculturae Scandinavica, 35 (4): 438 - 443.

Kirkegaard, J., O. Christen, J. Krupinsky and D. Layzell (2008). Break crop benefits in temperate wheat production. Field Crops Research, 107(3): 185-195.

Kopke, U. and T. Nemecek (2010). Ecological services of faba bean. Field Crops Research, 115 (3): 217-233.

Lal, R. (2004). Soil Carbon Sequestration Impacts on Global Climate Change and Food Security. Science, 304(5677): 1623-1627.

Li, Y., W. Ran, R. Zhang, S. Sun and G. Xu (2009). Facilitated legume nodulation, phosphate uptake and nitrogen transfer by arbuscular inoculation in an upland rice and mung bean intercropping system. Plant and Soil, 315(1): 285-296.

Martens, J. R. T., J. W. Hoeppner and M. H. Entz (2001). Legume cover crops with winter cereals in southern Manitoba: Establishment, productivity, and microclimate effects. Agronomy Journal, 93(5): 1086-1096.

Mathews, J. A. (2008). Carbon-negative biofuels. Energy Policy, 36(3): 940-945.

Moonen, A. C. and P. Barberi (2004). Size and composition of the weed seedbank after 7 years of different cover-crop-maize management systems. Weed Research, 44(3): 163-177.

Niggli, U., Fließbach, A., Hepperly, P. and Scialabba, N. (2009). Low Greenhouse Gas Agriculture: Mitigation and Adaptation Potential of Sustainable Farming Systems. FAO, April 2009, Rev. 2 – 2009.

Palmer, R., P. Perez, E. Ortiz-Perez, F. Maalouf and M. Suso (2009). The role of crop-pollinator relationships in breeding for pollinator-friendly legumes: from a breeding perspective. Euphytica, 170(1): 35-52.

Peigné, J., B. C. Ball, J. Roger-Estrade and C. David (2007). Is conservation tillage suitable for organic farming? A review. Soil Use and Management, 23(2): 129-144.

Peoples, M. B., D. F. Herridge and J. K. Ladha (1995). Biological nitrogen fixation: An efficient source of nitrogen for sustainable agricultural production? Plant and Soil, 174(1): 3-28.

Peoples, M. B., G. L. Turner, Z. Shah, S. H. Shah, M. Aslam, S. Ali, S. L. Maskey, S. Bhattarai, F. Afandi, G. D. Schwenke and D. F. Herridge (1997). Evaluation of the 15N natural abundance technique to measure N2 fixation in experimental plots and farmers' fields. International workshop on managing legume nitrogen fixation in the cropping systems of Asia, Andhra Pradesh, India, International Crops Research Institute for the Semi-Arid Tropics.

Peoples, M. B., H. Hauggaard-Nielsen and E. S. Jensen (2009a). The potential environmental benefits and risks derived from legumes in rotations. Nitrogen fixation in crop production. D. W. a. K. Emerich, H. B. 677 South Segoe Road, Madison, WI 53711-1086 USA, American Society of Agronomy, Inc. Crop Science Society of America, Inc. Soil Science Society of America, Inc.: 349-385.

Peoples, M. B., J. K. Ladha and D. F. Herridge (1995). Enhancing legume N2 fixation through plant and soil management. Plant and Soil, 174(1): 83-101.

Peoples, M. B., K. E. Giller, D. F. Herridge, J. K. Vessey and T. M. Finan (2002). Limitations to biological nitrogen fixation as a renewable source of nitrogen for agriculture. Nitrogen fixation: Global perspectives, CABI Publishing.

Peoples, M. B., M. J. Unkovich and D. F. Herridge (2009b). Measuring symbiotic nitrogen fixation by legumes. Nitrogen fixation in crop production. D. W. a. K. Emerich, H. B. 677 South Segoe Road, Madison, WI 53711-1086 USA, American Society of Agronomy, Inc. Crop Science Society of America, Inc. Soil Science Society of America, Inc.: 125-170.

Pimentel, D. (1996). Green revolution agriculture and chemical hazards. Science of the Total Environment, 188(Supplement): S86-S98.

Pimentel, D., P. Hepperly, J. Hanson, D. Douds and R. Seidel (2005). Environmental, Energetic, and Economic Comparisons of Organic and Conventional Farming Systems. BioScience, 55(7): 573-582.

Räisänen, J. (2007). How reliable are climate models? Tellus A, 59(1): 2-29.

Rochester, I. J., M. B. Peoples, N. R. Hulugalle, R. R. Gault and G. A. Constable (2001). Using legumes to enhance nitrogen fertility and improve soil condition in cotton cropping systems. Field Crops Research, 70(1): 27-41.

Saggar, S. (2010). Estimation of nitrous oxide emission from ecosystems and its mitigation technologies. Agriculture, Ecosystems & Environment, 136(3-4): 189-191.

Sainju, U. M., H. H. Schomberg, B. P. Singh, W. F. Whitehead, P. G. Tillman and S. L. Lachnicht-Weyers (2007). Cover crop effect on soil carbon fractions under conservation tillage cotton. Soil & Tillage Research, 96(1-2): 205-218.

Shearin, A. F., S. Chris Reberg-Horton and E. R. Gallandt (2009). Cover Crop Effects on the Activity-Density of the Weed Seed Predator Harpalus rufipes (Coleoptera: Carabidae). Weed Science, 56(3): 442-450.

Smil, V. (2001). Enriching the Earth, MIT Press, Cambridge.

Stehfest, E. and L. Bouwman (2006). N2O and NO emission from agricultural fields and soils under natural vegetation: summarizing available measurement data and modeling of global annual emissions. Nutrient Cycling in Agroecosystems, 74(3): 207-228.

Stehfest, E., L. Bouwman, D. P. v. Vuuren, M. G. J. d. Elzen, B. Eickhout and P. Kabat (2009). Climate benefits of changing diet. Climatic Change, 95: 83-102.

Stern, W. R. (1993). Nitrogen fixation and transfer in intercrop systems. Field Crops Research, 34(3-4): 335-356.

Stinner, W., K. Möller and G. Leithold (2008). Effects of biogas digestion of clover/grass-leys, cover crops and crop residues on nitrogen cycle and crop yield in organic stockless farming systems. European Journal of Agronomy, 29(2-3): 125-134.

Trenbath, B. R. (1993). Intercropping for the management of pests and diseases. Field Crops Research, 34(3-4): 381-405.

Unkovich, M. J. and J. S. Pate (2000). An appraisal of recent field measurements of symbiotic N2 fixation by annual legumes. Field Crops Research, 65(2-3): 211-228.

Unkovich, M. J., D. F. Herridge, M. B. Peoples, R. M. Boddey, G. Cadisch, K. E. Giller, B. Alves and P. Chalk (2008). Measuring plant-associated nitrogen fixation in agricultural systems. ACIAR Monography. C. o. Australia. Canberra, Australia. 136.

Unkovich, M. J., J. P. Pate, P. Sanford and E. L. Armstrong (1994). Potential precision of the δ15N natural abundance method in field estimates of nitrogen fixation by crop and pasture legumes in south-west Australia. Australian Journal of Agricultural Research, 45: 119-132.

Willey, R. W. (1979). Intercropping - Its importance and research needs. Field Crop Abstracts, 32: 1-10.

Willey, R. W. and M. R. Rao (1980). A Competitive Ratio for Quantifying Competition Between Intercrops. Experimental Agriculture, 16(02): 117-125.

# Appendix 3 - Appendix to the Report

## Suggested guidelines for appropriate estimations in $^{15}$N natural abundance technique: issues from our personal experience at Risø National Lab for Sustainable Energy (25th January - 24th March, 2010)

*Figure 1 - Physical separation of mills used for different $^{15}$N techniques*
*(Picture from Risø DTU Biosystems Department)*

*Figure 2 - From left to right: precision scale linked to PC, tin cups, firmly closed tin cups, final result*
*(Pictures from Risø DTU Biosystems Department)*

*Figure 3 - CHN Elemental analyzer (on the right) and mass spectrometer (on the left)*
*(Picture from Risø DTU Biosystems Department)*

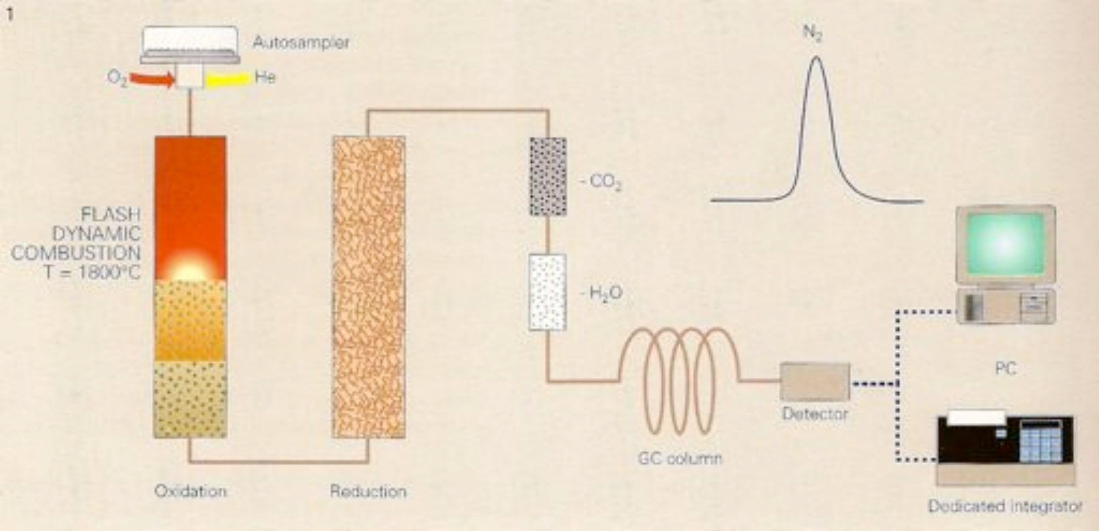


Figure 4 - Flash dynamic combustion in a Carlo Erba® NA-1500 Series-2 EA

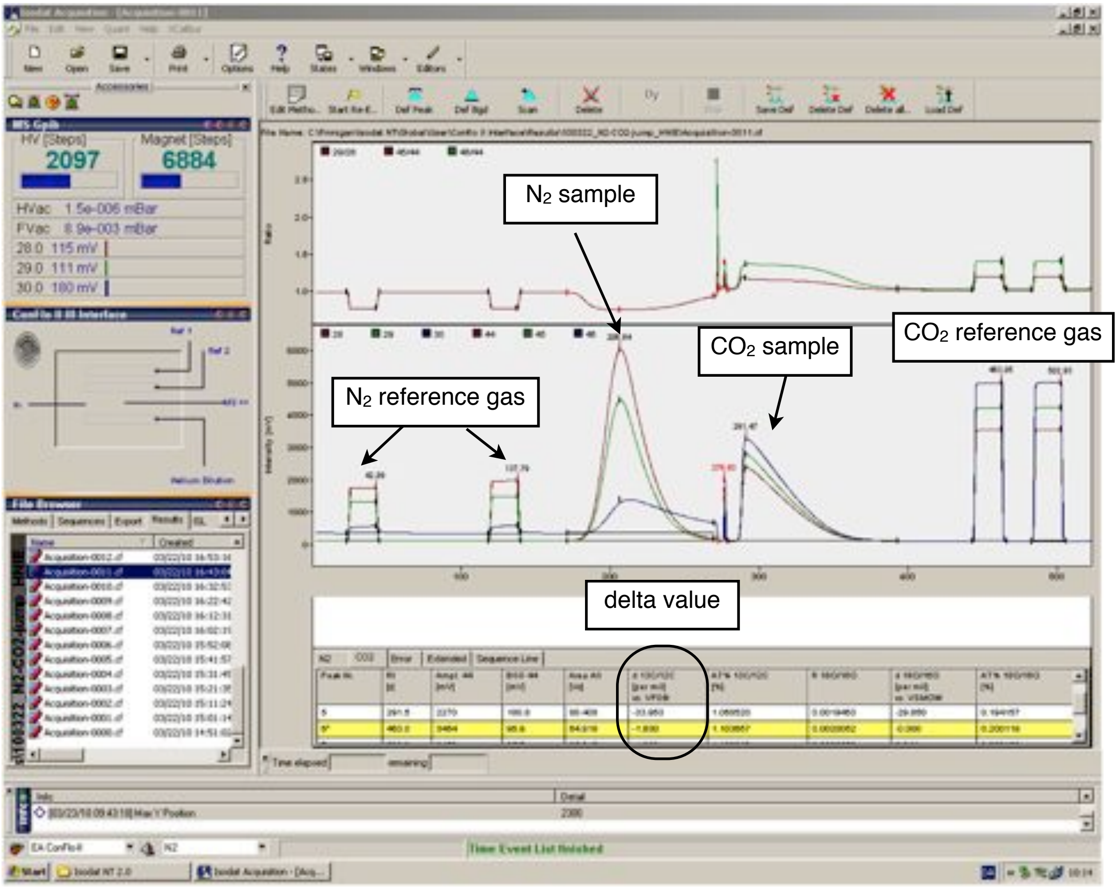


Figure 5 - Screen capture of MS results after the whole run of samples and standards
(Picture from Risø DTU Biosystems Department)

| **DISTANCE[h] (cm)** | **CROP** | | | *Mean* | **% OF VETCH IN** | | **LER** | | |
|---|---|---|---|---|---|---|---|---|---|
| | **RMC[a]** | **IC[b]** | **VMC[c]** | | **IC** | **Comb. MC[d]** | **IC** | **RIC[e]** | **VIC[f]** |
| 0-25 | 518.40 | 466.70 | 467.70 | *484.27 c* | 27 | 47 | 0.93 | 0.66 | 0.27 |
| 25-50 | 938.30 | 975.10 | 393.75 | *769.05 b* | 11 | 30 | 1.19 | 0.93 | 0.27 |
| 50-75 | 1170.65 | 1127.30 | 572.65 | *956.87 a* | 13 | 33 | 1.10 | 0.84 | 0.26 |
| >150 | 1137.85 | 1092.95 | 745.60 | *992.13 a* | 14 | 40 | 1.03 | 0.83 | 0.20 |
| *Mean* | *941.30 a* | *915.51 a* | *544.93 b* | | *16* | *37* | *1.06* | *0.81* | *0.25* |
| LSD | 223.52 | | | 173.55 | | | | | |
| CV[g] | 25.88% | | | | | | | | |

Table 1 - Aboveground dry weight production (g $m^{-2}$) of vetch and rye grown in mono- and intercrops (24/07/2008). Data from Bioconcens trial at Risø DTU, Denmark

[a] Rye Monocropping; [b] Rye-Vetch Intercropping; [c] Vetch Monocropping; [d] Combined Monocropping = [VMC/(VMC+RMC)]*100; [e] Rye grown in Intercropping; [f] Vetch grown in Intercropping; [g] Coefficient of variation (%)

[h] Distance of sampling areas from red clover strip

| **DISTANCE[h] (cm)** | **CROP** | | | *Mean* | **% OF VETCH IN** | | **LER** | | |
|---|---|---|---|---|---|---|---|---|---|
| | **RMC[a]** | **IC[b]** | **VMC[c]** | | **IC** | **Comb. MC[d]** | **IC** | **RIC[e]** | **VIC[f]** |
| 0-25 | 3.06 | 4.89 | 9.42 | *5.79 b* | 60 | 76 | 0.95 | 0.64 | 0.31 |
| 25-50 | 5.02 | 7.21 | 7.92 | *6.72 b* | 31 | 61 | 1.27 | 0.99 | 0.28 |
| 50-75 | 6.51 | 9.14 | 11.30 | *8.98 a* | 38 | 63 | 1.18 | 0.87 | 0.31 |
| >150 | 6.83 | 9.20 | 14.81 | *10.28 a* | 40 | 68 | 0.82 | 0.81 | 0.25 |
| *Mean* | *5.36 b* | *7.61 b* | *10.86 a* | | *42* | *67* | *1.05* | *0.29* | *0.83* |
| LSD | 2.55 | | | 1.63 | | | | | |
| CV[g] | 24.51% | | | | | | | | |

Table 2 - Aboveground N accumulation (g N $m^{-2}$) of vetch and rye grown in mono- and intercrops (24/07/2008).
Data from Bioconcens trial at Risø DTU, Denmark

[a] Rye Monocropping; [b] Rye-Vetch Intercropping; [c] Vetch Monocropping; [d] Combined Monocropping = [VMC/(VMC+RMC)]*100; [e] Rye grown in Intercropping; [f] Vetch grown in Intercropping; [g] Coefficient of variation (%)

[h] Distance of sampling areas from red clover strip

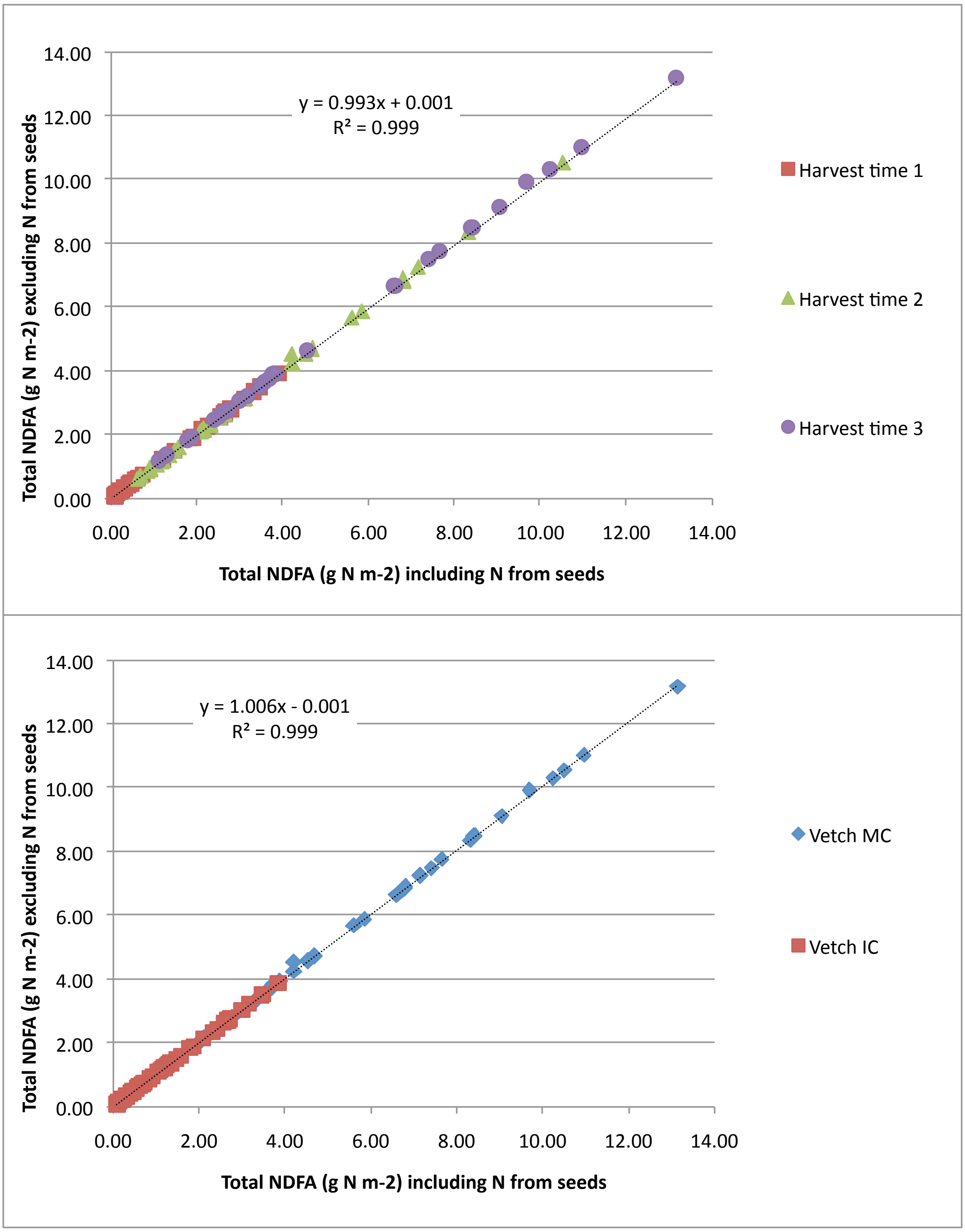


Figure 6 - Regression analysis of original and corrected *Ndfa* of hairy vetch split by sampling date (on the top) and treatment (on the bottom). Data from Bioconcens trial at Risø DTU, Denmark

| **Crop** | **Distance (cm)** | **δ $^{15}$N** | **δ $^{15}$N reference crop** | **Legume Ndfa (%)** |
|---|---|---|---|---|
| RMC[a] | 0-25 | 2.41 | 2.41 | - |
| | 25-50 | 3.16 | 3.16 | - |
| | 50-75 | 3.02 | 3.02 | - |
| | >150 | 3.88 | 3.88 | - |
| | *Mean* | *3.12 a* | *3.12* | - |
| RIC[b] | 0-25 | 2.77 | 2.41 | - |
| | 25-50 | 3.49 | 3.16 | - |
| | 50-75 | 3.56 | 3.02 | - |
| | >150 | 3.77 | 3.88 | - |
| | *Mean* | *3.40 a* | *3.12* | - |
| VIC[c] | 0-25 | 0.05 | 2.41 | 81.98 |
| | 25-50 | -0.05 | 3.16 | 88.43 |
| | 50-75 | 0.03 | 3.02 | 85.61 |
| | >150 | 0.02 | 3.88 | 88.83 |
| | *Mean* | *0.01 c* | *3.12* | *86.21 a* |
| VMC[d] | 0-25 | 0.52 | 2.41 | 65.73 |
| | 25-50 | 0.55 | 3.16 | 71.88 |
| | 50-75 | 0.78 | 3.02 | 64.17 |
| | >150 | 0.83 | 3.88 | 70.02 |
| | *Mean* | *0.67 b* | *3.12* | *67.95 b* |
| | LSD | 1.32 | | 15.03 |
| | CV[e] | 49.19% | | 12.20% |

Table 3 - $^{15}$N delta values of vetch and rye grown in mono- and intercrops and vetch N derived from the atmosphere (Ndfa%) (24/07/2008).
Data from Bioconcens trial at Risø DTU, Denmark

[a] Rye Monocropping; [b] Rye grown in Intercropping; [c] Vetch grown in Intercropping; [d] Vetch Monocropping; [e] Coefficient of variation (%)

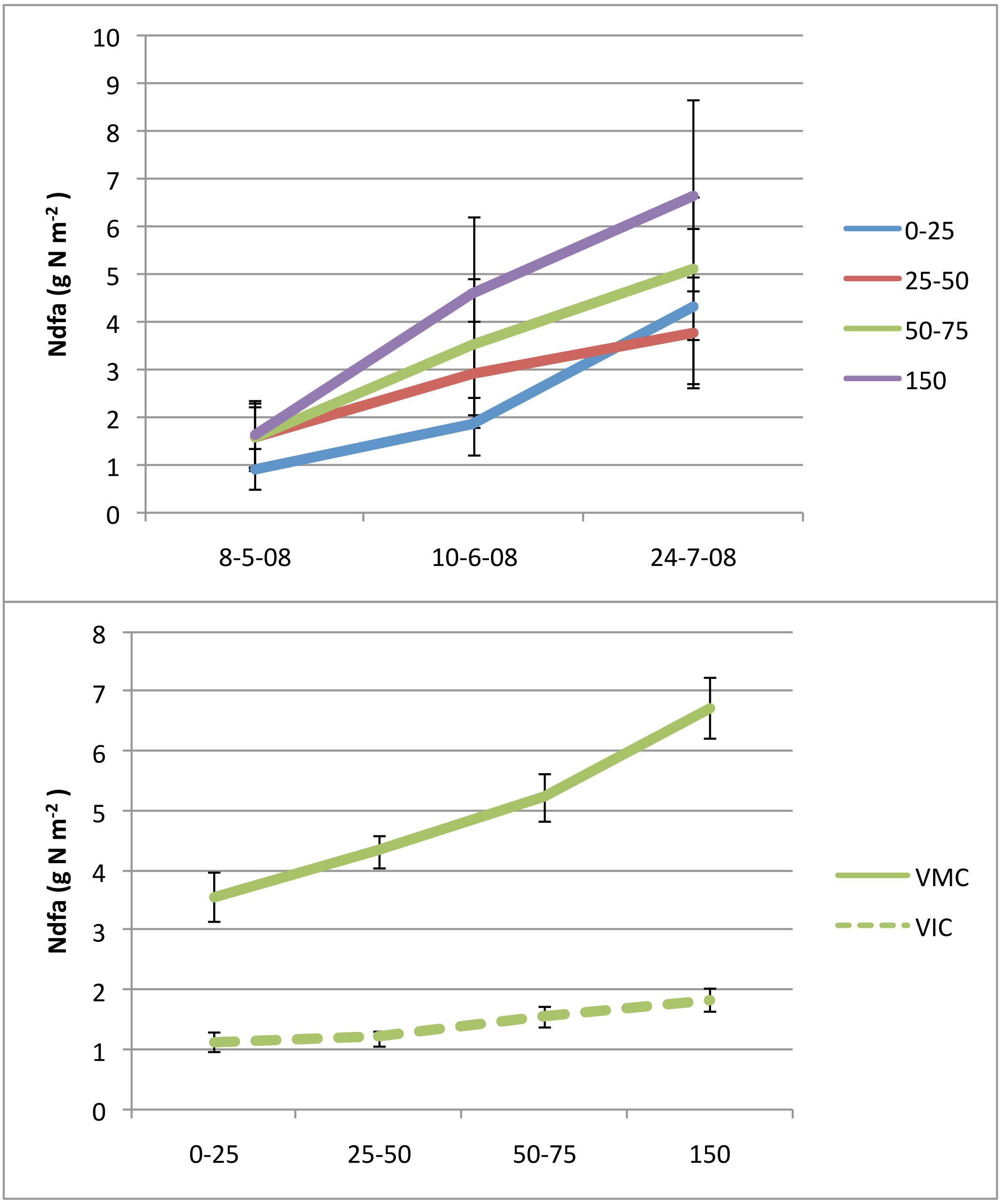


Figure 7 - a) Trend over time of Nitrogen derived from atmosphere (Ndfa) for hairy vetch sampled at different distances from monocropped strip, averaged over mono- and inter-cropping with rye. b) Trend over distance of vetch monocropping (VMC) and vetch intercropped with rye (VIC) Ndfa averaged over harvest times.
Data from Bioconcens trial at Risø DTU, Denmark

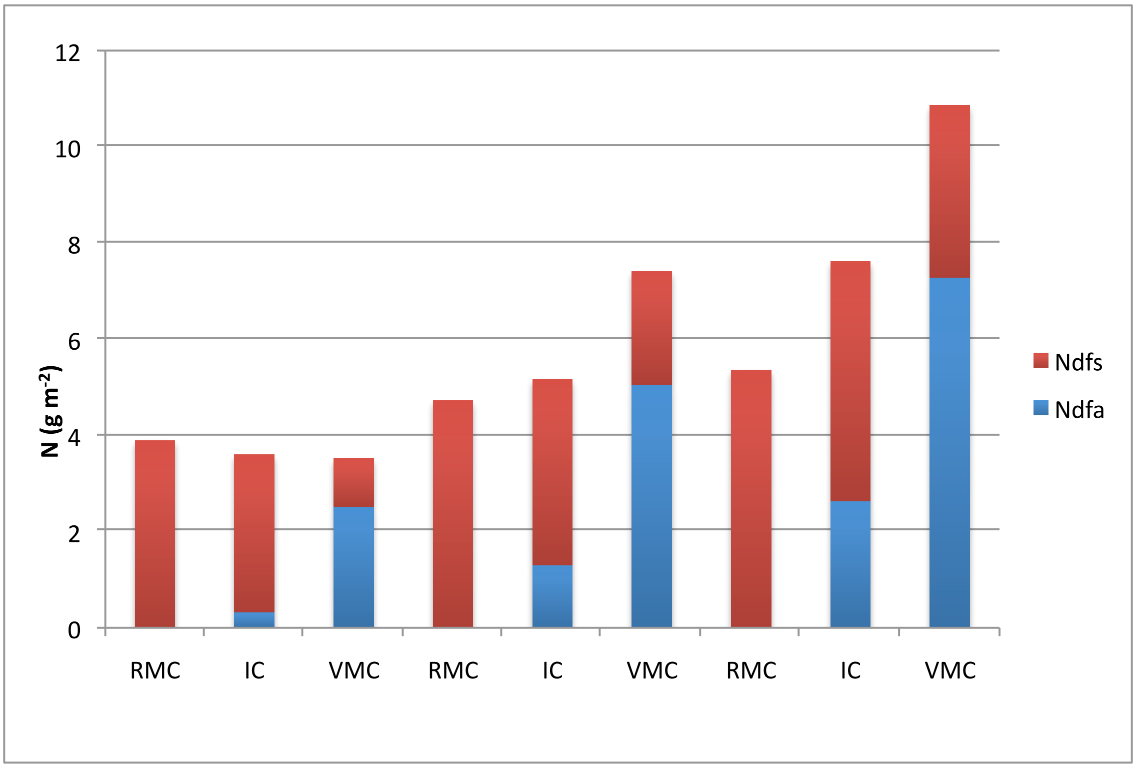


Figure 8 - Nitrogen derived from the atmosphere (Ndfa) and from the soil (Ndfs) of rye monocrop (RMC), rye-vetch intercropping (IC) and vetch monocropping (VMC) averaged over distances. Data from Bioconcens trial at Risø DTU, Denmark

| **Crop** | **Distance[b] (cm)** | **Aboveground DW production (g m$^{-2}$)** | **Aboveground N concentration (%)** | **Aboveground N accumulation (g N m$^{-2}$)** | **Ndfa (%)** | **Ndfa (g N m$^{-2}$)** |
|---|---|---|---|---|---|---|
| RMC | 0-25 | 518.40 | 0.63 | 3.27 | - | - |
| | 25-50 | 938.30 | 0.55 | 5.16 | - | - |
| | 50-75 | 1170.65 | 0.56 | 6.56 | - | - |
| | >150 | 1137.85 | 0.59 | 6.71 | - | - |
| | *Mean* | *941.30 a* | *0.58 c* | *5.46 b* | - | - |
| IC | 0-25 | 466.70 | 1.03 | 4.81 | 49.51 | 2.38 |
| | 25-50 | 975.10 | 0.75 | 7.31 | 26.94 | 1.97 |
| | 50-75 | 1127.30 | 0.83 | 9.36 | 31.74 | 2.97 |
| | >150 | 1092.95 | 0.85 | 9.29 | 34.77 | 3.23 |
| | *Mean* | *915.51 a* | *0.87 b* | *7.96 b* | *33.17 b* | *2.64 b* |
| VMC | 0-25 | 467.70 | 2.08 | 9.73 | 65.73 | 6.39 |
| | 25-50 | 393.75 | 1.99 | 7.84 | 71.88 | 5.63 |
| | 50-75 | 572.65 | 1.97 | 11.28 | 64.17 | 7.24 |
| | >150 | 745.60 | 1.99 | 14.84 | 70.02 | 10.39 |
| | *Mean* | *544.93 b* | *2.01 a* | *10.95 b* | *67.95 a* | *7.44 a* |
| | LSD | 223.52 | 0.08 | 2.55 | 15.03 | 4.29 |
| | CV[a] | 25.88% | 9.28% | 24.51% | 12.20% | 35.35% |

Table 4 - Aboveground dry weight production, N concentration, N accumulation, Ndfa (%, g N m$^{-2}$) of rye monocropping (RMC), rye-vetch intercropping (IC) and vetch monocropping (VMC) (24/07/2008). Data from Bioconcens trial at Risø DTU, Denmark

[a] Coefficient of variation (%); [b] Distance of sampling areas from red clover strip