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Growth, nitrogen uptake and maize nitrogen recovery of cover crops in conservation agriculture

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Preface

This PhD thesis comprises the research carried out in the Department of Agricultural and Environmental Sciences at the University of Milan. The thesis is organized in six chapters. The first Chapter is a general introduction that presents the state of art of cover crops. It summarizes the reviews and meta-analyses that document a number of cover crop effects: growth, nitrogen uptake, apparent nitrogen recovery and yield of the following cash crop, soil quality, weed and nitrogen emissions such as nitrogen leaching and nitrous oxide emissions.

We focused on winter cover crops that are sown between two main cash crops (from September to March). In our geographical area (Northern Italy), the use of cover crops is not common and we wanted to introduce this practice. The objective was to assess the winter-killed and winter-hardy cover crops growth and nitrogen uptake as influenced by plant species from three different botanical families and two sowing dates. In this context, we carried out a field experiment for two years and results are reported in Chapter 2.

After termination of cover crops, our objectives were to assess the effect of cover crops presence/absence (bare soil) on weed suppression and the productivity of the following cash crop. Results of this experiment are reported in Chapter 3.

From the cover crops and weed shoots grown in the field, we carried out a laboratory incubation experiment under controlled conditions of temperature and soil moisture. The objective was to establish the course of nitrogen mineralization of the different cover crops and weed shoots collected at different dates. The results of this laboratory incubation experiment are presented in Chapter 4.

In the context of reducing the use of chemicals (mainly glyphosate) for cover crops termination especially winter-hardy cover crops, we carried out a one year field experiment and the objective was to assess the effect of different managements of cover crops termination methods and control of weed in the cash crop on the cash crop productivity. The results of this experiment are reported in Chapter 5.

The last Chapter presents a general conclusion of the results obtained from the field and laboratory incubation experiments.

Abstract

Planting winter cover crops has several benefits compared to keeping the soil bare. The choice of the cover crop species and sowing date is crucial to have the best cover crops establishment and weed suppression. The seeds germination of cover crops is affected by the sowing date with a preference of early sowing. However, the appropriate date of cover crops sowing is not known. Also, cover crops nitrogen dynamics is variable among species. In a conservation agriculture context, we conducted two field experiments in Northern Italy and one laboratory experiment under controlled conditions of temperature and soil moisture. The objectives were to (i) assess the growth and nitrogen uptake of five pure winter cover crops (black oat, *Avena strigosa* Schreb.; cereal rye, *Secale cereale*. L.; white mustard, *Sinapis alba* L.; Egyptian clover *Trifolium alexandrinum* L.; and hairy vetch, *Viccia villosa* Roth) as influenced by plant species from three botanical families and two sowing dates (SD1 and SD2), (ii) assess the effect of cover crops presence/absence (bare soil) on weed suppression and maize productivity, (iii) estimate and assess the cover crops contribution to the following main crop (maize) in terms of nitrogen recovery and immediate availability, (iv) establish the course of nitrogen mineralization from pure cover crops in laboratory incubation conditions and (v) assess the effect of three managements of winter-hardy cover crops termination methods and control of weed in maize (chemical vs. mechanical) on maize productivity. The field experiments were carried out in Orzinuovi, Brescia, Italy. Relevant differences in cover crops growth were observed among species, with white mustard SD1 having the highest biomass in November (5.3 and 3.2 t ha^{-1}, respectively for the first and the second year) and Egyptian clover the lowest (less than 1 t ha^{-1}). Also, we demonstrated that hairy vetch SD1 had the highest nitrogen uptake in November (114 kg N ha^{-1}). The presence of cover crops reduced weed infestation compared to a bare soil. Sowing cover crops at end of August, instead of mid-September, had a positive effect on production, establishment, nitrogen uptake, and weed suppression. Maize yield following cover crops was not affected by the cover crop sowing dates and species during the two years of experiment. The maize nitrogen recovery was variable within years; the highest recovery was for maize following hairy vetch SD2 (+67%). The importance of sowing cover crops was demonstrated by the higher nitrogen recovery of maize following cover crops compared to maize following no cover crop treatment. In a laboratory incubation experiment of 84 days, cover crop shoots were collected from cover crops grown in the field, mixed with soil and kept under controlled temperature of 20 °C and soil moisture of 100% field capacity. We demonstrated differences in nitrogen mineralization among the five pure cover crops and weed shoots with hairy vetch, collected in March (C/N ratio of 10.1), having the highest and immediate net nitrogen mineralization from the beginning of incubation until 84 days after start of incubation. Black oat collected in March (C/N ratio of 19.8), had also an immediate net nitrogen mineralization during the whole incubation period but at a lower rate compared to hairy vetch. Egyptian clover collected in November (C/N ratio of 11.4), started nitrogen mineralization 7 days after start of incubation. White mustard collected in November (C/N ratio of 17.7), had a low rate of nitrogen mineralization. Shoots of weed, cereal rye, white mustard and black oat collected in March immobilized nitrogen during the whole incubation period at different rates; cereal rye had the highest rate of immobilization and was not able to start nitrogen mineralization 84 days after start of incubation. In the second field experiment our results indicated that a “post-glyphosate” scenario (mechanical termination of cover crops and chemical control of weed in maize) is the best management to produce the highest yield of maize compared to a “business-as-usual” management (chemical termination of cover crops and weed control in maize) and “organic” management (mechanical termination of cover crops and weed control in maize).

Key-words: Cover crops, Nitrogen uptake, Nitrogen recovery, Nitrogen mineralization, Maize, Weed suppression, Cover crops termination method.

Summary

Chapter 1. General introduction: state of art of cover crops

1. Introduction

Conservation agriculture (CA) is an alternative of conventional tillage (Jat et al. 2013; Wall et al. 2014). It is defined by the Food and Agriculture Organization (FAO, 2004) as a system based on three principles that are (i) minimal soil disturbance (no tillage/minimum tillage), (ii) permanent soil cover (mulch/crop residue) and (iii) diversified rotations with legumes. CA is a set of farming practices with the objectives of enhancing the sustainability of food and agriculture production by conserving and protecting the available soil (Hubbard et al., 1994; Karlen et al., 1994), water, and biological resources such that the need for external inputs can be kept minimal (Garcia-Torres et al., 2003). The CA feature is the maintenance of a permanent or semi-permanent soil cover that serves to protect the soil from sun, rain and wind and to feed soil biota.

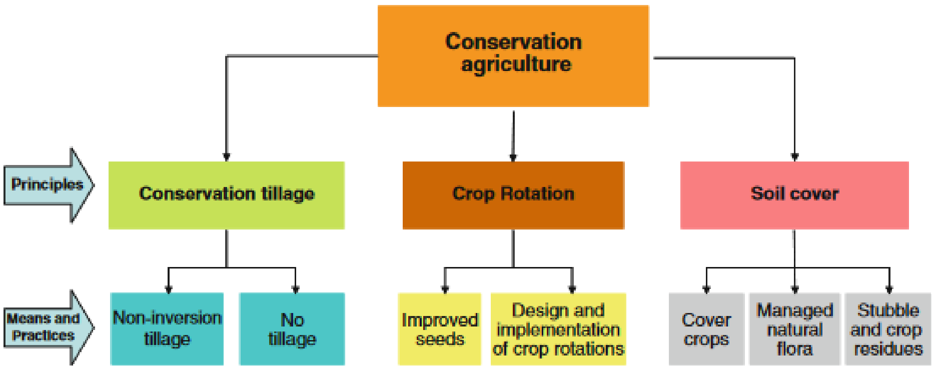


***Figure 1.1**. Conservation agriculture: principles, means and practices (Stagnari et al. (2009)).*

CA has become a priority in scientific and policy thinking on sustainable agricultural development. Several projects devoted to CA have emerged in leading international research and policy institutes such as CGIAR and FAO http://www.fao.org/ag/ca;http://www.cimmyt.org/en/programs-and-units/conservation agriculture-program

Emergence of CA was in 1970s in the United States of America (USA) and then it became an acceptable practice in the USA, Brazil, Argentina, Canada and Australia due to its ability to combat increased soil erosion and land degradation and because of lower fuel consumption (Dumanski et al., 2006; Harrington, 2008). CA reduces soil degradation and contributes to sustained agricultural production, in particular in areas of fragile soils and of high risk of declining soil quality (Hobbs et al., 2008). CA is efficient for practical implementation of sustainable crop production in terms of soil fertility management and soil productivity (Benites et al., 2003; Holland, 2004; Calegari et al., 2008).

Cover crops – known also as inter crops and catch crops – are crops cultivated to replace a bare fallow and that are ploughed under (or left on the soil surface) as green manure before sowing the successive main crop (Dabney et al., 2001). The purpose of cultivating cover crops is protecting and/or improving the soil rather than being harvested. Also, cover crops could provide Biological Nitrogen Fixation (BNF), weed or pest suppression or prevention of soil erosion. Cover crops increase the functional diversity and environmental suitability of cropping systems (Drinkwater and Snapp, 2007). Moreover, cover crops are used as an agroecological practice that limit fertilizer inputs and reduce risk of water contamination due to a decreased risk of leaching (Sanchez et al., 2004; Scholberg et al., 2010), and also to reduce soil or wind erosion.

The objective of this chapter is to summarize the reviews and meta-analyses that document a number of cover crop effects: growth, nitrogen (N) uptake, apparent N recovery and yield of the following cash crop, soil quality, weed and N emissions such as N leaching and nitrous oxide emissions. Reviews and meta-analysis were found in bibliographic databases such as Google scholar, Scopus and Web of Science using keywords: (“cover crop” or “catch crop” or “green manure”) and (“review” or “meta-analysis”).

While cover crop was used as a keyword, some of the papers found were not completely dedicated to these crops, but had a more general focus. Table 1.1 presents a general description of the papers collected that were totally on cover crops, sorted according to their main topic. The papers varied between meta-analysis, a statistical method used to combine numerical results from studies in a new statistical framework to test hypotheses (quantitative assessment of papers), and reviews, a systematic approach to identify relevant publications and summarize their results (qualitative assessment). Studies were mostly in the United States and Canada except for the paper of Valkama et al. (2015) which was conducted in European Nordic countries (Denmark, Sweden, Finland and Norway).

Table 1.1. *Summary of papers main topics, categories, geographical areas, number of studies and number of observations of published reviews and meta-analyses on cover crops.*

Main topic	Papers	Category	Geographical area	Number of studies	Number of observations
Cover crop productivity	Cherr et al. (2006)	Review	Mostly United States + Germany	31 (from 1970 to 2001)	62
Cash crop yield	Tonitto et al. (2006)	Meta-analysis	Mostly United States + Brazil + Canada	31 (from 1970 to 2002)	276
	Valkama et al. (2015)	Meta-analysis	European Nordic countries: Denmark, Sweden, Finland and Norway	35 studies (between 1988 and 2014)	43
	Miguez and Bollero (2005)	Review	United States and Canada	36 (between 1965 and 2004)	70
	Marcillo and Miguez (2017)	Meta-analysis	United States and Canada	65 (from 1965 to 2015)	268
Nitrogen Fertilizer Replacement Value (NFRV)	Ketterings et al. (2015)	Review	United States (Northeast)	23 (from 1987 to 2014)	37

Main topic	Papers	Category	Geographical area	Number of studies	Number of observations
Weed suppression	Osipitan et al. (2018)	Systematic review and meta-analysis	Asia, Europe, North America, and South America	46 (from 1990 to January 2017)	Not specified
	Osipitan et al. (2019)	Meta-analysis	Not specified	53 (from 1990 to 2018)	276
Carbon sequestration	Ruis and Blanco-Canqui (2017)	Review	Mostly United States+ Canada + India + Japan	25 (from 1986 to 2016)	108
	Blanco-Canqui et al. (2015)	Review	United States	13 (from 1989 to 2014)	43
	Poeplau and Don (2015)	Meta-analysis	Mostly United States + India + Germany +Canada	30 (from 1959 to 2013)	139
Nitrous oxide emissions	Basche et al. (2014)	Meta-analysis	Mostly United States + United Kingdom	26 (from 2000 to 2012)	106

2. Cover crops growth and nitrogen uptake

Cover crops growth and N uptake was assessed by variables of productivity, N fixation and uptake.

2.1. Productivity

Only one review was dedicated to the cover crop productivity before planting the cash crop. In general, cover crops had a good biomass growth. Cherr et al. (2006) summarized results for legumes and non-legumes cover crops. This paper indicated that the dry weight of legumes in temperate climates was variable according to the cover crop species, environment (soil texture and location), growing season, and management techniques.

We report some examples of cover crop productivity from Cherr et al. (2006). For hairy vetch (*Vicia villosa* Roth), the dry weight ranged between 5.6 and 8.9 t ha^{-1} in a sandy loam soil after 7 months of growth. In a sandy loam soil after 22 weeks of growth, it was ranging between 1.8 and 3.8 t ha^{-1}, for a cover crop that had been interseeded in sweet corn two weeks after its sowing.

In a silty clay loam soil berseem clover (*Trifolium alexandrinum* L.) was cultivated for 14-16 weeks. At the end of this period, the dry weight was 9.2 t ha^{-1} (not mowed) and ranged between 6.7 and 10.2 t ha^{-1} (for partly mowed clover after 7 weeks of growth). In a loam soil, 13 weeks after sowing, the dry weight was 4.1 t ha^{-1} (uncut) and 1.9 t ha^{-1} (partially cut for forage at 60 days).

In a sandy loam soil, the crimson clover (*Trifolium incarnatum* L.) dry weight ranged between 4.2 and 5.7 t ha^{-1} in Maryland 8 months after sowing and was higher (ranged between 5.8 and 7.3 t ha^{-1}) in Maine, 3.5 months after sowing. In a silty clay soil in Germany, the crimson clover dry weight ranged between 4.0 and 10.5 t ha^{-1} after 4 months of growth. The dry weight was less (it ranged between 2.1 and 2.2 t ha^{-1}) in a loam soil. For three different seeding mixtures, in a silty clay soil in Germany, the crimson clover and rye dry weight ranged between 6.0 and 12 t ha^{-1}.

For non-legume cover crops, the same factors were influencing the cover crops dry weight. In fact, for oat (*Avena sativa* L.), the dry weight was between 3.3 and 4.3 t ha^{-1} after 3 months of growth in a sandy loam soil in Maine. For rye (*Secale cereale* L.), it ranged between 4.1 and 6.6 t ha^{-1}, after 9 months of growth in Maine, and between 1.0 and 6.1 t ha^{-1} in a loam soil in Ontario after 8 months.

In general, cover crops had a higher dry weight when cultivated in silty clay soils. Moreover, keeping the cover crop not mowed is better than mowing. Within cover crops, berseem clover (*Trifolium alexandrinum* L.) had the highest dry weight.

2.2. Nitrogen fixation and uptake

Cover crops N fixation and uptake was reviewed in two papers: Tonitto et al. (2006) and Ketterings et al. (2015). Compared to control treatments, cover crops increase N fixation and uptake. According to Tonitto et al. (2006), who used 53 datapoints of non-legume cover crops, the average non-legume cover crops uptake was 37 kg N ha^{-1} and 50% of studies reported a cover crop N uptake that ranged between 20 and 60 kg N ha^{-1}. This variability in N uptake data is due to different cash crop fertilization levels, which impacts soil mineral N concentration immediately before cover crop sowing. Cover crops can scavenge a significant proportion of post-harvest surplus inorganic N. Tonitto et al. (2006) found that 47% of the cover crops scavenged N at rates equivalent to >20% of applied fertilizer N on previous cash crop and 26% of the cover crops took up the equivalent of 10-20% of applied fertilizer N on previous cash crop.

Cover crop seeding time effect on N uptake was reviewed by Ketterings et al. (2015). These Authors reviewed winter cover crops (WCCs) that were either inter-seeded into standing wheat or seeded in August directly after spring wheat harvest. They indicated a great impact of cover crop seeding time on cover crops ability of N accumulation. Legume cover crops N uptake before winter period averaged 59 kg N ha^{-1}. For hairy vetch grown in Pennsylvania in 1990-1991 (seeded after wheat harvest in late July and early August) N uptake in the fall was 59 kg N ha^{-1}, 44 to 98 kg N ha^{-1} for medium red clover seeded into wheat in March in Ontario, Canada, and 118 kg N ha^{-1} for red clover seeded mid-April in standing wheat in Pennsylvania.

Large fall N cover crop uptake was reported for Pennsylvania and Ontario studies in a two-year study (on average 138 kg N ha^{-1}), ranging between 50 kg N ha^{-1} for various legume-containing mixtures seeded in August and 190 kg N $ha^{-1}$for radish seeded in August.

For a mixture of legume-cereal biculture terminated in the spring, total N content ranged between 57 kg N ha^{-1} for a vetch and cereal rye mixture seeded after corn silage harvest, and 77 kg N ha^{-1} for a mixture of medium red clover and cereal rye inter-seeded at corn side-dressing time (Ketterings et al., 2015).

3. Effect of cover crops on cash crops

The effect of cover crops on cash crops is evaluated through the nitrogen fertilizer replacement value and the cash crop yield.

3.1. Nitrogen Fertilizer Replacement Value

Nitrogen Fertilizer Replacement Value (NFRV), also called cash crop nitrogen recovery, was calculated using the traditional method. It consists on calculating NFRV as the fertilizer N application required for continuous corn to obtain a yield equal to that of corn after a WCC with no fertilizer N applied to the corn crop. NFRV was reviewed only in the paper of Ketterings et al. (2015). A limitation of this review is that the literature base and inconsistencies in reporting field histories (e.g., crop rotations, manure use), timing of WCCs seeding, above- and belowground biomass, termination dates in relation to corn planting, and weather conditions made it difficult to quantify NFRVs. An important factor that affects NFRV is the decomposition pattern of cover crop biomass in relation to its C/N ratio. N immobilization on termination of high C/N ratio cover crops is one of the factors that imply low NFRVs of cereal grains that were lower than for legumes. Cereal rye showed an insignificant to negative NFRV.

In most studies summarized by Ketterings et al. (2015), a positive effect on yield and N supply to the following corn crop was noticed for legume WCCs. NFRVs ranged from 0 to 235 kg N ha^{-1} across a variety of environments and crop management systems with most sites showing NFRVs between 50 and 100 kg N ha^{-1}. Ketterings et al. (2015) reported several factors that can influence the effect of legume cover crops on cash crop nitrogen recovery. These are detailed in the next paragraphs.

3.1.1. Climate

A regional difference in NFRVs of legumes for corn was identified in several studies. For legume WCCs, N accumulation values ranged between 67 and 170 kg ha^{-1} in the humid temperate environments of the eastern and southeastern regions of the United States, and between 34 and 45 kg ha^{-1} in Iowa and Nebraska (Midwestern). This variability is due to cover crop mineralization affected by the differences in the temperatures and in the distribution of precipitations among the years.

3.1.2. Tillage decisions

Time of cover crop establishment, time of turnover/termination vs. time of corn planting and tillage system are the three management decisions that affect NFRVs of cover crops.
Hairy vetch (*Vicia villosa* Roth) NFRVs varied between 17 and 149 kg N ha^{-1}. For more than 50% of the studies conducted in the Northeast of the United States, NFRVs were higher than 78 kg N ha^{-1}; for 80% of the studies NFRV was approximately higher than 56 kg N ha^{-1}. The highest value was 149 kg N ha^{-1}.
NFRV might not have been affected by the rate of N accumulation due to weather that impacted N mineralization dynamics and non-N rotation (without legumes). This effect is evident in studies accomplished in southern US climates and suggests that NFRVs might exceed the N content of the vetch cover crop at the time of termination. An increase in the soil organic matter levels, an improvement of soil physical conditions and water relations are the main effects of non-N rotation.
In addition, NFRV varied according to the tillage method. In one study made by Dou and Fox (1994), in the first year, NFRV was 149 kg N ha^{-1} under no tillage (NT) and 103 kg N ha^{-1} under conventional tillage (CT), while, in the second year, it was 15 and 30 kg N ha^{-1}under NT and CT, respectively. In another study made by Sarrantonio and Scott (1988), it was 17 and 52 kg N ha^{-1} in the second year.
N losses in conventional and no tillage systems complicated the NFRVs prediction. In a conventional tillage system, inorganic N is exposed to early season leaching or denitrification losses. However, under no tillage system, inorganic N is lost via NH_3 volatilization or denitrification.

3.1.3. Maturity stage of winter cover crop

Maturity stage of WCCs at termination time has an impact on NFRV of legume cover crops. There is no increase in the amount of N available to subsequent crops for legumes beyond flowering due to limited N uptake during reproductive growth stages and slower mineralization of cover crops vegetative biomass and reduced N release from hard seeds.
For red clover, NFRVs vary between 29 and 235 kg N ha^{-1} compared to 78 kg N ha^{-1} for crimson clover in Ontario, Canada. For yellow sweet clover (*Melilotus officinalis* L. Pall) cultivated in New York, the NFRV ranged between 56 and 112 kg N ha^{-1} and it was between 10 and 77 kg N ha^{-1} for sweet clover cultivated in Wisconsin.

3.2. Cash crop yield

Cover crops effects on cash crop yield were summarized in several papers such as Miguez and Bollero (2005), Tonitto et al. (2006), Valkama et al. (2015), and Marcillo and Miguez (2017). Most of these studies indicated that planting cover crops instead of keeping the soil bare had a neutral effect on cash crops yield. For example, Tonitto et al. (2006) indicated that the corn yield was not statistically different between a fertilized bare soil (7 t ha^{-1}; n=146) and a soil with legume cover crop as N source (6.4 t ha^{-1}; n=228). The same remark is valid for sorghum yield that was 5.2 t ha^{-1} (n=44) and 4.6 t ha^{-1} (n=60) under a fertilized and a legume nitrogen source, respectively.
Factors that can influence the effects of cover crops on cash crops yield are discussed below.

3.2.1. Cover crop type

A clear evidence exists of legume cover crop benefits on cash crop yield. Marcillo and Miguez (2017) updated a previous meta-analysis by Miguez and Bollero (2005). In this update, they confirmed significant differences in cash crop yield response for the three WCCs groups, with significant lower yield after grasses than legumes and mixtures. Miguez and Bollero (2005) indicated a significant positive effect of **legume** WCCs (weighted total sums of squares Qt = 293.5, df = 81, p < 0.0001): a yield increase by 24% (based on 80 observations in 30 studies) has been registered for corn following legume WCCs compared to following non cover crop; a similar increase (21%, n=101) was reported by Marcillo and Miguez (2017). Differences between studies accounted for 70% of total variation in yield response for legume observations. According to Tonitto et al. (2006), a small decline (10%) was noticed in unfertilized cash crop yield (n=206 comparisons) following legume cover crops cultivated in conventional system compared to cash crop managed at recommended fertilizer level. In particular for corn, yield was lower by 12% (n=165 comparisons). Valkama et al. (2015) mentioned an equal distribution between positive and negative yield responses due to legumes (n=11 observations).
Under **non-legume** cover crops compared to keeping the soil bare, the change in yield was equally distributed between slight positive and slight negative effect (n=69 comparisons; Tonitto et al., 2006). Valkama et al. (2015) mentioned a slight significant negative grain yield difference (-3%, n=19) of spring cereals due to non-legume catch crops compared with legume cover crops.
For **mixtures**, a positive significant difference was reported by Miguez and Bollero (2005) (+21%, n=10) and by Marcillo and Miguez (2017) (+13%, n=28). A mixture of WCCs has a positive effect on their biomass production, a reduction of the soil erosion and an improvement of the weed control. The biomass production of cover crop mixtures depends on the termination date due to its impact on composition and quality of cover crops residue. The additional observations allowed the detection of a significant difference for biomass and termination date. Mixture WCCs observations were not homogeneous (weighted total sum of squares for log-RR Q = 92.00, n = 28, p < 0.001). Between-studies variance was estimated at 0.015 (unitless, as it is calculated on standardized effects) and explained 70% of total variability.

Grass WCCs has a neutral effect on corn yields (Marcillo and Miguez, 2017). Even if the number of observations in the updated review was the double compared to the original review (Miguez and Bollero, 2005), the weighted mean response was 0.99 and 1.00. Between-studies variance was estimated at 0.002 and accounted for 32% of total variability in grass observations.

3.2.2. Fertilizer level

When reviewing studies in which the cash crop following the cover crop had been fertilized, Tonitto et al. (2006) divided the fertilizer level in three categories: low, recommended and high application. No significant difference was noticed between the yield of cash crops following legume cover crops relative to cash crop following no cover crops, under either high applications of fertilizer (n=94) or recommended fertilization (n=207). An improvement of the yield by 5% (n=115) was noticed in case of low inorganic fertilizer application (Tonitto et al. 2006). Similar results were obtained also by Marcillo and Miguez (2017), who found yield increase due to legume cover crops for N fertilization rates lower than 200 kg ha^{-1}, and no differences for N rates higher than 200 kg ha^{-1}. These findings are similar to those reported by Miguez and Bollero (2005) and relate to lower yield response at high N rates because considerable N mineralization and N release following legume residue decomposition provide amounts of N to the crop that are similar to those applied with the high N fertilizer rates.

Marcillo and Miguez (2017) have used mixed models and mentioned that intercepts for mixture or legume cover crops were statistically different from 1 and unfertilized corn yield was greater by 18 to 42% for legume or mixture WCCs, compared to no cover crop. As the N rates increase, the Relative Ratio (RR) for mixture WCCs remains unchanged but it decreases for legume WCCs. Miguez and Bollero (2005) indicated that the highest difference in corn yield between WCCs and no cover crops was identified at low NFR. For legume WCCs, at low NFR, the contribution to higher yields could be related to N mineralization. However, according to other studies, it was noticed that legume cover crops contributed to higher yields compared to keeping the soil bare or even when a mixture of cover crops was cultivated (Blanco-Canqui et al., 2015). Miguez and Bollero (2005) indicated that, after grass WCCs, corn had a similar N availability compared to corn following no cover crops. Cultivation of grass WCCs did not increase soil N, which is either retained in the WCC biomass or immobilized by microbes that decompose high C/N ratio residues. The synchronization between N recycling and the crop demand thus did not occur.

3.2.3. Legume N input rate

Tonitto et al. (2006) and Valkama et al. (2015) discussed the effect of legume nitrogen input rate on cash crops and mentioned two different results. The change in cash crop yield relative to conventional systems was positive (+5%; n=23) for legume-derived nitrogen inputs exceeding 180 kg N ha^{-1} and negative for moderate (-15%; n=22; 80-110 kg N ha^{-1}) and low (-19%; n=44; < 80 kg ha^{-1}) legume inputs. It is important also to notice that no change was observed by Tonitto et al. (2006) for a legume input ranging between 110 and 180 kg ha^{-1} (n=54). However, Valkama et al. (2015) mentioned that, at low nitrogen rates, catch crops had a positive effect. In fact, at 60 kg N ha^{-1}, the yield rose to 8% over that of the controls with no catch crop. In contrast, at 95 kg N ha^{-1}, the yield response declined compared to that of the controls, and at 120 kg N ha^{-1}, it dropped to –6%.

3.2.4. Grain quality

Valkama et al. (2015) indicated that legume and mixed catch crops increased grain quality in terms of grain N uptake by 6% over that of the controls (n =7), whereas non-legumes had a small (2%), non-significant increase (n = 4).

3.2.5. Winter cover crops termination and biomass

A significant effect was noticed for WCCs termination and WCCs biomass for mixture WCCs. The Relative Ratio (RR) of cash crop yield increased as termination date was delayed. The RR was less than 1 for winter cover crops terminated more than two weeks ahead the subsequent corn crop and above 1 for mid termination (7 to13 days before subsequent corn) of corn following a mixture of WCCs or no cover crops. However, a late termination (less than six days before subsequent corn) had a significant increase in corn yield by 30%. Late and mechanically terminated mixtures have been shown to result in higher corn grain yields in relation to no cover crop treatments (16% to 22%) because of increased biomass that reduced early-season weed (Marcillo and Miguez, 2017).

The same authors indicated that, using mixed models, no significant difference was identified for the regression coefficients of the WCCs termination model for cash crop yield, and no significant difference was observed between cultivating cover crops or keeping the soil bare whatever the date of termination of the cover crop was (early or late termination). The RR was higher for legume and mixture WCCs rather than for grass WCCs at late termination. In case of early termination, cultivating legume and mixture WCCs had a higher but non-significant effect on corn yield compared to cultivating grass WCCs. As a summary, the cash crop yield was affected positively only by legume and mixture WCC's terminated late ((less than six days before subsequent corn).

3.2.6. Objective of corn production

The RR of cash crop yield was variable according to the corn production objective. In fact, grass WCCs neither increased nor decreased corn yields, although corn grown for grain yielded relatively higher than silage corn after grass WCCs (Marcillo and Miguez 2017). This variability could be explained by extended growing periods for silage corn compared to grain corn or the decrease of quantity and quality of ears of grain corn. Planting corn for silage production late allows the delay of harvest but increases the risk of erosion and nitrate leaching that impacts yield. For example, keeping rye for four weeks more (late termination) compared to its early termination leads to yield penalties for grain corn. However, neutral impact was recorded for silage corn. Moreover, the choice of winter killed species such as oat increased silage corn yield by 41%, with no-added fertilizers, compared to keeping the soil bare due to long time of cover crop decomposition and greater N release.

3.2.7. Soil texture

Only Valkama et al. (2015) discussed the effect of soil texture. They reported that, following non legume cover crops, the cash crop grain yield was variable according to the soil texture: the effect was a decrease by 8% for loamy soils (n=11), slight negative (-1%; n=3) for clay soils, and was not statistically significant for sandy soils (3%; n=4). No significant difference was observed between the soil textures for legume cover crops.

3.2.8. Period when the experiment was conducted

Valkama et al. (2015) have classified the experiments according to the decade when they had been realized. They found that the cash crop grain yield difference between a cover crop and a bare soil increased across the decades. It was negative (-12%) in the 1980s and increased up to a positive effect (+9%) in the 2000s. This increase could be due to the reduction in nitrogen applied to the cash crop (140 vs 85 kg ha^{-1}). No significant differences were identified between ryegrass species, clover species or between Nordic countries. The effect of green manures on grain yield was not changed due to the amount of annual precipitation or seed rates.

3.2.9. Region

A significant difference in yield response to WCCs compared to no cover crops was identified for the region factor. The difference between the first review elaborated by Miguez and Bollero (2005) and the updated meta-analysis of Marcillo and Miguez (2017) is that this latter has increased the sample size. In fact, it has gone from 11 to 77 observations and it included new areas not covered in the first review. The RR confidence interval encompassed 1 for Great Plains, Canada and North Central regions, indicating that there were no significant differences between yield of catch crops cultivated after WCCs and those cultivated after no cover crops. This result could be explained by the fact that, in these regions, the period of cultivation was too short so the yield benefit was limited. In much of the North Central regions, grass WCCs predominate. In Southeast and Northeast regions, the WCCs effect was positive and significant, with a yield increase by 12 and 14%, respectively. These results are similar to those found by Miguez and Bollero (2005). In Southern regions, the climate is warmer and heat-tolerant species were established in better conditions, have grown rapidly, controlled weed efficiently and responded favorably to irrigation. WCCs cultivation succeeded to restore eroded soil in humid Southern regions, conserved the soil productivity and so applied one of the conservation agriculture principles. In Alabama, grasses and legumes were successfully cultivated, winter rye had a neutral effect on the biomass yield of the subsequent corn crop and an increase of the yield and N content of the subsequent grain corn was noticed after cultivation of dense tropical legumes (Marcillo and Miguez, 2017).

4. Effect of cover crops on soil

4.1. Carbon sequestration

Most of studies indicated that cover crops increased Soil Organic Carbon (SOC). The effect of cover crops is significant. In their review, Ruis and Blanco-Canqui (2017) found that it can range from 0 to 3.5 Mg $ha^{–1}$ $yr^{–1}$; on average, the SOC increase was 0.45 g $kg^{–1}$ $yr^{–1}$ and 0.49 Mg $ha^{–1}$ $yr^{–1}$ for SOC stocks in the first 30 cm of the soil.

Another meta-analysis based on 137 observations (Poeplau and Don, 2015) indicated that cover crops can sequester on average 0.32 ± 0.08 Mg $ha^{–1}$ $yr^{–1}$ of C to the 22 cm soil depth, with an annual change rate above 1 Mg ha^{-1} yr^{-1} for 24 cases, between 0 and 1 Mg ha^{-1} yr^{-1} for 102 cases, and a SOC stock depletion for 13 cases.

The extent to which cover crops (CCs) increase soil C is site-specific and depends on: (i) CC biomass input, (ii) years in CCs, (iii) antecedent soil C level, (iv) soil type, (v) CC species, (vi) tillage management, and (vii) climate. Some of these factors are discussed below.

4.1.1. Tillage system

In no-till systems, cover crop allows to accumulate soil organic C of 0.1 to 1 Mg ha^{-1} yr^{-1}. In fact, hairy vetch (*Vicia villosa* Roth) and cereal rye (*Secale cereale* L.) cultivated as cover crops had sequestrated different rates of C according to the tillage system: it was 0.88 Mg ha^{-1} yr^{-1} under no-till, 0.49 Mg ha^{-1} yr^{-1} under chisel plow, and only 0.10 Mg ha^{-1} yr^{-1} under moldboard plow for the 0 to 75 cm depth after 12 years of management (Blanco-Canqui et al., 2015).

Also Ruis and Blanco-Canqui (2017) have found significant differences of SOC concentration gain due to tillage in response to use of cover crops: 0.49 ± 0.35 g kg^{-1} yr^{-1} for no-till, 0.11 ± 0.09 g kg^{-1} yr^{-1} for conventional till, and 0.47 ± 0.52 g kg^{-1} yr^{-1} for other tillage practices. The same authors indicated that cover crops do not accumulate SOC stocks at different rates under different tillage systems. Tillage does not affect SOC gain under CC. The mean annual SOC stock gain was 0.54 ± 0.17 for no-till, 0.29 ± 0.05 Mg ha^{-1} yr^{-1} for chisel plow, and 0.77 ± 0.27 Mg ha^{-1} yr^{-1} for conventional till.

4.1.2. Time

Short term cover crops cultivation effect on soil organic C is not detectable. Then, after a certain period, it becomes detectable and cover crops increase the soil organic C concentration (Blanco-Canqui et al., 2015). However, only the rate of SOC stock gain with CC was moderately and linearly correlated with duration (n =71) and so as the growing period of cover crops increases, the SOC gain increases, while there was no correlation between cultivation of cover crops and duration of the experiment on SOC concentration gain (n=79) (Ruis and Blanco-Canqui, 2017). It was noticed that only 21% of the variability in SOC stock gain under cover crop was explained in the correlation between duration and SOC stock gain.

4.1.3. Cover crop classification

Effectiveness of cover crops ability to increase soil C levels is variable according to the cover crop species. In fact, grass cover has a greater ability to increase the soil C level compared with legume cover crops because the process of decomposition of grass cover crops is slow (Blanco-Canqui et al., 2015). Also, soil organic C increases more in case of cultivation of a mixture of cover crops rather than cultivating one single species due to the greater above and belowground biomass production. For example, a mixture of Austrian winter pea (*Pisum sativum* L.) and radish (*Raphanus sativus* L.) induced greater soil organic C concentration (19.4 g kg^{-1}) than winter pea (15.9 g kg^{-1}) or radish (17.6 g kg^{-1}) cultivated as single species.

Similar but not significant effects were documented by Ruis and Blanco-Canqui (2017), who found mean annual SOC concentration gains of 0.81 ± 0.75 g kg^{-1} yr^{-1} for brassicas (n=5), 0.50 ± 0.38 g kg^{-1} yr^{-1} for grasses (n=13), 0.36 ± 0.32 g kg^{-1} yr^{-1} for legumes (n=17), and 0.61 ± 0.20 g kg^{-1} yr^{-1} for mixes (n=3).

No clear differences were observed for the mean annual stock gains between different groups of cover crops. It was 0.67 ± 0.29 Mg ha^{-1} yr^{-1} for grasses, 0.43 ± 0.15 Mg ha^{-1} yr^{-1} for legumes (n=12), and 0.42 ± 0.28 Mg ha^{-1} yr^{-1} for mixes (n=11) (Ruis and Blanco-Canqui, 2017).

4.1.4. Climate

Precipitation is one of the climatic parameters that affect the cover crops biomass production and thus C inputs (Blanco-Canqui et al., 2015). In fact, soil organic C increases slowly in regions with low precipitations (semiarid regions) compared with regions with high precipitation amount (>500 mm). A comparison of the effect of winter and spring triticale (×*Triticosecale* Wittm.) and spring lentil (*Lens culinaris* Medikus) on soil organic C pool in Garden City, KS, a region having a mean annual precipitation of 489 mm, was done by Blanco-Canqui et al. (2015). Results show that after 5 years of management winter and spring triticale (×*Triticosecale* Wittm.) - grown in between two wheat crops in a wheat-fallow system - increased the soil organic C pool by 0.56 Mg ha^{-1} yr^{-1} in the 0 to 7.5 cm depth relative to fallow. However, the same authors indicated that the SOC stock increase was lower when spring lentil was cultivated (0.44 Mg ha^{-1} yr^{-1}). In semiarid climates, cover crops had no effect on soil organic C.

5. Weed suppression

Cover crops effects on weed suppression were assessed in three papers: review of Blanco-Canqui et al. (2015), a systematic review and meta-analysis of Osipitan et al. (2018) and a meta-analysis of Osipitan et al. (2019). Cover crops can suppress weeds by leaving residues after termination that will modify the micro-environment (physical competition) and interfere and affect nutrient availability for emerging weeds (chemical competition); cover crops can also affect weed seed germination by releasing allelopathic compounds or by outcompeting weeds that would otherwise produce seed and increase the potential for weed–crop competition in the succeeding cropping cycles (direct competition). Osipitan et al. (2019) indicated differences between cover crop species in weed suppression at termination (n=276 observations), with buckwheat (*Fagopyrum esculentum* Moench) and radish (*Raphanus raphanistrum* L.) having the highest response ratio (less effective weed suppression; Response Ratio (RR) =0.71; n=4 observations) and cereal rye the lowest response ratio (0.06; n=18 observations). Also, they identified that not all cover crop species were able to suppress weeds at termination (95% Confidence Interval of RR was higher than 1), such as black medic (*Medicago lupulina* L.), buckwheat, mustard (*Sinapis alba* L.), pea, radish, and sunflower (*Helianthus annuus* L). In general, weed suppression was greater in grass cover crop species than in broadleaf cover crops species. In the same meta-analysis prepared by Osipitan et al. (2019), the authors demonstrated that cover crop biomass was inversely related to the level of weed biomass (r^2=0.67; n=40 observations) and weed density (r^2=0.64; n=32 observations). A late termination (a two week interval before cash crop sowing) of cover crops provided a greater weed suppression (n=8 observations) compared to cover crops terminated earlier (n=8 observations). Also, the response ratio increased when the main crop planting date was delayed 1 to 4 weeks after termination of cover crops (n=24 observations). Ospitian et al. (2019) mentioned that the response ratio of cover crops weed suppression increased under reduced tillage (n=8 observations) compared to no tillage (n=19 observations). No difference was identified in early weed suppression (up to 5 weeks after main crop planting) by cover crops intercropped (n=76 observations) or in sequence with main crop (n=21 observations).

The paper of Blanco-Canqui et al. (2015) discussed cover crops effects on weed and indicated a positive effect. One of the cover crops benefits is their ability to suppress weed. In fact, some management practices such as dates of establishment and termination of the cover crop, method of termination, cover crops decomposition rate are so important to succeed in suppressing weed. Several factors can influence the cover crop effects on weed suppression. In fact, Blanco-Canqui et al. (2015) indicated that fall-sown winter annual cover crops are chosen due to their benefits on weed provided before termination in temperate regions. In other climatic conditions where water is a limiting factor for cultivation (northern Great Plains), cereal rye is chosen due to its large quantities of biomass that allow a better weed suppression and an allelopathic effect. Blanco-Canqui et al., 2015 discussed also the effect of method of termination on weed suppression. They mentioned a greatest effect. In fact, the use of a sweep-plow under cutter allowed subsequent weed suppression, whereas disking generally enhanced weed growth.

6. Effect of cover crops on nitrogen emissions

Cover crops effects on nitrogen emissions can be evaluated by nitrogen leaching and nitrous oxide emissions.

6.1. Nitrogen leaching

Two papers were discussing the cover crops effects on nitrogen leaching (Tonitto et al., 2006; Valkama et al., 2015). Both indicated that cover crops decrease nitrogen leaching. According to Tonitto et al. (2006) the reduction was 70% (n=69 comparisons) under non-legume cover crops compared to keeping the soil bare. A reduction of 40% (n=8 comparisons) in nitrate leaching was noticed for fertilized cash crops following legume cover crops. The post-harvest soil inorganic N pools ranged from -50% to +80% compared to no cover crop (n=10 comparisons); this indicates that the leaching reduction is achieved thanks to fall and winter N uptake by the cover crop, and not by lower soil nitrate levels in the fall after the cash crop.

Also Valkama et al. (2015) mentioned a reduction of nitrate leaching by 50% in the case of non-legume cover crops (n=27); their controls averaged 46±29 kg ha^{-1} yr^{-1}. No statistical differences were observed between Italian ryegrass and perennial ryegrass. No effects were found by Valkama et al. (2015) among soil texture groups (n=29), autumn and spring ploughing (n=18), fertilizer types (n=28) and Nordic countries (n=29). Neither the amount of fertilizer (0 – 160 kg ha^{-1}) nor the amount of annual precipitation (480 – 1040 mm) nor the duration of the experiments (2-7 years) modified the effect of catch crops on N leaching loss.

6.2. Nitrous oxide emissions (N_2O)

The effects of cover crops on nitrous oxide emissions during the cover crops growth period were reviewed in the paper of Basche et al. (2014). Studies did not arbitrate if cover crops decreased N_2O emissions or no. In fact, 40% of studies indicated a negative natural log response ratio (LRR), i.e. cover crops decreased N_2O emissions. However, 60% of studies indicated a positive LRR and so an increase of N_2O emissions.

Factors that can influence the effect of cover crops on N_2O are discussed below.

6.2.1. N rate

According to Basche et al. (2014), a significant interaction was identified between N rate as a fertilizer N applied to the cash crop and cover crop type. As an example, when no amount of N was applied, a decrease of N_2O emissions was noticed in legume cover crops compared to non-legume cover crops. C:N ratios less than 25 of legume cover crop residues stimulated N mineralization rates in maize systems cultivated without N applied. Mineralized N is subsequently nitrified and increases NO_3 substrate for N_2O production. In case of non legume cover crops, the N_2O emissions increased slightly as N fertilizer rates increases, showing the importance of both C and N.

6.2.2. Tillage system

A significant interaction was noticed between N rate and tillage system (Basche et al., 2014). Mechanical soil disturbances stimulated C and net N mineralization by disrupting soil aggregates so the organic C was decomposed by microbial process. As N rates increased, the N_2O emissions from systems managed with cover crops slightly increased for no-till systems. For conventionally tilled systems, low N rates tended to increase N_2O emissions from system managed with cover crops; at higher N rates, cover crops reduced the N_2O emissions compared to control treatment. Negative N_2O emissions from system managed with cover crops could not reflect a large reduction in the overall magnitude of N_2O emissions. At negligible N rates or up to 2 kg, cover crops reduced N_2O emissions. At higher N rates, cover crops increased N_2O emissions by 2 to 4 kg ha^{-1}. At time of cover crop decomposition, the release of N_2O emissions was high (40 kg N_2O).

6.2.3. Type of cover crop

There are three types of cover crops: (i) legume such as clover, vetch (*Vicia villosa*), field bean and pea, (ii) non-legume such as cereal rye (*Secale cereale*), annual ryegrass (*Lolium multiflorum*), oats (*Avena sativa*), wheat (*Triticum aestivum*) and radish and (iii) biculture species such as vetch and rye mixes. N_2O emissions from system managed with non-legume cover crops and biculture species were close to zero and positive for legume cover crops. A significant difference was noticed for the three groups of cover crops. Cover crops may increase soil N availability during decomposition and, thus, may increase the available NO_3^- substrate for denitrification and N_2O emissions within agricultural fields (Basche et al., 2014).

6.2.4. Period of measurement

The effect of cover crops on N_2O emissions was evaluated during different periods of the year. In fact, period of measurement was significant (Basche et al., 2014). The average response ratio of measurements made during the entire year was close to zero in comparison to other periods of measurement. This indicated that cover crops, measured over long timescales, had a neutral effect on N_2O emissions.

Measurements made during decomposition period of the cover crop showed the highest LRRs. In non-fertilized plots, cover crops decreased N_2O emissions due to temporary N immobilization from the C contribution. The interaction between N availability and C input was essential in the determination of amount of N_2O emissions during the cover crop decomposition period. At low C:N ratios, a positive LRR (N_2O emissions increased) was noticed.

For legume cover crops, N_2O emissions were positively correlated with residue N content. During growth period of the cash crop, legume cover crops showing a positive LRR indicates that the cover crop decomposition is still in progress.

The lowest mean LRR registered during growth period of the cover crop was due to the cover crop N uptake and the weather during winter period (low temperature). Temperature was an important factor that influenced microbial process such as N mineralization, nitrification and denitrification. In fact, as temperature decreased, the microbial process exponentially declined.

6.2.5. Soil Incorporation

Emissions after incorporating cover crop residues were significantly higher than leaving the residues on the soil surface. Incorporation of cover crops residues increased N_2O emissions, N mineralization rates of soil organic matter and cover crop residues, soil temperature and the potential for denitrification and contributed to higher NO_3 availability.

Anaerobic conditions for denitrification of N content in the cover crops tissues is more likely to occur if the residues are incorporated with tillage rather than left on the surface. Incorporating aboveground cover crop residues led to relative increases in N_2O emissions through a variety of mechanisms. Incorporated residues with tillage compared to be left on the soil surface facilitate the anaerobic conditions for denitrification of the cover crop N.

6.2.6. Precipitation

Large or intense precipitation events are important due to their impact on denitrification for anaerobic soil conditions and so N_2O emissions. Cover crops decreased soil evaporation, increased rainfall infiltration and transpiration of stored soil water.

7. Conclusion

After reviewing existing meta-analyses and reviews, we can conclude that the effect of cover crops is dependent on the variable assessed. In fact, cover crops had a good biomass growth, decreased nitrogen leaching, increased soil organic carbon and suppressed weed. However, we consider that the effect of cover crops on the cash crop yield was neutral and existing reviews did not confirm either a strong positive or negative effect on nitrous oxide emissions.

Also, we can mention that there are not reviews and meta-analyses that summarizes the cover crops effects on other variables such as the cover crop root biomass and the nitrogen recovery by the following cash crops. Even, for some variables like cover crops productivity and weed suppression and nitrogen recovery of cash crops, literature is still scant.

The above discussion shows that existing reviews and meta-analyses do not address the topic of nitrogen recovery of cover crops. For this reason, we tried to prepare a meta-analysis to summarize the results of nitrogen recovery of cover crops in temperate climate (the climate of our study area). The work flow consisted on searching papers from the bibliographic database

Scopus and to set the criteria to decide if the paper shall be kept or rejected. We started by defining the search query.[1]

The result of this literature search was a list of 617 papers. As indicated in Figure 1.2, 519 papers were eliminated (i) because the abstract was not available or information available in the abstract indicated that it was a review, a meta-analysis or a survey not related to the topic of N recovery in cash crops (n=129 papers) and (ii) due to the absence of information on N recovery (n=390 papers). Later, only papers on maize, tomato and sorghum were kept, while papers on rice, cotton and soybean were eliminated (n=47 papers) for reasons that for example, cotton cannot be cultivated in a temperate climate and for soybean, we cannot calculate the N recovery due to that soybean fixes N. Among the remaining 51 papers, only 11 papers were presenting results of experiments conducted in temperate climate (Cf and Cs classes of Köppen classification). Finally, five papers were eliminated due to the absence of control treatment that is essential to calculate the response ratio. In conclusion, it was not possible to prepare a meta-analysis on N recovery in cash crops with only six papers. This number represented less than 1% of the output of the literature search from bibliographic database Scopus.

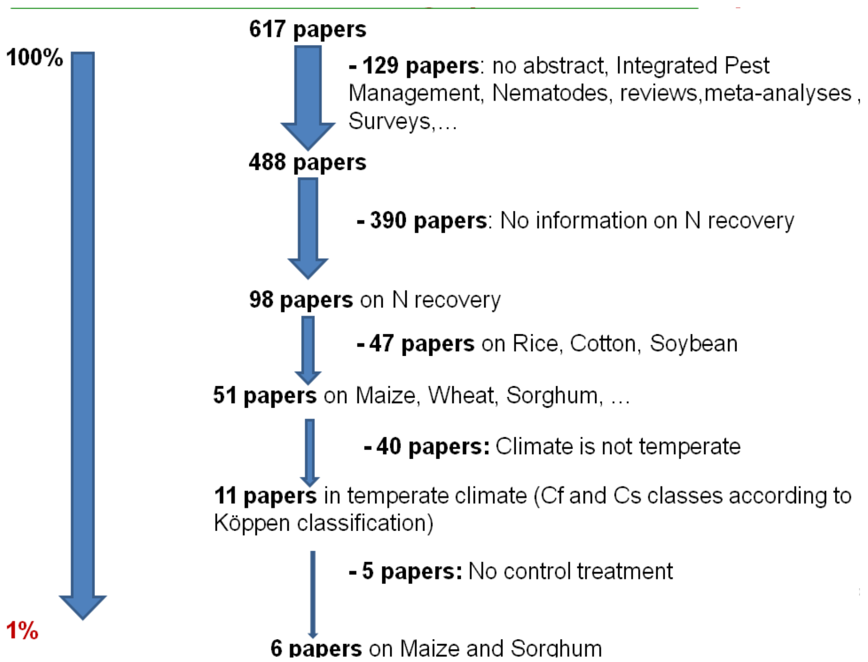


Figure 1.2. *Steps of papers selection for the meta-analysis of nitrogen recovery in cash crops.*

Therefore, in order to improve the knowledge of cover crops benefits in CA, in this PhD thesis, we conducted several experiments, under field and laboratory conditions, to assess the effects of different factors on the cover crops growth, N uptake and effects on N dynamics in

[1] Key-words chosen were (“cover crop” OR “catch crop” OR “green manure”) **AND [**(“nitrogen balance” OR “N balance” OR “nitrogen budget” OR “N budget”) **OR** ("nitrogen recovery" OR "N recovery" OR "NFRV" OR "ANR" OR "Nitrogen fertiliser replacement value" OR "Nitrogen fertilizer replacement value" OR "N fertiliser replacement value" OR "N fertilizer replacement value" OR "Nitrogen fertiliser value") **OR** ("nitrogen efficiency" OR "N efficiency" OR "cash crop" OR "N release" OR "nitrogen release")**].**

the soil and the following main crop. Research objectives, material and methods, results and discussion of the obtained results of the experiments conducted are presented in the following chapters.

Chapter 2. Cover crop growth and nitrogen uptake as affected by sowing date and species

1. Introduction

Cover crops are cultivated to replace a bare fallow and are ploughed under (or left on the soil surface) as green manure before sowing the successive main crop (Dabney et al., 2001). Cover crops provide a green manure service by releasing in the soil part of acquired nitrogen (N), which can be used by the subsequent cash crop (Kramberger et al., 2009; Justes et al., 2012). The agronomic decisions such as sowing date, seed rate and the choice of cover crop species are crucial for a successful establishment of cover crops. Planting of crops under no-till or reduced tillage and selecting the best main crop, cover crop species, and planting date for a given region or environment will aid in optimizing weed suppression and crop productivity and reducing costs of energy, labor, and machinery (Chauhan et al., 2006; Lawley et al., 2011). According to Abdalla et al. (2019), it is important to adjust timings and dates of the planting and kill of the cover crops, to avoid competition with the primary crop, to improve their effectiveness and avoid trying to establish cover crops when soil conditions are suboptimal (potentially increasing soil erosion losses, and nitrate leaching). Conservation agriculture is emerging as a more sustainable way for soil conservation in Northern Italy. However, application is still scant and it is not easy to convince farmers to adopt new practices. University of Milan, in collaboration with Condifesa, a local technical consortium for crop protection, had initiated some experiments to test some cover crops and determine their contribution in improving soil fertility with nitrogen that will be available for the subsequent main crop (mostly maize in this geographical area). Choice of cover crops was made based on their behavior in winter as winter-hardy or winter killed with more interest on the latest due to low temperatures registered in December and January. Also, compared to keeping soil bare, sowing cover crops have several benefits such as preventing soil erosion (Kaspar et al., 2001), preventing nutrient leaching (Dabney et al., 2001), increasing soil carbon inputs (Moore et al., 2014), and suppressing soil diseases and pests. Sowing date is a key factor for a successful establishment of the cover crop. It is known that cover crops should be sown as early as possible to have a better establishment of the cover crops and weed suppression. However, an early sowing, immediately after harvest of the previous main cash crop, can be done in unfavorable climatic conditions where the soil is still dry before the first precipitations in September that will influence on the germination of cover crops seeds. In this context, a two-year field trial was conducted in Northern Italy (Brescia, Lombardy region) to quantify the combined effects of two consecutive cover crops sowing dates (end August vs. mid-September) and five cover crop species (black oat, *Avena strigosa* Schreb.; cereal rye, *Secale cereale*. L.; white mustard, *Sinapis alba* L.; Egyptian clover *Trifolium alexandrinum* L.; and hairy vetch, *Viccia villosa* Roth) plus one control treatment (no cover (-), plot left with spontaneous growth of weed) on above-ground dry matter accumulation (of cover crops and weed), N concentration and N uptake of cover crops in autumn (November) and spring (cover crop termination, March).

2. Material and methods

2.1. Field presentation

The experiment was carried out in the framework of the CoCrop project funded by the Lombardy region Program of Rural Development 2014-2020, Measure 16.2.01 (https://sites.unimi.it/cocrop/). The field was located in Orzinuovi, Brescia, Italy (45°23’55.2”N, 9°54’30.2”E). The soil texture was sandy loam. Organic matter was 4%, carbon to nitrogen ratio was 9.1 and pH in soil: water was 6.8.

2.2. Experimental design

The trial (Figure 2.1) has two factors: sowing date and cover crop species. The sowing date factor is presented in two levels. For the first year cover crops were sown in August, 30th 2017 (SD1) and September, 14th 2017 (SD2). For the second year, sowing dates were September, 5th 2018 (SD1) and September, 18th 2018 (SD2). The second factor is presented as seven levels that are five cover crop species: black oat (cv. “Saia 6”), cereal rye (cv. “Stanko”), white mustard (cv. “Architect”), Egyptian clover (cv. “Mario”) and hairy vetch (cv. “Villana”). In addition to the five cover crop species, we had two control treatments: one control treatment left without weed control (growth of spontaneous weed; no cover (-)) and a second control treatment with weed control (application of herbicides; no cover (+)). These treatments were replicated four times (4 blocks) and therefore in total there were 56 plots (6 m wide × 8 m long). Factors were arranged in a split-plot design, with the sowing date in the main plot and the soil cover in the sub-plot. Each plot hosted the following treatments during the two years of experiment:

- A cover crop treatment/plot kept bare (no cover treatment) from September 2017 to March 2018;
- Maize from April 2018 to August 2018;
- A cover crop treatment/plot kept bare (no cover treatment) from September 2018 to March 2019;
- Maize from March 2019 to August 2019.

For each plot, the same cover/no cover treatment was applied in both years.

The cover crop species can be grouped according to their behavior in winter:

- Winter-hardy cover crops: cereal rye and hairy vetch
- Winter-killed cover crops: black oat, Egyptian clover and white mustard

Also, these cover crops can be grouped according to their N fixation:

- Legume cover crops: hairy vetch and Egyptian clover
- Non-legume cover crops: cereal rye, black oat and white mustard.

Block 1 Sowing date 1	cereal rye	hairy vetch	white mustard	black oat	Egyptian clover	no cover (+)	no cover (-)
Block 1 Sowing date 2	no cover (+)	cereal rye	Egyptian clover	hairy vetch	black oat	no cover (-)	white mustard
Block 2 Sowing date 2	no cover (-)	no cover (+)	black oat	Egyptian clover	hairy vetch	white mustard	cereal rye
Block 2 Sowing date 1	Egyptian clover	white mustard	black oat	cereal rye	no cover (+)	hairy vetch	no cover (-)
Block 3 Sowing date 1	no cover (+)	Egyptian clover	white mustard	hairy vetch	cereal rye	no cover (-)	black oat
Block 3 Sowing date 2	hairy vetch	cereal rye	no cover (-)	no cover (+)	black oat	Egyptian clover	white mustard
Block 4 Sowing date 2	cereal rye	Egyptian clover	no cover (-)	hairy vetch	black oat	white mustard	no cover (+)
Block 4 Sowing date 1	black oat	white mustard	cereal rye	no cover (-)	hairy vetch	Egyptian clover	no cover (+)

8m ***6m***

Figure 2.1. *Experimental design. No cover (-): plots (bare soil) left without weed control (growth of spontaneous weed) and no cover (+): plots (bare soil) with weed control (application of herbicides).*

Table 2.1 presents the main activities of crop and soil management and above-ground biomass sampling dates. In mid-March, cover crops were terminated and ten days later soil was tilled as a seedbed preparation for maize sowing in spring.

Table 2.1. *Crop and soil management and above-ground biomass sampling dates.*

Year	Date	Activity	Notes
	30/08/2017	Cover crop sowing	First sowing date
	14/09/2017	Cover crop sowing	Second sowing date
1	22/11/2017	Biomass sampling	
	14/03/2018	Biomass sampling + cover crop termination	
	25/03/2018	Soil tillage (seedbed preparation for maize)	
	05/09/2018	Cover crop sowing	First sowing date
	18/09/2018	Cover crop sowing	Second sowing date
2	22/11/2018	Biomass sampling	
	12/03/2019	Biomass sampling + cover crop termination	
	22/03/2019	Soil tillage (seedbed preparation for maize)	

Table 2.2 presents the sowing rates of the different cover crop species at the first and second sowing dates.

Table 2.2. *Seed rates of cover crop species.*

Cover crop species	Seed rates (kg ha^{-1})
cereal rye	150
hairy vetch	40
white mustard	15
black oat	50
Egyptian clover	25

2.3. Measurements

Samples of cover crops and weed above-ground biomass (AGB) were taken in November, before winter low temperatures, (all treatments) and March, at cover crops termination, (for the first year, only cereal rye and no cover (-) samples were taken while for the second year, samples were taken for all treatments). A sample (1 m^2) was taken from each plot and then dried in the stove at 105 °C. Fresh and dry weights were taken and then the above-ground biomass was calculated.

Each biomass sample could contain cover crops and weed. In the first year, if weed was less than 10% of total above-ground biomass, a unique "weed + cover" crop sample was created by mixing the two components; otherwise, cover crop and weed samples were kept separate for further analysis.
Above-ground biomass samples were grinded at 0.2 mm using Ultra Centrifugal Mill ZM 200 (Retsch, Haan, Germany). Then, nitrogen concentration was determined using an elemental analyzer Carlo Erba NC Thermoquest, model NA 1500 series 2 (Carlo Erba, Milano, Italy).
N uptake was calculated as the product of AGB by nitrogen concentration.
The nitrogen dilution curve of the different cover crop species was determined with data recorded during the two years of cover crops cultivation.

2.4. Precipitation and growing degree days

Average daily temperature and daily precipitation were registered from an ARPA weather station close to the field.
Growing Degree Days (GDD) were calculated according to the averaging method.

GDD = (max + min temperature) / 2 - Base temperature

If answer was negative, GDD were counted as 0.
Cover crops base temperatures are presented in Table 2.3.

Table 2.3. *Cover crops base temperatures (Tribouillois et al., 2016).*

Cover crop species	Base temperature (°C)
cereal rye	0.6
hairy vetch	1.4
white mustard	1.2
black oat	4.8
Egyptian clover	6.1

2.5. Statistical analysis

Analysis of variance was conducted to determine the effect of factors (cover crop species and sowing date) on cover crops and weed AGB, nitrogen concentration and uptake using SPSS 25. The analysis was carried out for both years. If a significant interaction year x cover crop sowing date x cover crop species was found, the least significant difference (LSD) was calculated. Homogeneity of variance and normality of distribution of data were checked before analysis of variance. Post-hoc comparisons were made using SIDAK tests.

3. Results

3.1. Precipitation and Growing Degree Days

Cumulative precipitations and GDD of the five cover crop species during two years are presented in Figure 2.2. Cumulative precipitation (Figure 2.2 a) was variable within years, with higher cumulative precipitation for the first year (394 mm) than the second year (277 mm). Variability of cumulative precipitation within years can be presented by intervals as:

- higher precipitation for the first year than the second year from sowing (first sowing date) to 45 days after sowing (85 vs. 21 mm, respectively);
- higher precipitation for the second year than the first year between 45th and 60th day after sowing (130 vs. 2 mm, respectively);
- higher precipitation for the first year than the second year 60 to 135 days after sowing with variability within dates: (i) 105 vs. 57 mm, respectively, during the third month of cover crops cultivation (60 to 90 days after sowing); (ii) 59 vs. 21 mm from 90 to 120 days after sowing and; (iii) 44 vs. 5 mm between 120 and 135 days after sowing;
- higher precipitation for the second year than the first year between 135 and 150 days after sowing (20 vs. 6 mm, respectively);
- very low precipitation for the second year (less than 1 mm) compared to the first year (73 mm) during the last 40 days before cover crops termination (150 to 190 days after sowing).

As a summary, precipitation has sustained the initial growth of cover crops in the first year with 83 mm in the first 21 days after sowing (SD1) then it was very low (4 mm) during the following 45 days while in the second year, during the first 52 days after sowing (SD1), precipitation was 21 mm and then we recorded an important precipitation (136 mm) in 12 days.

Cumulative GDD of cover crop species were variable within years and cover crops sowing dates. Temperatures were lower in the first year than in the second year. All cover crop species had a higher GDD in the second year compared to the first year. Cereal rye, white mustard and hairy vetch, comparatively to black oat and Egyptian clover, had in general a continuous accumulation of GDD from sowing to termination for both years. Egyptian clover had the lowest cumulative GDD in the first year compared to the second year (-15.5 and -19.4% for the first and the second sowing date, respectively) and was followed by black oat (-12.7 and -15.7% for the first and the second sowing date, respectively) while cereal rye had a 5.7 and 7.2% (for the first and the second sowing date, respectively) less cumulative GDD in the first year compared to the second year and this variability is explained by the differences in the base temperature of the different cover crop species as presented in Table 2.3. For the first year, average temperature does not exceeded, in general, 4.8° C from December to March and then cumulative GDD of black oat and Egyptian clover was stable starting from 90 days after sowing to cover crops termination, while for the second year, average temperature exceeded 4.8° C from mid–February to cover crops termination (mid-March) and in consequence cumulative GDD of both cover crops (Egyptian clover and black oat) increased, after a stable period, from 166 days after sowing to cover crops termination. Delaying sowing dates by 15 days has affected the cumulative GDD of cover crop species in the first year more than the second year. In fact, Egyptian clover was more affected by the delay of sowing date for both years than other cover crops with 26.3 and 22.8% less cumulative GDD for the first and the second year, respectively. Cereal rye was the less affected cover crop by the delay of sowing dates for both years. It has accumulated 17.6 and 16.3% less cumulative GDD for both years.

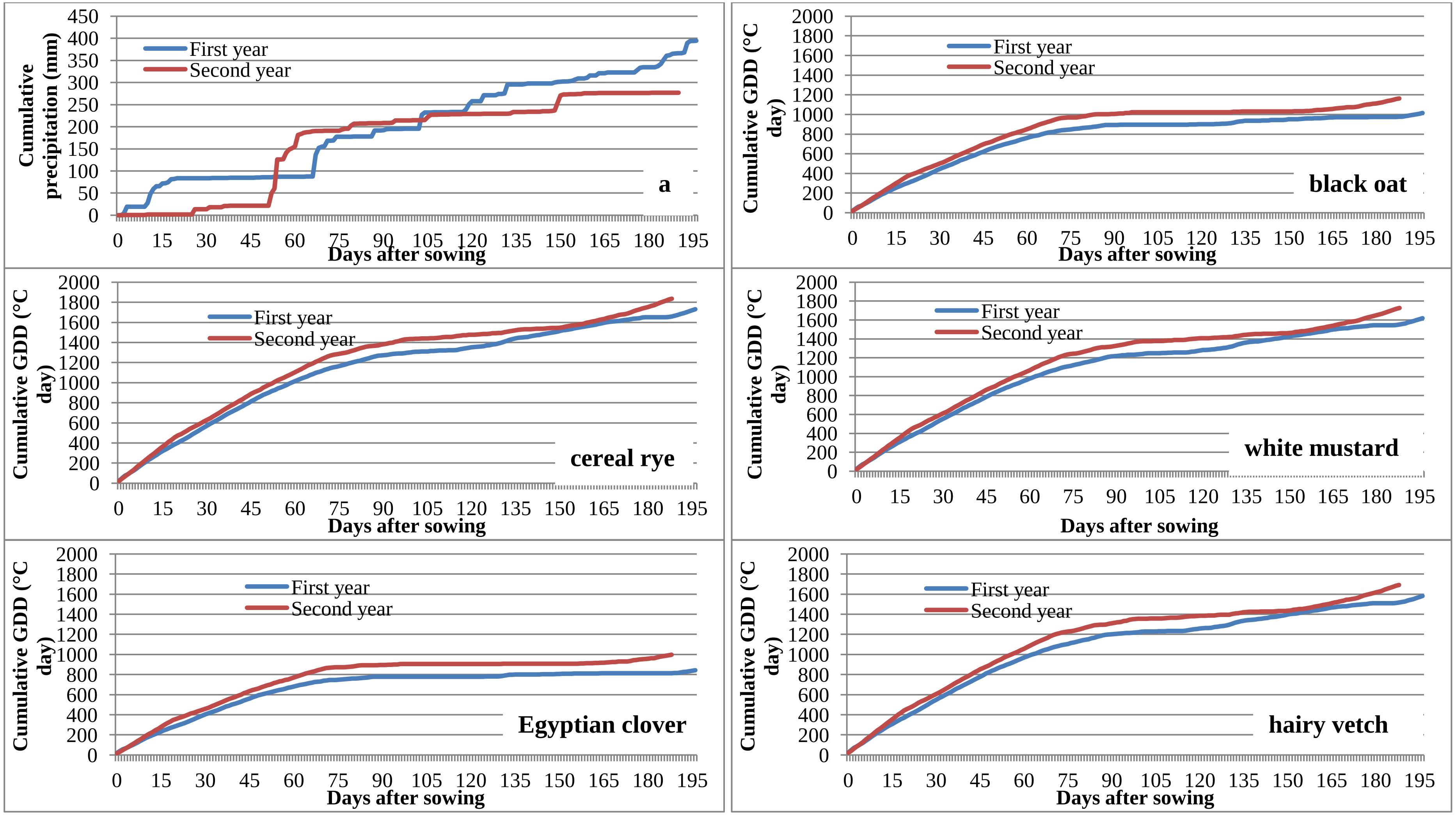


Figure 2.2. *Cumulative precipitation (a) and cumulative Growing Degree Days (GDD) of five cover crop species for two years of cover crop cultivation from SD1 to cover crops termination. SD2 corresponds to the 15th and the 13th day after sowing for the first and the second year, respectively.*

3.2. Cover crops and weed above-ground biomass

3.2.1. Cover crops above-ground biomass

For the first year, November AGB of cover crops (without weed) ranged in most cases between 1 and 3 t ha^{-1} (Table 2.4). For the second year, November AGB values were in general lower compared to the first year. For both years, mustard SD1 reached the highest AGB (5.3 and 3.3 t ha^{-1} for first and second year, respectively). A highly significant interaction between sowing date and cover crop species was detected (P < 0.001) for both years. While rye and clover were not affected by sowing date, the AGB of mustard, oat and vetch was significantly lower for SD2 compared to SD1, for the first year. Biomass of mustard was significantly higher than all other species for both SDs. Clover and rye (1.0 and 1.5 t ha^{-1}, respectively) were significantly lower than the other cover crops for SD1, while for SD2 this was true for clover, rye and vetch (0.8, 1.4 and 1.4 t ha^{-1}, respectively). For the second year, all cover crops but rye showed a significant (P < 0.05) decrease of AGB for SD2 compared to SD1; for rye, the opposite occurred, because the AGB for SD1 was close to zero (20 kg ha^{-1}), due to an attack of *Duponchelia fovealis* Zeller larvae in the few days after sowing. Mustard sown in SD1 had a significantly higher AGB than all the other species, while no differences among species were found for SD2.

For the first year, the biomass was sampled in March only for rye and the no cover (-) treatment (with spontaneous weed vegetation). For the second year, March AGB of cover crops (without weed) was less than 2 t ha^{-1} (Table 2.4); a significant interaction between sowing date and cover crop species was detected (P<0.01). March AGB of all cover crop species was affected by sowing date, except rye. For SD1, mustard, oat and vetch had a significant higher AGB (2.1, 2.0 and 1.9 t ha^{-1}, respectively) compared to rye and clover (0.3 and 0.7 t ha^{-1}). However, for SD2, mustard was significantly higher than rye, clover and vetch.

3.2.2. Weed above-ground biomass

For the first year, November weed AGB ranged in most cases between 0.1 and 0.4 t ha^{-1}, and no cover (-) treatment reached 1.9 t ha^{-1}, while for the second year, November weed AGB ranged between 0.3 and 1.9 t ha^{-1} (Table 2.4). A highly significant interaction was identified between the year, the sowing date and the cover crop species (P<0.001). For the first year, for SD1, weed in no cover (-) and clover (1.8 and 1.3 t ha^{-1}) was significantly higher than in all other cover crops, while for SD2 only no cover (-) treatment (1.9 t ha^{-1}) remained significantly higher than all cover crop species. For the second year, a significant difference was found only for clover between SD1 and SD2 (P<0.05). For SD1, no cover (-) AGB (1.8 t ha^{-1}) was significantly higher than mustard (0.3 t ha^{-1}) and no significant differences were observed between other treatments and for all treatments belonging to SD2.

For the second year, March weed AGB ranged between less than 0.1 (0.04 t ha^{-1}) and 1.8 t ha^{-1}. For both SDs, no cover (-) weed AGB was significantly higher than weed AGB in rye (1.0 and 1.8 t ha^{-1} compared to 0.04 and 0.4 t ha^{-1}).

Table 2.4. *Cover crops and weed AGB (above-ground biomass) in November 2017, March 2018, November 2018 and March 2019. Each value represents the mean of four measurements. SE: Standard Error. The averages of each value followed by the same letter do not differ statistically between cover crop species within the same sowing date. No cover (-): plots (bare soil) left without weed control (growth of spontaneous weed).*

		Cover crops AGB (t ha^{-1})				Weed AGB (t ha^{-1})			
	Cover crop	Sampling dates				Sampling dates			
Sowing date	**Species**	**November 2017**	**March 2018**	**November 2018**	**March 2019**	**November 2017**	**March 2018**	**November 2018**	**March 2019**
SD1	**black oat**	3.10 [b]		2.12 [b]	2.05 [a]	0.18 [b]		0.54 [a]	0.10 [b]
	cereal rye	1.48 [c]	2.09	0.02 [c]	0.25 [b]	0.22 [b]	0.04 [b]	1.36 [a]	0.76 [a]
	white mustard	5.29 [a]		3.30 [a]	2.14 [a]	0.06 [b]		0.30 [b]	0.04 [b]
	no cover (-)					1.82 [a]	1.00 [a]	1.79 [a]	0.79 [a]
	Egyptian clover	1.04 [c]		1.71 [b]	0.72 [b]	1.28 [a]		0.95 [a]	0.43 [a]
	hairy vetch	2.86 [b]		1.35 [b]	1.90 [a]	0.29 [b]		0.99 [a]	0.22 [b]
SD2	**black oat**	2.05 [b]		0.86 [ab]	0.68 [ab]	0.17 [b]		0.86 [a]	0.34 [b]
	cereal rye	1.37 [bc]	2.12	0.33 [b]	0.54 [ab]	0.21 [b]	0.01 [b]	1.26 [a]	0.62 [ab]
	white mustard	3.15 [a]		1.15 [a]	0.68 [ab]	0.05 [b]		1.06 [a]	0.36 [b]
	no cover (-)					1.89 [a]	1.79 [a]	1.89 [a]	0.90 [a]
	Egyptian clover	0.84 [c]		0.35 [b]	0.08 [b]	0.37 [b]		1.84 [a]	0.62 [ab]
	hairy vetch	1.41 [bc]		0.53 [b]	0.97 [a]	0.37 [b]		1.61 [a]	0.40 [b]
	SE	0.36		0.32	0.23	0.86		0.15	0.08

3.3. Relationship between cumulative Growing Degree Days and cover crops/weed above-ground biomass

3.3.1. Cover crops Above-Ground Biomass

Figure 2.3 a shows the relationship between cover crops cumulative GDD and AGB in November and March of both years of cultivation. In November, we notice that different cover crop species sown at SD1 had a higher GDD accumulated in the second year than those sown at SD2 in the first year but in both cases the AGB was very close. It is the example of black oat which was had a biomass of 2.05 t ha^{-1} in the first year when it was sown at SD2 and 2.12 t ha^{-1} in the second year when it was sown in SD1 but with 53% more of GDD cumulated in the second year. The second example is white mustard: a higher accumulation of GDD (+42%) in the second year (mustard sown at SD1) allowed a similar AGB to mustard sown at SD2 in the first year. Hairy vetch is the third cover crop that had a similar AGB with higher cumulative GDD (+34%) in the second year (SD1) compared to the first year (SD2). At cover crops termination (March), in the second year (we did not collect samples in the first year), the AGB of black oat, white mustard and Egyptian clover decreased compared to the AGB in November due to that this three cover crops are winter-killed while for cereal rye and hairy vetch (two winter-hardy cover crops) the AGB increased from November to March. For SD1, cereal rye had the highest rate of AGB increase due to the attack of *Duponchelia fovealis* Zeller larvae in the few days after sowing while for hairy vetch the AGB increased by 64%. For SD2, the AGB recorded in March was 41 and 83% higher than in November, for cereal rye and hairy vetch, respectively.

3.3.2. Weed Above-Ground Biomass

Figure 2.3 b shows the relationship between cumulative GDD and weed AGB. It shows that the weed AGB was higher in the second year compared to the first year for samples collected in November, for all cover crop species. Also, for the same sampling date (November), the weed AGB was higher in SD2 compared to SD1 for both years, except Egyptian clover in the first year. Compared to November, the weed AGB in March decreased for all cover crop species in the second year.

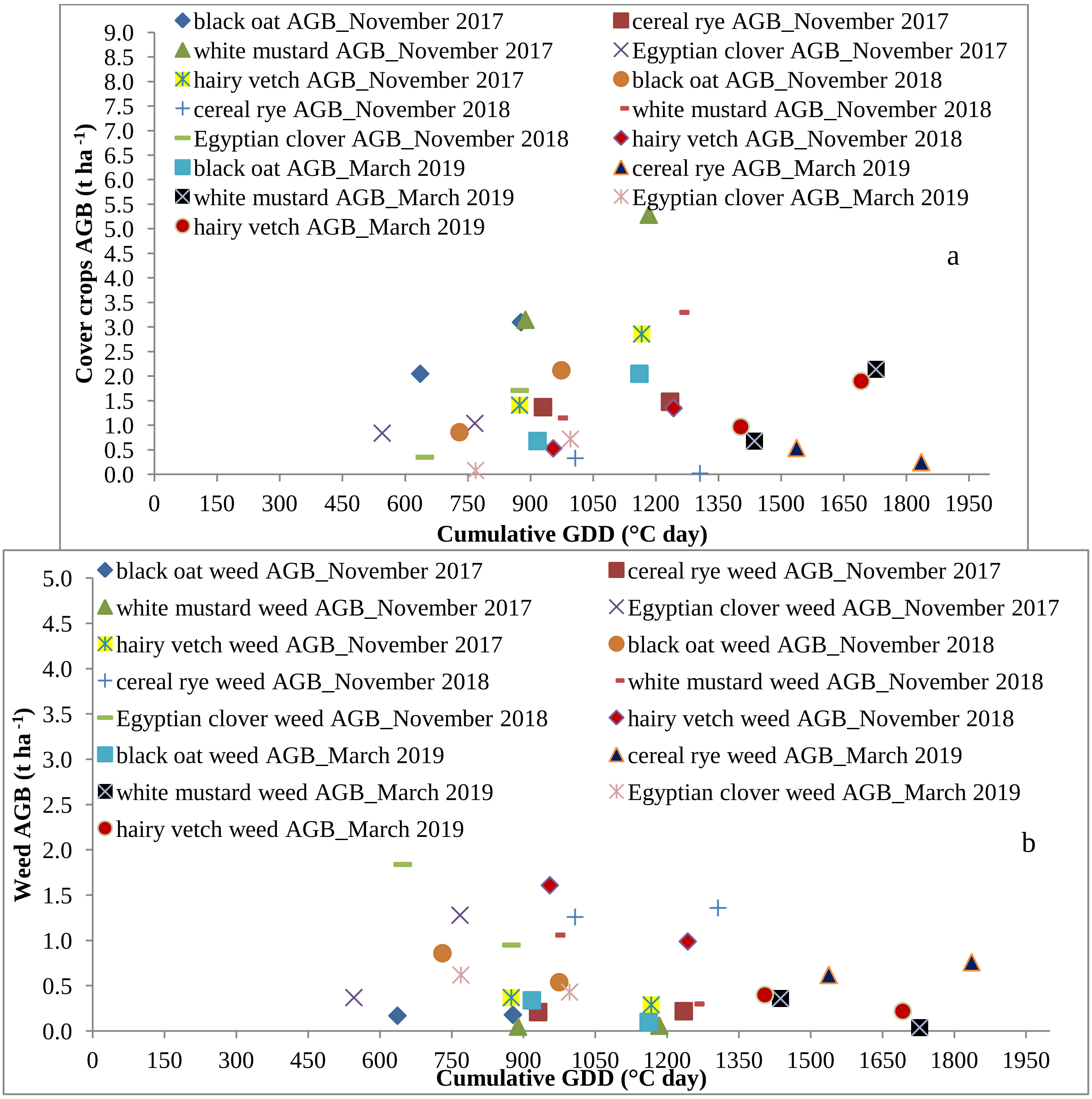


Figure 2.3. *Relationship between cumulative Growing Degree Days and cover crops (a) / weed (b) Above ground biomass during two years of experiment.*

3.4. Cover crops and weed nitrogen concentration

3.4.1. Cover crops nitrogen concentration

November N concentration of cover crops was similar for both years. It ranged between 1.7 and 4.0% (Table 2.5). A significant interaction between sowing date and cover crop species was detected in both years (*P*<0.01). For the first year, N concentration was significantly higher for SD2 than SD1 only for non-legume cover crops For SD1, vetch was significantly higher (4%) than all other cover crops, followed by clover (3%) significantly higher than rye, oat and mustard (2.4, 1.9 and 1.7 %, respectively). Oat and mustard had significantly lower N concentration than other studied cover crops. For SD2, legume cover crops had significantly higher N concentration than non-legume cover crops. Compared to SD1, for SD2 N concentration of rye was not significantly different than oat and mustard. For the second year, November nitrogen concentration of rye and vetch was not affected by sowing date, while oat, mustard and clover had significantly lower nitrogen concentration for SD1 than SD2. For SD1, vetch N concentration was significantly higher (3.6%) than clover, mustard and oat (2.8, 2 and 1.9%, respectively). For SD2, nitrogen concentration of vetch was significantly higher (3.8%) than all cover crops. Vetch was followed by rye and clover (3.3%).

For the second year, cover crops March N concentration ranged between 1.4 and 3.9% (Table 2.5). A significant interaction between sowing date and cover crops species was detected ($P<0.01$). Only mustard was significantly affected by sowing date ($P<0.001$). For both sowing dates, legume cover crops had significantly higher nitrogen concentration than non-legume cover crops. For SD1, vetch had the highest nitrogen concentration (3.9%) and was significantly higher than clover (2.9%). For SD2, difference in nitrogen concentration between vetch and clover was lower than SD1 (3.7 and 3.1%, respectively) but a significant difference between both cover crops was maintained.

3.4.2. Weed nitrogen concentration

For the first year, November weed nitrogen concentration was on average 2.4 % (Table 2.5). For SD1, weed N concentration under clover was significantly higher than in rye. No significant different N concentration in weed biomass was identified among cover crops for SD2. For the second year, November weed nitrogen concentration ranged between 1.8 and 2.5% and was not affected by sowing date. For both sowing dates, no significant differences were identified between cover crops.

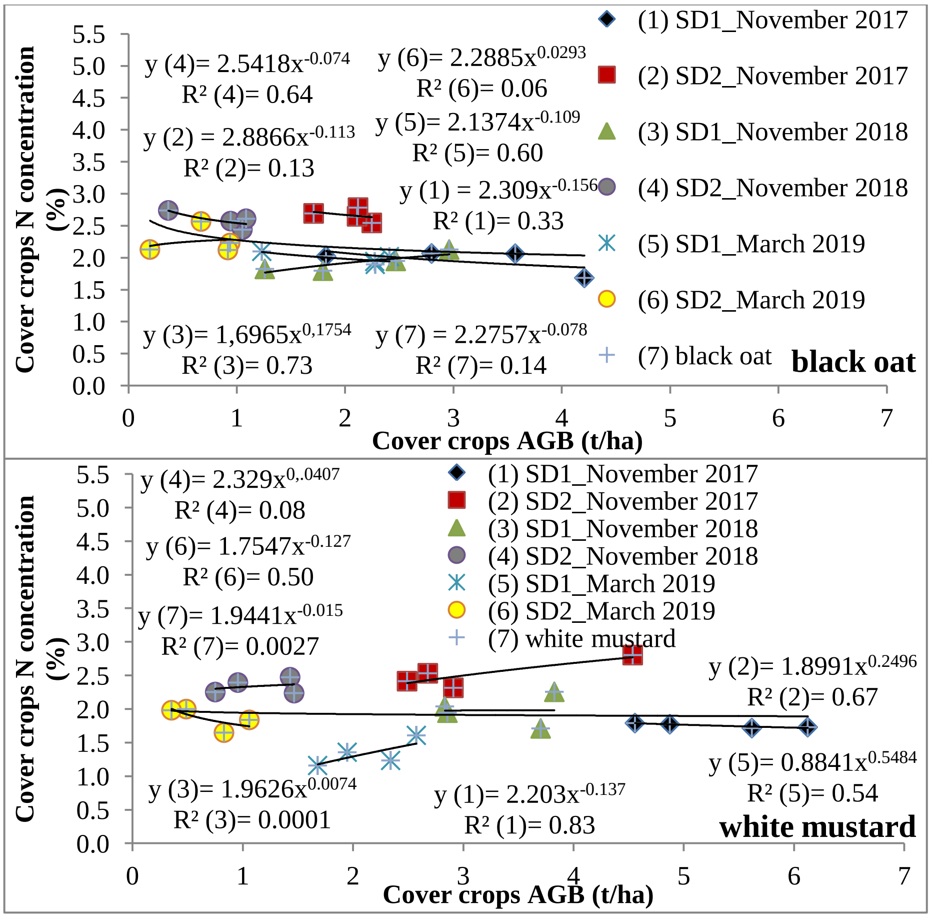
For the second year, March weed nitrogen concentration ranged between 1.3 and 2.6%. Weed of mustard was significantly affected by sowing date, while weed of other cover crops was not. Cover crops weed nitrogen concentration was lower for SD2 than for SD1. Weed nitrogen concentration in mustard and oat (2.6 and 2.4%, respectively) were significantly higher than in rye and no cover (-) (1.6 and 1.3%, respectively) for SD1. For SD2, oat weed nitrogen concentration was significantly higher than rye and no cover (-), while mustard was not.

Table 2.5. *Cover crops and weed N concentration in November 2017, March 2018, November 2018 and March 2019. Each value represents the mean of four measures. SE: Standard Error. The averages of each value followed by the same letter do not differ statistically between cover crop species within the same sowing date. No cover (-): plots (bare soil) left without weed control (growth of spontaneous weed).*

		Cover crops nitrogen concentration (%)				Weed nitrogen concentration (%)			
		Sampling dates				Sampling dates			
Sowing date	**Cover crop Species**	**November 2017**	**March 2018**	**November 2018**	**March 2019**	**November 2017**	**March 2018**	**November 2018**	**March 2019**
SD1	**black oat**	1.83 ^{d}		1.92 ^{c}	2.00 ^{c}			1.75 ^{a}	2.46 ^{a}
	cereal rye	2.38 ^{c}	2.21	3.25 ^{ab}	2.56 ^{b}	1.65 ^{b}	2.21 ^{a}	2.10 ^{a}	1.66 ^{ab}
	white mustard	1.69 ^{d}		1.97 ^{c}	1.38 ^{d}			1.91 ^{a}	2.63 ^{a}
	no cover (-)					2.18 ^{a}	1.97 ^{a}	1.74 ^{a}	1.34 ^{b}
	Egyptian clover	3.04 ^{b}		2.78 ^{b}	2.90 ^{b}	2.53 ^{a}		2.47 ^{a}	1.94 ^{ab}
	hairy vetch	3.98 ^{a}		3.60 ^{a}	3.85 ^{a}	2.48 ^{a}		2.18 ^{a}	2.04 ^{a}
SD2	**black oat**	2.57 ^{c}		2.65 ^{c}	2.26 ^{c}	1.85 b		1.82 ^{a}	2.23 ^{a}
	cereal rye	2.72 ^{c}	2.28	3.33 ^{b}	2.26 ^{c}	2.10 ^{b}		2.04 ^{a}	1.42 ^{b}
	white mustard	2.41 ^{c}		2.36 ^{c}	1.84 ^{c}			2.10 ^{a}	1.81 ^{ab}
	no cover (-)					2.12 ^{ab}	1.85	1.83 ^{a}	1.48 ^{b}
	Egyptian clover	3.28 ^{b}		3.25 ^{b}	3.11 ^{b}	2.61 ^{a}		2.50 ^{a}	1.81 ^{ab}
	hairy vetch	4.03 ^{a}		3.72 ^{a}	3.71 ^{a}	2.59 ^{a}		2.16 ^{a}	1.67 ^{ab}
	SE	0.25	0.04	0.20	0.25	0.11	0.11	0.07	0.12

3.5. Nitrogen dilution curve

The fitting of the power function N concentration (%) = a DM^b (Justes et al., 1994). For black oat, white mustard and Egyptian clover, nitrogen dilution curve (Figure 2.4) shows that delaying the sowing date by 15 days, the cover crops AGB decreases and the nitrogen concentration increases for both years in November. For hairy vetch, a delay of the cover crop sowing date induced a decrease in the biomass but nitrogen concentration was constant in November and March of both years. For cereal rye, a 15 days delay in the sowing date has not affected the AGB and the nitrogen concentration in the first year (November and March) while for the second year, the cereal rye AGB has increased in the second sowing date compared to the first year due to the attack of *Duponchelia fovealis* Zeller larvae in the few days after the first sowing. In general, cereal rye presented the highest coefficient of determination (R^2) among all cover crop species (R^2=0.35).



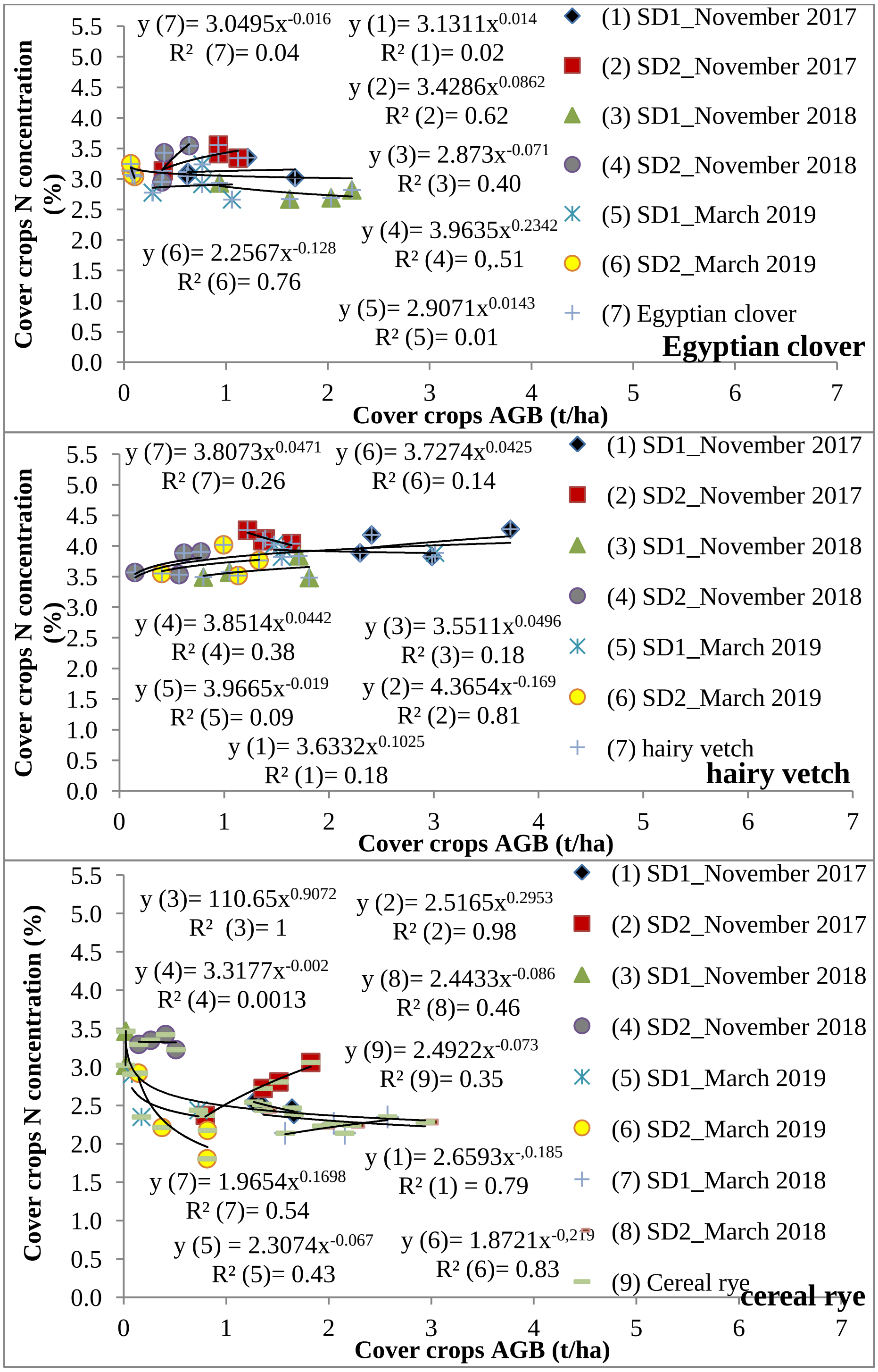


Figure 2.4. *Nitrogen dilution curve of cover crop species as affected by sowing dates and sampling dates during two years of cultivation.*

3.6. C/N ratio of cover crop and weed biomass

3.6.1. Cover crops C/N ratio

For the first year, November cover crops C/N ratio ranged between 10.1 (minimum of legume cover crops) and 23.4 (maximum of non-legume cover crops), while for the second year, it ranged between 10.2 (minimum of legume cover crops; vetch SD1) and 20.8 (maximum of non-legume cover crops; oat SD1). A highly significant interaction between sowing date and cover crop species was detected (P<0.001) for both years (Table 2.6). Legume cover crops were not affected by sowing date, while non-legume cover crops were affected and C/N ratio was significantly lower for SD2 than for SD1. Mustard and oat had a significant higher C/N ratio (23.4 (SD1) and 15.9 (SD2) for mustard and 22.1 (SD1) and 20.8 (SD2) for oat, respectively) than rye (17.2 and 15.1 for SD1 and SD2, respectively), clover (13.6 and 12.3 for SD1 and SD2, respectively) and vetch (10.6 and 10.1 for SD1 and SD2, respectively) for both sowing dates. Compared to the first year, clover C/N ratio was highly affected by sowing date (P<0.01) for the second year. Vetch C/N ratio was significantly lower than all cover crops for SD1, while for SD2, it was significantly lower than clover, oat and mustard.

For the second year, March C/N ratio ranged in most cases between 13 and 22, with mustard SD1 reaching 33. A highly significant interaction between sowing date and cover crop species was detected (P<0.001). Among all cover crops, only mustard was affected by sowing date. For SD1, mustard was significantly higher than all cover crops, while for SD2 it was significantly higher than legume cover crops.

3.6.2. Weed C/N ratio

For the first year, November weed C/N ratio ranged between 13.9 and 23.6 (Table 2.6), with weed of rye SD1 having the highest C/N ratio; weed C/N ratio in rye plots was affected by sowing date (significantly higher for SD1 than SD2). Rye weed C/N ratio was significantly higher than legume weed for SD1, while for SD2 was not. For the second year, November weed C/N ratio ranged between 16 and 23.5 and no significant differences were identified within sowing date or cover crop species.

For the second year, March weed C/N ratio ranged between 14.1 and 29.6. A highly significant interaction between sowing date and cover crop species was detected (P<0.01). Mustard and vetch weed C/N ratio were significantly affected by sowing date. Weed C/N ratio of plots with no cover (-) treatment was significantly different than oat, mustard and legume cover crops for SD1, while for SD2 no significant differences were identified.

Table 2.6. *Cover crops and weed C/N ratio in November 2017, March 2018, November 2018 and March 2019. Each value represents the mean of four measures. SE: Standard Error. The averages of each value followed by the same letter do not differ statistically between cover crop species within the same sowing date. No cover (-): plots (bare soil) left without weed control (growth of spontaneous weed).*

	Cover crop	Cover crops C/N ratio				Weed C/N ratio			
		Sampling dates				Sampling dates			
Sowing date	**Species**	**November 2017**	**March 2018**	**November 2018**	**March 2019**	**November 2017**	**March 2018**	**November 2018**	**March 2019**
SD1	**black oat**	22.06 ^{a}		20.78 ^{a}	22.14 ^{b}			23.28 ^{a}	16.92 ^{b}
	cereal rye	17.15 ^{b}	19.31	12.52 ^{a}	17.07 ^{bc}	23.64 ^{a}		18.29 ^{a}	24.54 ^{ab}
	white mustard	23.41 ^{a}		20.37 ^{a}	33.01 ^{a}			20.28 ^{a}	14.11 ^{b}
	no cover (-)					17.44 ^{ab}	20.16	23.45 ^{a}	29.63 ^{a}
	Egyptian clover	13.56 ^{c}		14.92 ^{b}	15.31 ^{cd}	15.45 ^{b}		16.03 ^{a}	22.78 ^{b}
	hairy vetch	10.62 ^{d}		10.24 ^{c}	11.57 ^{d}	14.89 ^{b}		19.72 ^{a}	19.86 ^{b}
SD2	**black oat**	16.02 ^{a}		15.55 ^{a}	19.73 ^{a}	21.03 ^{a}		19.83 ^{a}	19.29 ^{b}
	cereal rye	15.09 ^{a}	18.71	11.49 ^{bc}	19.94 ^{a}	17.85 ^{b}		19.67 ^{a}	28.79 ^{a}
	white mustard	15.94 ^{a}		16.95 ^{a}	23.62 ^{a}			19.01 ^{a}	24.26 ^{ab}
	no cover (-)					15.20 ^{bc}	21.28	21.47 ^{a}	26.05 ^{ab}
	Egyptian clover	12.28 ^{b}		12.59 ^{b}	13.97 ^{b}	14.52 ^{c}		17.58 ^{a}	23.77 ^{ab}
	hairy vetch	10.14 ^{c}		10.39 ^{c}	12.12 ^{b}	13.90 ^{c}		18.52 ^{a}	26.29 ^{ab}
	SE	1.40		1.21	2.04	1.10		0.63	1.35

3.7. Above-ground cover crop nitrogen uptake

For the first sampling (November of the first year), samples of cover crops and weed were mixed in a unique "weed + cover" sample if weed biomass was less than 10% of total above-ground biomass.

A significant interaction was identified between the year, the sowing date and the cover crop species (P<0.05). For the first year, November N uptake ranged between 28 and 114 kg N ha^{-1} (Figure 2.5), with hairy vetch SD1 reaching the highest uptake (114 kg N ha^{-1}), while, for the second year, November N uptake ranged between 0.3 (cereal rye SD1 affected by the attack of *Duponchelia fovealis* Zeller larvae in the few days after sowing) and 65.7 kg N ha^{-1} (mustard SD1). A significant interaction was identified between sowing date and cover crop species (P<0.01 and P<0.001 for the first and the second year, respectively). For the first year, hairy vetch and white mustard SD1 had significantly higher N uptake (114.1 and 88.9 kg N ha^{-1}, respectively) compared to other cover crops while for the second year no significant differences were identified between all the cover crop species. For the first year, sowing date factor was significant only for hairy vetch (that reached higher N uptake for SD1 than for SD2), and not significant for other cover crops. For the second year, all cover crop species had a significantly higher N uptake for SD1 than for SD2, except rye.

March N uptake ranged between 2 and 74 kg N ha^{-1} (hairy vetch SD1). A significant interaction was identified between sowing date and cover crop species (P<0.05). Legume cover crops and oat were significantly affected by sowing date, with higher N uptake for SD1 than for SD2, while non-legume cover crops (cereal rye and white mustard) were not affected by sowing date For SD1, vetch had a significantly higher N uptake than all cover crop species, while for SD2, it was significantly higher than clover N uptake only.

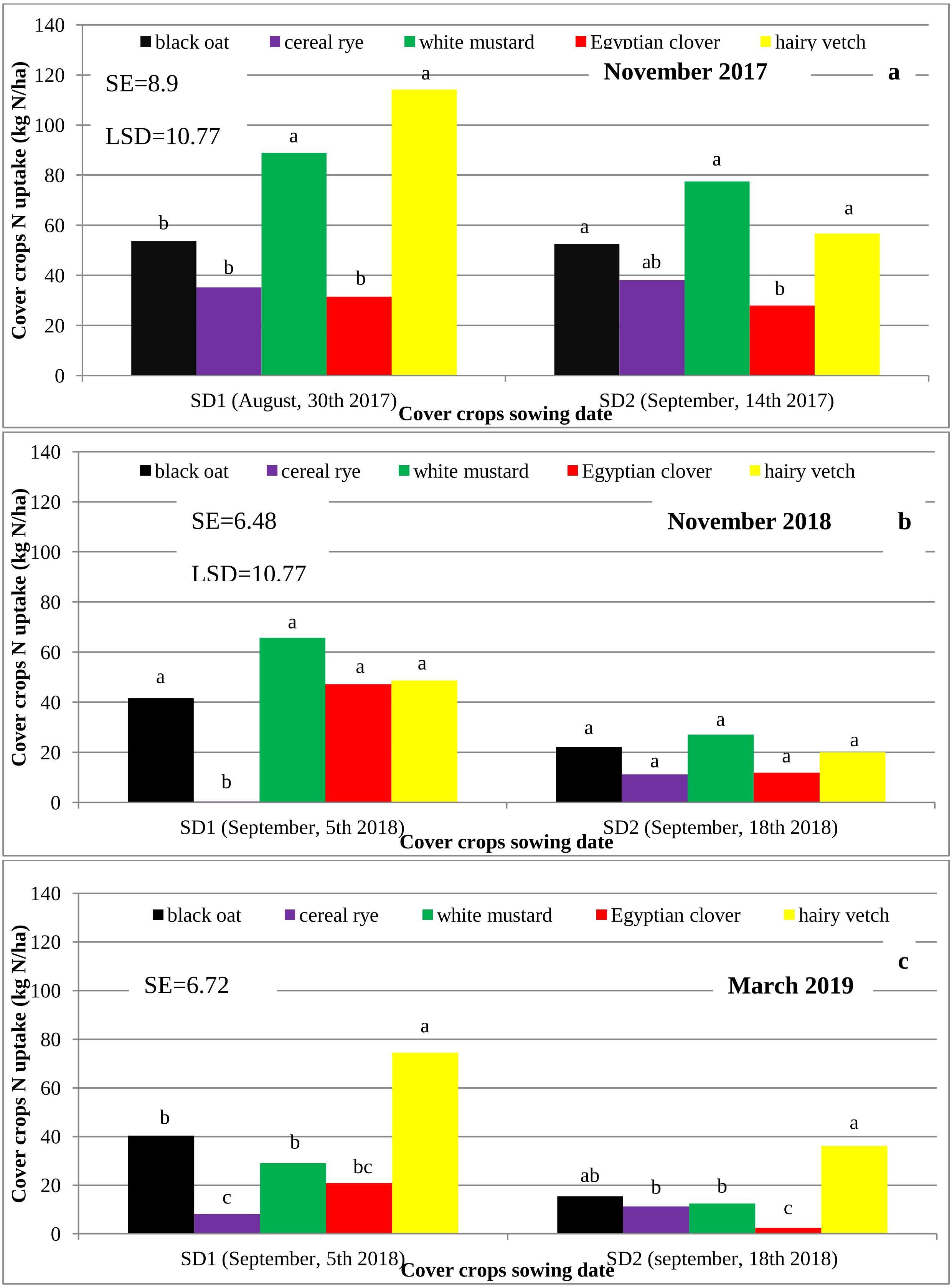


Figure 2.5. *Cover crops N uptake in November 2017 (a), November 2018 (b) and March 2019 (c). Each value represents the mean of four measures. SE: Standard Error. LSD: Least Significant Difference. The average of each value followed by the same letter do not differ statistically between cover crop species within the same sowing date.*

4. Discussion

4.1. Cover crops productivity

The variability of cover crops productivity between the different species was identified especially in the first year of cover crops cultivation SD1. According to Dorsainvil et al. (2005) and Constantin et al. (2015), germination is totally influenced by temperature and water availability in the soil. Also, Baskin and Baskin (1988) and Gummerson (1986) indicated that seed germination and seedling emergence are crucial processes that depend on meteorological conditions and are the first key steps for plant establishment. Rapid plant development after sowing is more important than the final cover crop biomass (Baraibar et al, 2018a; Brennan and Smith, 2005; Dorn et al., 2015) due to that we are more interested in the agroecological services and benefits of cover crops to soil and environment rather than final biomass at termination. In fact, cover crops could provide Biological Nitrogen Fixation (BNF), weed or pest suppression or prevention of soil erosion. Cover crops increase the functional diversity and environmental suitability of cropping systems (Drinkwater and Snapp, 2007). Moreover, cover crops are used as an agroecological practice that limit fertilizer inputs and reduce risk of water contamination due to a decreased risk of leaching (Sanchez et al., 2004; Scholberg et al., 2010), and also to reduce soil or wind erosion. In case of winter cover crops, establishment starts from late summer when cover crops are sown and a successful establishment allows to obtain the expected services of cover crops within few months (Tribouillois et al., 2016). The same authors demonstrated an immediate germination (8 to 18 h after sowing) of Brassicaceae species (mustard); 22 to 42 h after sowing, the crop reached a high final germination percentage. The low biomass of cover crops in the second year compared to the first year could be explained by weather conditions after sowing, that were unfavourable for the cover crops establishment, especially precipitation. Rainfall was close to zero in the second year (less than 1 mm, 10 days after sowing for SD1 and no precipitations during the first 10 days after sowing for SD2) while for the first year, precipitation was 19 mm, 4 days after sowing for SD1 and 10 mm, 5 days after sowing for SD2. The establishment of cover crop species is closely related to soil moisture. The lack of precipitations influenced directly the soil moisture and in consequence a difference in establishment was identified between cover crop species. Also, Tribouillois et al. (2016) mentioned the differences of base water potential of the cover crop species as a key factor for the establishment of the cover crops with Egyptian clover had a base water potential of -0.8 MPa, white mustard and hairy vetch (-1.1 MPa), black oat (-1.5 MPa) and cereal rye (-2.6 MPa). In our experiment, for both years, the effect of temperature was not revealed as a determinant factor responsible for lower biomass of cover crops because similar GDD were accumulated for both years. In fact, in November, black oat, white mustard and hairy vetch accumulated higher GDD (+53, +42 and 34%, respectively) in the second year (crops sown in SD1) compared to the first year (crops sown in SD2) but the AGB difference was negligible. Moreover, precipitation at samples collection date (November) was higher in the second year (195 mm for SD1) compared to the first year (107 mm for SD2). However, we recorded a very low accumulation of GDD for Egyptian clover from November to March (<150°C). It could be explained by the high base temperature of this cover crop (6.1°C) and the low temperatures recorded in the period between November and March. De Notaris et al. (2018) mentioned that cover crops growth is positively correlated with cumulated growing degree days (GDD). Sturm et al. (2017) indicated that lower precipitation with higher mean temperature during the vegetation period of treatments led to lower oilseed radish germination, which resulted in a reduced biomass production.

Delaying the sowing date by 15 days has affected the AGB of white mustard, black oat and hairy vetch. In fact, lower cumulative GDD was quantified by, -18.2, -18.5 and -23.8% for white mustard, hairy vetch and black oat, sown in SD2 compared to SD1, respectively for the first year while for the second year, lower cumulative GDD was for the same three cover crop species with -16.8, -17.0 and -21.0 %. Our results confirmed those of Hashemi et al. (2013), conducted in South Deerfield, Massachussets, United States, who indicated that, after 3 years of experiment, postponing the sowing date from beginning of September to mid-September induced a decrease of the oat biomass by 30 to 50%. A late sowing (mid-October) reduced the oat biomass, determined in December, by 95%, compared to oat sown in beginning of September. Also, Hashemi et al. (2013) recorded a reduction of rye biomass by 10 to 60% for a sowing in mid-September and 60 to 90% for a sowing in mid-October, compared to sowing rye early (beginning of September). They explained the significant interaction between year and cover crop sowing date on cover crops biomass by the changes in soil and air conditions. In our experiment, the increase of rye AGB was detected in the second year for SD2 respect to SD1 is due to the very low rye AGB (20 kg ha^{-1}) for the first sowing date.

4.2. Weed suppression

Our results indicated a concrete effect of cover crops in weed AGB suppression, especially for the first year. As indicated previously, the first step for a successful establishment of the cover crop within few months is seed germination. During two years of experiment, our results indicated that cover crops can produce a high amount of biomass (white mustard) and reduce weed AGB compared to keeping the soil bare. This was confirmed by a highly significant interaction year x cover crop sowing date x cover crop species and the opposite trend between cover crops and weed biomass in the second year. These findings confirmed those of Osipitan et al. (2019) who indicated that cover crop biomass was inversely related to the amount of weed biomass (r^2 = 0.67) and weed density (r^2 = 0.64). Mustard SD1 had the highest AGB and the lowest weed AGB, while clover SD1 had the lowest AGB and the highest weed AGB within cover crop species but always lower than weed AGB of the no cover (-) treatment. Our result confirm that cover crops with favorable establishment and above-average biomass yields tended to suppress weed by showing lower weed dry matter and weed numbers (Schappert et al., 2019). Precipitation, as discussed for cover crops productivity, is a determinant factor also for weed AGB. In addition, the cover crop species intrinsic characteristics are important in weed suppression. For example, mustard, as a Brassicaceae, has a high content of glucosinolates in its tissues, enzymatically hydrolyzed to active compounds as isothiocyanates, ionic thiocyanates and organic cyanates (Petersen et al., 2001, Haramoto and Gallandt, 2004). Melander et al. (2017) and Rueda-Ayala et al. (2015) explained the ability of cover crops in weed suppression by the different mechanisms that can use, such as the direct suppression through resource competition as light, water, space and nutrients. Cover crops can severely hamper the development of weed or even prevent them from emerging (Brennan and Smith, 2005) and even continue affecting weed from sowing date until a certain time of subsequent main crop establishment (Falquet et al., 2015). In fact, it seems that weed were more resistant to unfavourable climatic conditions during the second year than in the first year and then cover crops had penalities in their biomass. In fact, Werle et al. (2018) indicated that cereal rye reduced winter annual weed density and biomass by >90% at time of spring termination, showing potential as a component of an integrated weed management program. Also, the sowing date is important in weed AGB reduction. In fact, according to Vos and Van der Putten (1997) and Anugroho et al. (2009), delaying cover crop sowing date can provide a decreased weed suppression, due to lower cover crop nitrogen accumulation and biomass production. Efficiency of cover crops in weed suppression is variable within cover crop species. Legume cover crops had the highest weed AGB compared to non-legume cover crops. Baraibar et al. (2018b) indicated a classification of cover crops according to their botanical family, N fixation ability or monocultures/mixtures, showing that grasses and mixtures are the most weed suppressive and legumes are the least. Grass cover crop species provided greater weed suppression than broadleaf species (Osipitan et al., 2019). The high weed AGB of clover (especially for SD1 in both years), compared to other cover crops, confirmed the findings of Akemo et al. (2000), Snapp et al. (2005), Flower et al. (2012) and Hayden et al. (2012) who indicated that cereal cover crops such are often stronger competitors and more winter-hardy than legumes such as crimson clover. The high weed AGB of rye (especially for SD1) in the second year can be explained by the attack of *Duponchelia fovealis* Zeller larvae in the few days after the first sowing so the cover crop was not able to establish properly and reduce weed AGB. The early-season functional traits of grass species such as rapid emergence, growth, and soil coverage are most important in weed suppression rather than the total biomass they produce (Dorn et al. 2015).

4.3. Nitrogen concentration and uptake

As indicated in the Material and Methods section, samples of cover crops and weed, collected in November 2017, were mixed in a unique “weed+cover” crop sample when weed biomass was less than 10% of total above-ground biomass. Our results showed a significant interaction year x cover crop sowing date x cover crops species in November. Cover crops N concentration was higher for legume rather than non-legume cover crops. Also, taking into consideration the highest biomass registered for SD1 cover crops compared to SD2, N was diluted in the higher biomass of SD1 and concentrated for SD2. After establishing the N dilution curves of the five cover crop species, we notice that our curves had a lower a and b coefficients compared to the coefficients proposed for C3 crops by Greenwood et al. (1990). The low N concentration in the second year compared to the first year can be related to the high amount of precipitation registered before the sampling date (November, 22nd). In the second year, 135 mm were accumulated in the interval of 25 days before sampling while in the first year, 90 mm were accumulated within 18 days before sampling. In the second year, high amounts of precipitation have probably accelerated the leaching of N available in the soil. Our results indicate that, in general, hairy vetch accumulated the highest N amount compared to other cover crops and at both sowing dates. In a meta-analysis prepared by Thapa et al. (2018), above-ground N content in hairy vetch and cereal rye monocultures averaged 122 (range: 3–236) and 51 (range: 6–124) kg ha^{-1}, respectively. Assessment of cover crops contribution to soil depends on C/N ratio of the cover crop species due to its influence on mineralisation/immobilisation processes and therefore on the synchrony of soil N mineralized from legume cover crops with cash crop N demand (Drinkwater and Snapp, 2007). Cover crop C/N ratios can determine net mineralization or immobilization as ratios >25 to 30:1 limit plant available nitrogen due to microbial immobilisation. Legume cover crop species are known to impact N availability for up to 8 weeks following termination (Parr et al., 2014). Increasingly, the link between C/N ratio and biomass production is being used to predict multi-faceted ecosystem services such as N retention, inorganic N supply, and yield production (Finney et al., 2016). For the second year, hairy vetch C/N ratio at cover crops termination was similar to that found by Parr et al. (2011) (11 to 15:1).

Our results of N uptake confirmed those of Akbari et al. (2019), where hairy vetch accumulated the largest N uptake, compared to oat, radish and rye. However, in our experiment, hairy vetch sown early accumulated less N (114 kg N ha^{-1}) compared to results of Akbari et al. (2019) where hairy vetch was sown on September, 9^{th} and accumulated 141 kg N ha^{-1}. Our results confirmed those of Hashemi et al. (2013) who indicated that, after 3 years of experiment, as planting oat was postponed from beginning of September to mid-September, the nitrogen uptake decreased by 35 and 45%, respectively for the first and the second year. The nitrogen uptake decreased dramatically (-60, -91 and -95%, respectively for the first, the second and the third year experiment) when it was delayed by 6 to 7 weeks from beginning of September to mid-October. For the first year, rye nitrogen uptake in our experiment was not affected by the sowing date, while the results of Hashemi et al. (2013), conducted during three years, revealed a higher nitrogen uptake (samples collected in late December) in the first planting date (beginning of September) compared to the ultimate planting date (35 to 42 days later than the first sowing date). We demonstrated that delaying the sowing date by 15 days from end-August to mid-September the N uptake was reduced by 50 and 59% for the first and the second year, respectively, while Akbari et al. (2019) mentioned that reduction of N uptake was by 15% for a delay of the sowing date from September, 9^{th} to September, 23^{rd}. The same authors indicated a reduction of N uptake by 7% for oat and radish and 13% for rye for the same sowing dates. Delaying sowing date by 7 weeks from September, 9^{th} to October, 14^{th} has implied a reduction of the N uptake by 29, 31, 33 and 51% for radish, oat, rye and vetch, respectively (Akbari et al., 2019).

5. Conclusions

In this experiment conducted for two years, we classified the five cover crop species tested according to their AGB with mustard SD1 having more than 5 t ha^{-1} and clover having the lowest AGB (less than 1 t ha^{-1}). Also, AGB of all cover crops, except rye, was lower when sowing date was delayed by 15 days. Cover crops biomass was lower in the second year compared to the first year. Weed AGB was inversely related to cover crops AGB demonstrating that were able to reduce weed AGB compared to keeping the soil bare. Legume cover crops had the highest N concentration compared to non-legume cover crops. Therefore, non-legume cover crops had the highest C/N ratio compared to legume cover crops. Nitrogen uptake was higher for hairy vetch than for other cover crops, except mustard, with the maximum uptake (114 kg N ha^{-1}) demonstrated in the first year at the first sampling date (November).

Chapter 3. Comparison of maize productivity following cover crops and bare soil in conservation agriculture

1. Introduction

Cover crops are considered one of the three principles of conservation agriculture (FAO, 2004). Importance of cover crops is due to their benefits in improving soil health, reducing soil erosion, and weed suppression (Osipitan et al., 2018). Benefits of cover crops are related to soil fertility, nitrogen emissions and the following cash crop. Tonitto et al. (2006), Valkama et al. (2015), Miguez and Bollero (2005) and Marcillo and Miguez (2017) indicated a neutral effect of cover crops on the following cash crops. Several factors were discussed (Chapter 1) as potential reasons for higher cash crop yield after cover crop compared to after a winter bare fallow, such as cover crop type, fertilizer level, legume nitrogen input rate, winter cover crops termination and biomass, soil texture and geographical region. Nitrogen (N) is the most limiting nutrient in crop production. Legumes can provide N through biofixation. Securing nitrogen in soil for subsequent crop production must also be considered. Variety selection and management of cover crop can influence soil mineral nitrogen (SMN) availability (McKenna et al., 2018). Information on productivity and nitrogen recovery of maize following cover crops in conservation tillage systems of Northern Italy is scarce. Therefore, the objectives of this chapter were to identify the effects of previous soil management before sowing maize (with vs. without cover crops and early vs. late sowing of cover crops that are described in details in the previous chapter) on maize productivity and N recovery.

2. Material and methods

2.1. Field presentation

The trial presented was carried out in the framework of the CoCrop project. It started after cover crops termination (results are reported in the previous chapter). The field was located in Orzinuovi, Brescia, Italy (45°23’55.2”N, 9°54’30.2”E). Soil analyses are reported in Chapter 2.

2.2. Experimental design

Table 3.1 presents the main activities done for crop and soil management, and for biomass sampling. Experimental design is the same presented in Chapter 2 with a second control treatment (no cover +) consisting on keeping soil bare and managing weed with herbicides. After cover crops termination, seedbed for maize sowing was prepared. Then, maize cv. Pioneer 2105 (FAO class 600) was sown between the last week of March and beginning of April at a row spacing of 0.7 m and at a density of 8 plants/m². The first maize samples were taken at V6 growth stage in order to assess the maize biomass as influenced by N released by the different cover crop species sown in two dates (early sowing: end of August vs. late sowing: mid-September). Nitrogen fertilizer was applied at 100 kg N ha^{-1} after V6 growth stage. The choice of 100 kg N ha^{-1} was to assess more the effect of nitrogen released by cover crop species on maize yield and quantify differences in N supply between cover crop species to maize. A higher amount of fertilizers could be responsible of different growth of maize and in consequence we will not be able to identify differences in maize yield based on the cover crop species N supply. Maize was harvested in the first week of August at the R5 stage (Dent maturity). For both years, maize irrigation was performed from end of May to harvest every two weeks. The irrigation amount was 231.4 mm.

Table 3.1. *Crop management and sampling.*

Year	Date	Activity	Notes
1	25/03/2018	Soil tillage (Seedbed preparation for maize)	
	01/04/2018	Maize sowing	
	17/05/2018	Biomass sampling	Six leaves (V6 growth stage)
	19/05/2018	Maize top dress fertilization	100 kg N ha^{-1}
	30/05/2018	Maize Irrigation	231.4 mm
	13/06/2018		231.4 mm
	28/06/2018		231.4 mm
	12/07/2018		231.4 mm
	27/07/2018		231.4 mm
	07/08/2018	Maize harvest	Dent maturity (R5 growth stage)
2	22/03/2019	Soil tillage (Seedbed preparation for maize)	
	26/03/2019	Maize sowing	
	22/05/2019	Biomass sampling	Six leaves (V6 growth stage)
	30/05/2019	Maize irrigation	231.4 mm
	03/06/2019	Maize top dress fertilization	100 kg N ha^{-1}
	13/06/2019	Maize irrigation	231.4 mm
	28/06/2019		231.4 mm
	12/07/2019		231.4 mm
	27/07/2019		231.4 mm
	05/08/2019	Maize harvest	Dent maturity (R5 growth stage)

2.3.Precipitation and growing degree days

Calculation of maize growing degree days (GDD) was made using the same method described in Chapter 2. Maize base temperature is 7.3°C (Sanchez et al., 2014).

2.4.Sampling and calculations

For May sampling, 15 plants were taken from each plot and then dried in the stove at 105 °C. Fresh and dry weights were taken and then the above-ground biomass was calculated. At maize harvest, 20 plants were taken to determine the above-ground biomass (AGB). All biomass data are reported as t ha^{-1}.

Above-ground biomass samples were grinded at 0.2 mm using Ultra Centrifugal Mill ZM 200 (Retsch, Haan, Germany). Then, samples were analyzed using an elemental analyzer Carlo Erba NC Thermoquest, model NA 1500 series 2 (Carlo Erba, Milano, Italy).

Nitrogen uptake was calculated as the product of AGB by nitrogen concentration.

Apparent Nitrogen Recovery (ANR) was calculated after maize harvest as the ratio of difference of nitrogen uptake between the treatment after cover and after no cover crops, to nitrogen applied with cover crop (de Boer et al., 2008).

ANR (%) = *(Nitrogen uptake of maize after cover crop – Nitrogen uptake of maize after no cover crop (+)) / (Nitrogen applied with cover crop) * 100*

2.5. Statistical analysis

Analysis of variance was conducted to determine the effect of factors (cover crop species and sowing date) on cover crops and weed AGB, nitrogen concentration and uptake using SPSS 25. The analysis was carried out for both years. If a significant interaction year x cover crop sowing date x cover crop species was found, the least significant difference (LSD) was calculated. Homogeneity of variance and normality of distribution of data were checked before analysis of variance. Post-hoc comparisons were made using SIDAK tests.

3. Results

3.1. Precipitation and Growing Degree Days

Cumulative GDD of maize between sowing and harvest was 11% higher in the first year (1829 °C) than in the second year (1654 °C) (Figure 3.1 a). Low temperatures, responsible for lower GDD, were noticeable in the second year between 30^{th} and 60^{th} day after sowing, a period that corresponds to the month of May. However, we recorded low temperatures in the first year between June, 15^{th} and first week of July (75 to 100 days after maize sowing). During the last month of maize cultivation, temperatures were similar in both years and in consequence differences in cumulative GDD between the two years were due to low temperatures recorded in the first period of cultivation of maize (from sowing to the third week of May). Cumulative precipitation (Figure 3.1 b) was higher in the first year (419 mm) than in the second year (390 mm). Precipitations were higher in the second year than the first year between 15^{th} and 30^{th} day after maize sowing (1^{st} and 3^{rd} week of April), between 45^{th} and 60^{th} day after maize sowing (1^{st} and 3^{rd} week of May) and between 115^{th} and 128^{th} day after maize sowing (last ten days of July).

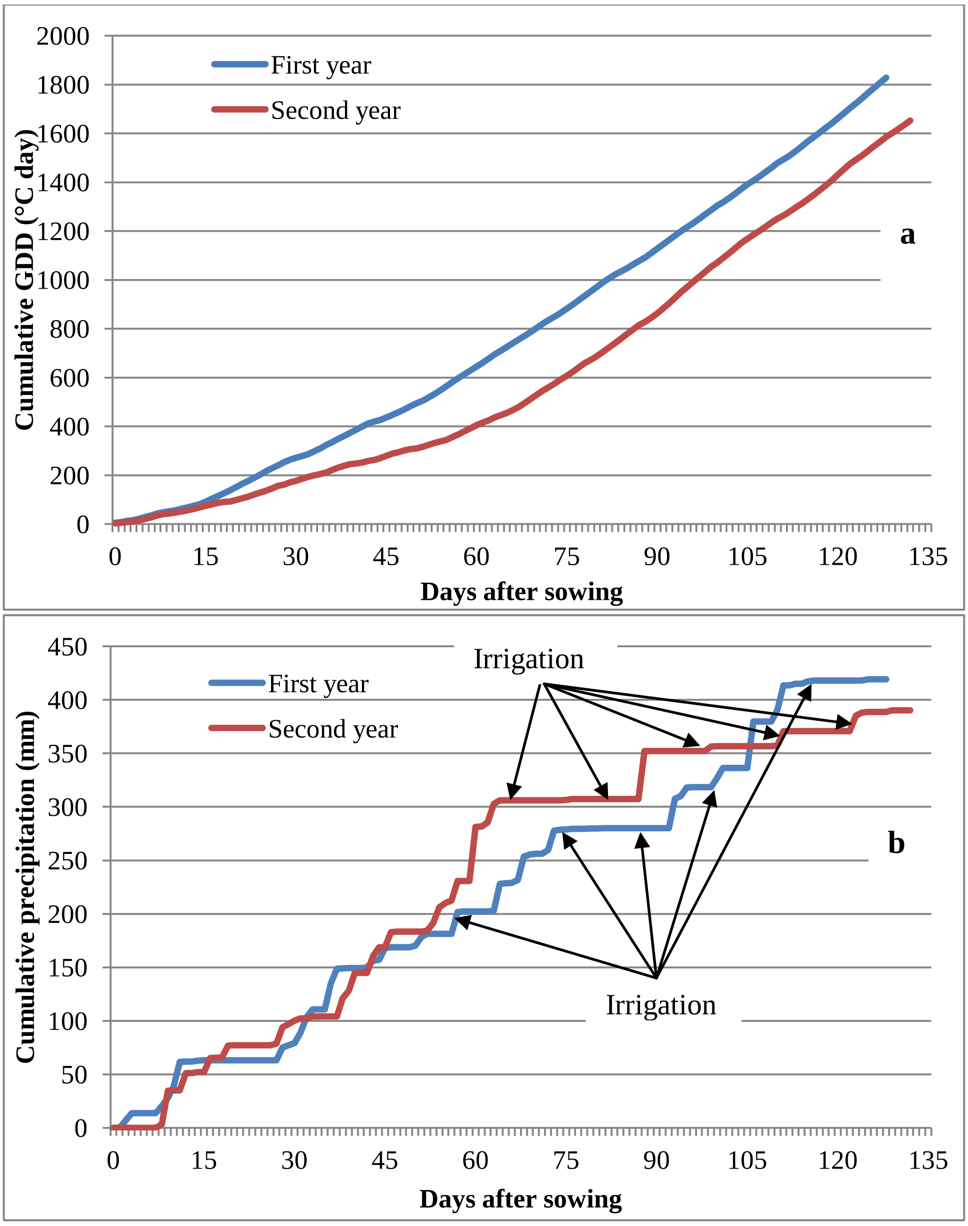


Figure 3.1. *Cumulative Growing Degree Days (GDD) (a) and cumulative precipitation of maize (b) during the two years of experiment.*

3.2. Maize above-ground biomass at V6 growth stage

Table 3.2 shows maize Above-Ground Biomass (AGB) at V6 growth stage. For the first year, maize AGB at V6 growth stage ranged between 0.75 and 1.00 t ha^{-1}, and was higher than values of the second year (0.19 to 0.32 t ha^{-1}). For the first year, only maize following no cover (-) had a significant difference in the AGB between both sowing dates. For SD1, maize AGB following no cover (-) (1.0 t ha^{-1}) was significantly higher than maize AGB following rye (0.75 t ha^{-1}). For the second year, only maize following rye was significantly affected by cover crops sowing date. For SD2, maize following mustard and no cover (+) treatment (0.32 and 0.29 t ha^{-1}, respectively) was significantly higher than maize following rye (0.19 t ha^{-1}).

Table 3.2. *Maize Above-Ground Biomass (AGB) at V6 growth stage for the first and the second year of experiment (May 2018 and May 2019, respectively). Each value represents the mean of four measurements. SE: Standard Error. Averages with the same letters are not significantly different at P < 0.05 within sowing date. No cover (-): plots (bare soil) left without weed control (growth of spontaneous weed) and no cover (+): plots (bare soil) with weed control (application of herbicides).*

Cover crop		**Maize AGB (t ha^{-1})**	
Sowing date	**Species**	**May 2018**	**May 2019**
SD1	**no cover (-)**	1.00 [a]	0.29 [a]
	no cover (+)	0.93 [ab]	0.32 [a]
	black oat	0.86 [ab]	0.25 [a]
	cereal rye	0.75 [b]	0.27 [a]
	white mustard	0.86 [ab]	0.27 [a]
	Egyptian clover	0.88 [ab]	0.28 [a]
	hairy vetch	0.86 [ab]	0.24 [a]
SD2	**no cover (-)**	0.82 [a]	0.26 [ab]
	no cover (+)	0.89 [a]	0.29 [a]
	black oat	0.90 [a]	0.26 [ab]
	cereal rye	0.76 [a]	0.19 [b]
	white mustard	0.84 [a]	0.32 [a]
	Egyptian clover	0.95 [a]	0.26 [ab]
	hairy vetch	0.92 [a]	0.24 [ab]
	SE	0.02	0.01

3.3. Maize nitrogen concentration at V6 growth stage

Figure 3.2 shows maize N concentration at V6 growth stage. For the first year, maize N concentration at V6 growth stage ranged between 2.40 (maize following black oat SD1) and 3.41%, (maize following vetch SD2). Maize N concentration was higher for the second year than the first year with values ranging between 3.24 and 3.91% (maize following vetch SD1). For the first year, maize N concentration was in general higher for SD2 than for SD1, while for the second year, it was in general slightly higher for SD1 than for SD2. For the first year, maize following hairy vetch had a significantly higher N concentration than maize following oat and mustard. Also, maize following rye had a significantly higher N concentration than maize following oat. For SD2, maize following vetch had a significant higher N concentration than maize following oat, mustard, no cover (-) and no cover (+). For the second year, for SD2, maize following hairy no cover (-) had a significantly lower N concentration than maize following hairy vetch and white mustard.

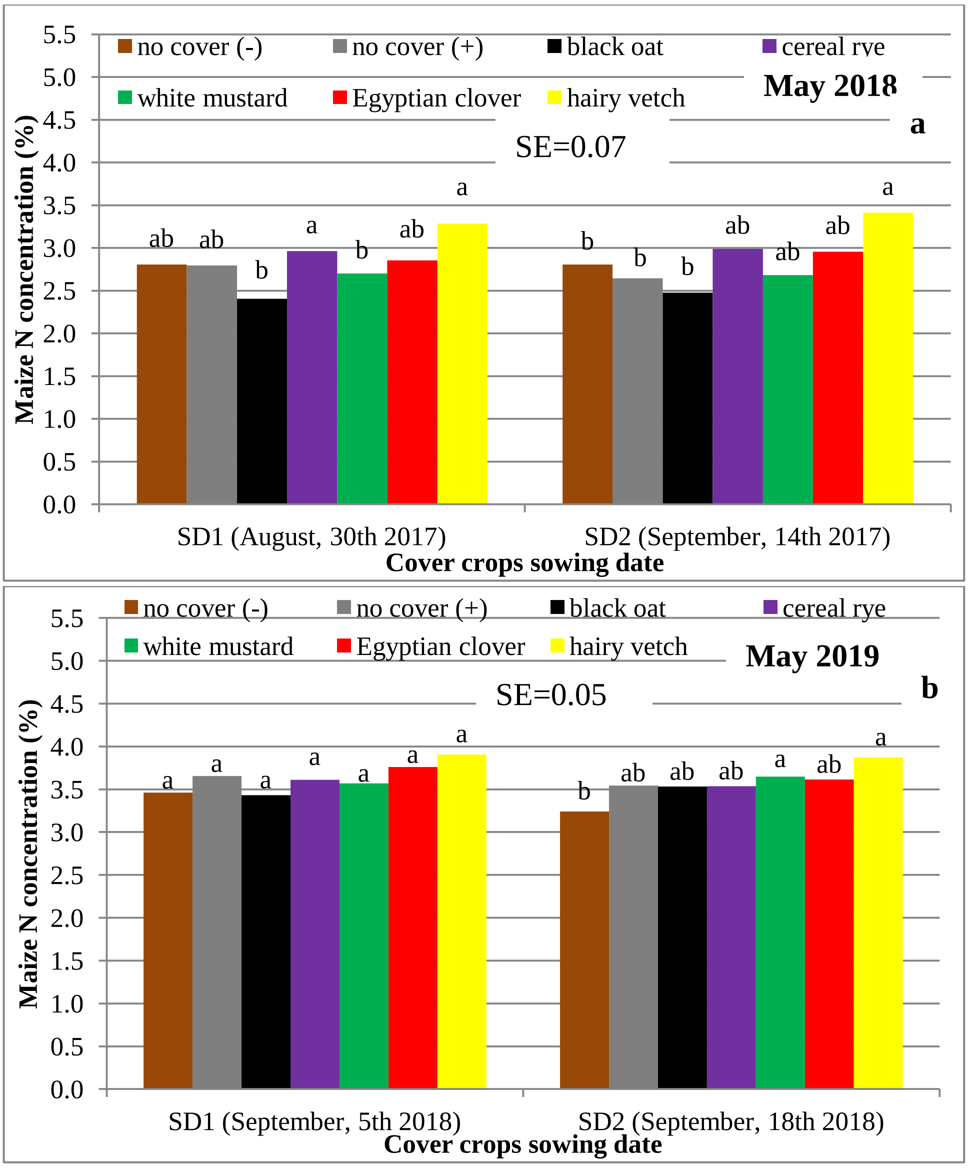


Figure 3.2. *Maize N concentration at V6 growth stage for the first (a) and the second year (b). Each value represents the mean of four measurements. SE: Standard Error. Averages with the same letters are not significantly different at P < 0.05 within sowing date. No cover (-): plots (bare soil) left without weed control (growth of spontaneous weed) and no cover (+): plots (bare soil) with weed control (application of herbicides).*

3.4. Maize nitrogen dilution curve at V6 growth stage

Figure 3.3 shows the maize N dilution curve as affected by cover crop species and sowing dates at V6 growth stage during two years of maize cultivation. The fitting of the power function N concentration (%) = a DM^b (Justes et al., 1994). We notice that, for both years, the coefficient of determination (R^2) is very low (0.015 and 0.0012 for the first and the second year, respectively). We notice also differences among the cover crop species and sowing dates.

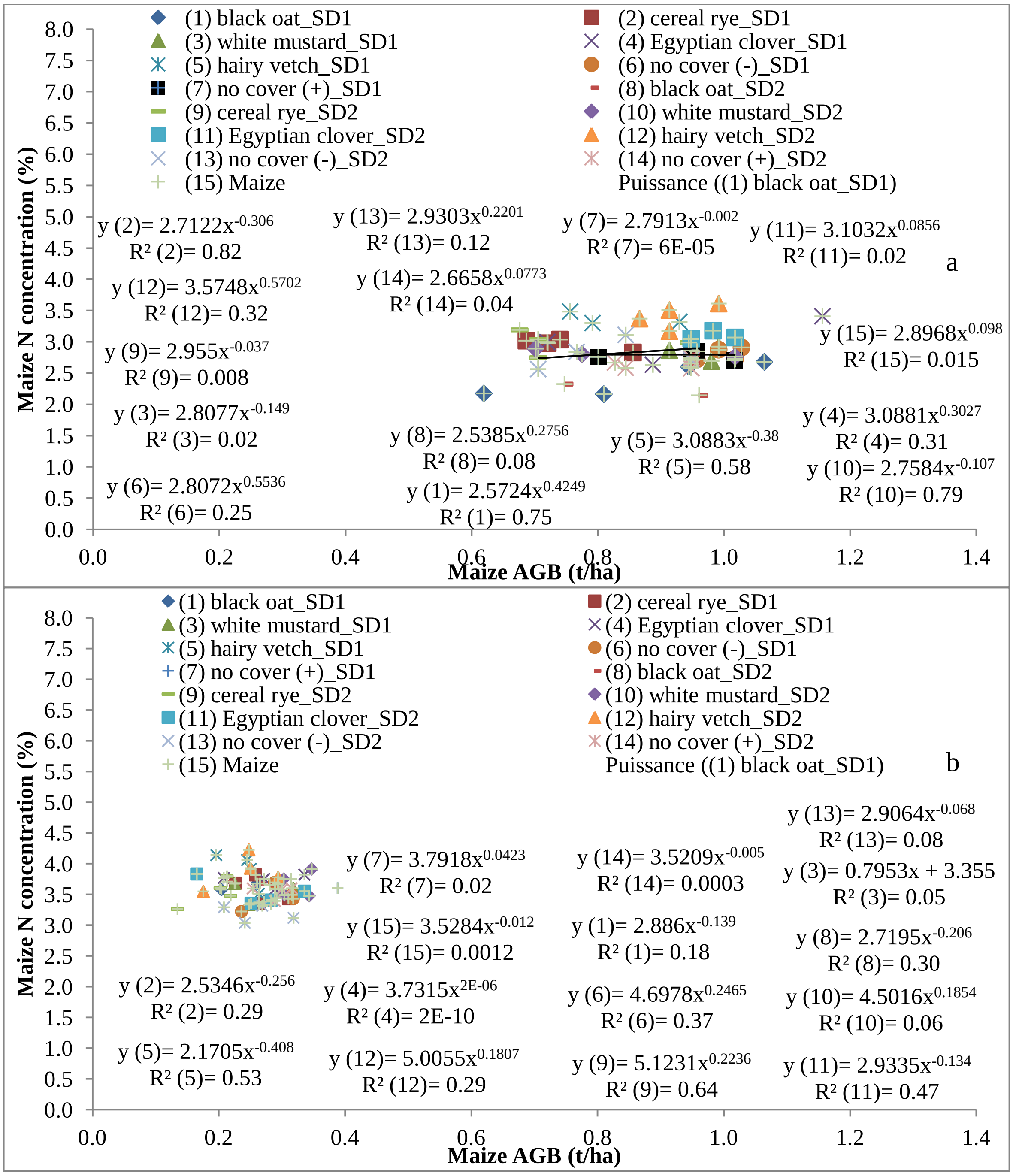


Figure 3.3. *Maize nitrogen dilution curve as affected by cover crop species and sowing dates at V6 growth stage in the first (a) and the second (b) year of maize cultivation. No cover (-): plots (bare soil) left without weed control (growth of spontaneous weed) and no cover (+): plots (bare soil) with weed control (application of herbicides).*

3.5. Maize nitrogen uptake at V6 growth stage

For the first year, maize N uptake at V6 growth stage ranged between 21.0 and 31.7 kg N ha^{-1}, while for the second year, it was lower and ranged between 6.9 and 11.9 kg N ha^{-1} (Table 3.3). For the second year, maize following rye was affected by the cover crop sowing date. For the second year, for SD2, maize following white mustard had a significantly higher N uptake than maize following no cover (-) and rye.

Table 3.3. *Maize N uptake at V6 growth stage for the first and the second year of experiment (May 2018 and May 2019, respectively). Each value represents the mean of four measurements. SE: Standard Error. Averages with the same letters are not significantly different at P < 0.05 within sowing date. No cover (-): plots (bare soil) left without weed control (growth of spontaneous weed) and no cover (+): plots (bare soil) with weed control (application of herbicides).*

Cover crop		Maize Nitrogen uptake (kg N ha^{-1})	
Sowing date	**Species**	**May 2018**	**May 2019**
SD1	**no cover (-)**	28.06 [a]	10.06 [a]
	no cover (+)	26.02 [a]	11.66 [a]
	black oat	21.04 [a]	8.76 [a]
	cereal rye	22.23 [a]	9.43 [a]
	white mustard	23.78 [a]	9.70 [a]
	Egyptian clover	25.72 [a]	10.32 [a]
	hairy vetch	28.04 [a]	9.29 [a]
SD2	**no cover (-)**	23.44 [a]	8.26 [b]
	no cover (+)	23.76 [a]	10.27 [ab]
	black oat	23.20 [a]	9.35 [ab]
	cereal rye	22.67 [a]	6.86 [b]
	white mustard	22.72 [a]	11.88 [a]
	Egyptian clover	27.99 [a]	9.10 [ab]
	hairy vetch	31.71 [a]	9.42 [ab]
SE		0.80	0.34

3.6. Maize yield

Table 3.4 shows maize yield. For the first year, maize yield at harvest ranged between 19.4 and 28.8 t ha^{-1} (maize following vetch SD1). For the second year, maize yield at harvest was lower than the first year and ranged between 17.1 and 20.6 t ha^{-1}. For the first year, for SD1, maize following vetch (28.8 t ha^{-1}) was significantly higher (P<0.001) than maize following no cover (-) (19.4 t ha^{-1}).

Table 3.4. *Maize yield at harvest for the first and the second year of experiment (August 2018 and August 2019, respectively). Each value represents the mean of four measurements. SE: Standard Error. Averages with the same letters are not significantly different at P < 0.05 within sowing date. No cover (-): plots (bare soil) left without weed control (growth of spontaneous weed) and no cover (+): plots (bare soil) with weed control (application of herbicides).*

Cover crop		Maize yield (t ha^{-1})	
Sowing date	**Species**	**August 2018**	**August 2019**
SD1	**no cover (-)**	19.38 [b]	17.14 [a]
	no cover (+)	23.85 [ab]	20.60 [a]
	black oat	22.92 [ab]	17.33 [a]
	cereal rye	23.88 [ab]	18.44 [a]
	white mustard	24.44 [ab]	18.83 [a]
	Egyptian clover	23.08 [ab]	20.10 [a]
	hairy vetch	28.79 [a]	20.04 [a]
SD2	**no cover (-)**	23.45 [a]	17.77 [a]
	no cover (+)	23.35 [a]	18.09 [a]
	black oat	25.07 [a]	18.97 [a]
	cereal rye	25.30 [a]	19.05 [a]
	white mustard	23.14 [a]	19.82 [a]
	Egyptian clover	25.29 [a]	19.13 [a]
	hairy vetch	27.87 [a]	20.14 [a]
SE		0.60	0.29

3.7. Maize nitrogen concentration at harvest

For the first year, maize N concentration ranged between 0.79 and 0.93 % while for the second year, it ranged between 0.87 and 1.04% (Figure 3.4). A significant interaction (P<0.01) was revealed between cover crop sowing date and cover crop species for the first year and maize following oat, rye and mustard was significantly affected by cover crop sowing date, for the same year. For SD1, N concentration of maize following vetch was significantly higher (0.93%) than maize following black oat (0.65%), no cover crops (0.83 and 0.79% for maize following no cover (+) and no cover (-), respectively) and maize following rye, mustard and clover was significantly higher than maize following no cover (-). For SD2, maize N concentration following vetch and oat was significantly higher (0.90% for both treatments) than following no cover (+) and mustard (0.79 and 0.80%, respectively).

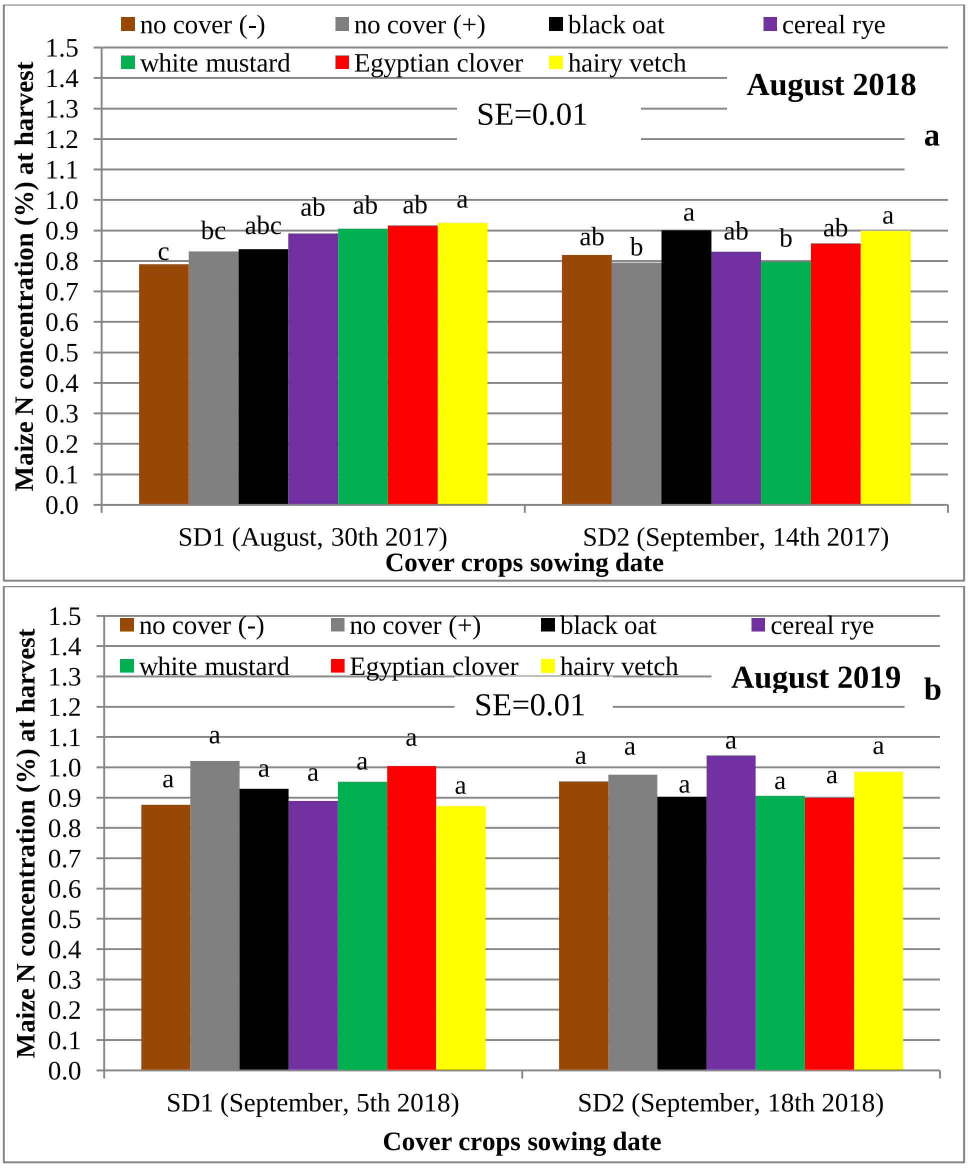


Figure 3.4. *Maize N concentration at harvest for the first year (a) and the second year (b). Each value represents the mean of four measurements. SE: Standard Error. Averages with the same letters are not significantly different at P < 0.05 within sowing date. No cover (-): plots (bare soil) left without weed control (growth of spontaneous weed) and no cover (+): plots (bare soil) with weed control (application of herbicides).*

3.8. Maize nitrogen dilution curve at harvest

Figure 3.5 shows the maize N dilution curve as affected by cover crop species and sowing dates at harvest (R5 growth stage) during two years of maize cultivation. The fitting of the power function N concentration (%) = a DM^b (Justes et al., 1994). We notice that the coefficient of determination (R^2) was higher in the second year compared to the first year.

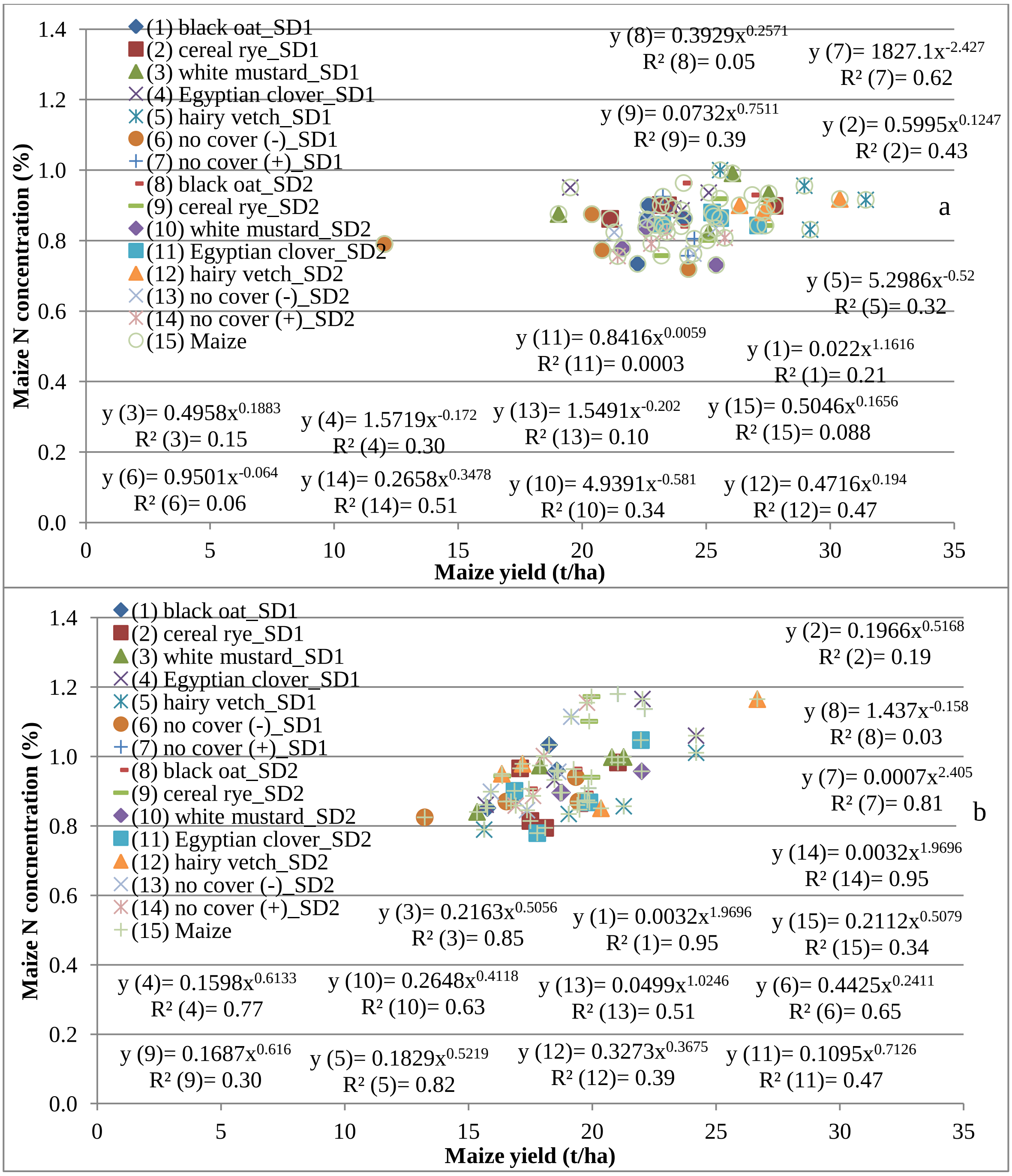


Figure 3.5. *Maize nitrogen dilution curve as affected by cover crop species and sowing dates at harvest (R5 growth stage) in the first (a) and the second (b) year of maize cultivation. No cover (-): plots (bare soil) left without weed control (growth of spontaneous weed) and no cover (+): plots (bare soil) with weed control (application of herbicides).*

3.9. Maize nitrogen uptake at harvest

Table 3.5 presents maize N uptake at harvest. For the first year, maize N uptake ranged between 153.2 (maize following no cover (–) SD1) and 267.0 kg N ha^{-1}, (maize following hairy vetch SD1) while for the second year, N uptake was slightly lower than the first year and ranged between 151.1 (maize following no cover (-) SD1) and 211.7 kg N ha^{-1} (maize following no cover (+) SD1). A significant interaction (P<0.05) was identified between cover crop sowing date and cover crop species only in the first year. For the same year, maize following oat, mustard and no cover (-) was significantly affected by the cover crop sowing date factor. For SD1, maize following hairy vetch was significantly higher than maize following no cover (-), no cover (+), oat, rye and clover. Also, maize following mustard, rye and clover was significantly higher than maize following no cover (-). For SD2, maize following hairy vetch was significantly higher than maize following mustard, no cover (-) and no cover (+).

Table 3.5. *Maize N uptake at harvest for the first and the second year of experiment (August 2018 and August 2019, respectively). Each value represents the mean of four measurements. SE: Standard Error. Averages with the same letters are not significantly different at $P < 0.05$ within sowing date. No cover (-): plots (bare soil) left without weed control (growth of spontaneous weed) and no cover (+): plots (bare soil) with weed control (application of herbicides).*

Cover crop		**Maize Nitrogen uptake (kg N ha^{-1})**	
Sowing date	**Species**	**August 2018**	**August 2019**
SD1	**no cover (-)**	153.20 ^{c}	151.05 ^{a}
	no cover (+)	198.10 ^{bc}	211.74 ^{a}
	black oat	193.58 ^{bc}	161.67 ^{a}
	cereal rye	215.53 ^{b}	164.52 ^{a}
	white mustard	224.64 ^{ab}	180.75 ^{a}
	Egyptian clover	212.13 ^{b}	205.16 ^{a}
	hairy vetch	266.97 ^{a}	177.28 ^{a}
SD2	**no cover (-)**	193.18 ^{b}	170.32 ^{a}
	no cover (+)	187.46 ^{b}	177.63 ^{a}
	black oat	229.95 ^{ab}	171.23 ^{a}
	cereal rye	209.95 ^{ab}	198.81 ^{a}
	white mustard	185.21^{b}	179.84 ^{a}
	Egyptian clover	217.37 ^{ab}	173.26 ^{a}
	hairy vetch	253.25 ^{a}	201.86 ^{a}
SE		7.72	4.75

3.10. Apparent Nitrogen Recovery

Figure 3.6 shows the ANR for species in each year for each SD was higher for the first than the second year. The importance of using cover crops instead of keeping the soil bare was demonstrated by the higher nitrogen recovery of maize following cover crops compared to maize following no cover crops. In fact, for both years and both sowing dates, the lowest nitrogen recovery was recorded for maize following no cover crops (-53.6, -105.8 and -46.8% for maize following no cover (-) in SD1 of the first year, SD1 of the second year and SD2 of the second year, respectively). Among the cover crop species, maize following hairy vetch SD2 had the highest nitrogen recovery for both years (+67.3 and+6.4% for the first and the second year, respectively). Also, for the first year, maize following black oat SD2 (52.2%) and Egyptian clover SD2 (41.0%) had a higher recovery compared to maize following the same cover crops SD1 (1.0 and 18.8% for black oat and Egyptian clover, respectively). For the second year, the highest nitrogen recovery was recorded for maize following Egyptian clover SD1 (11.6%).

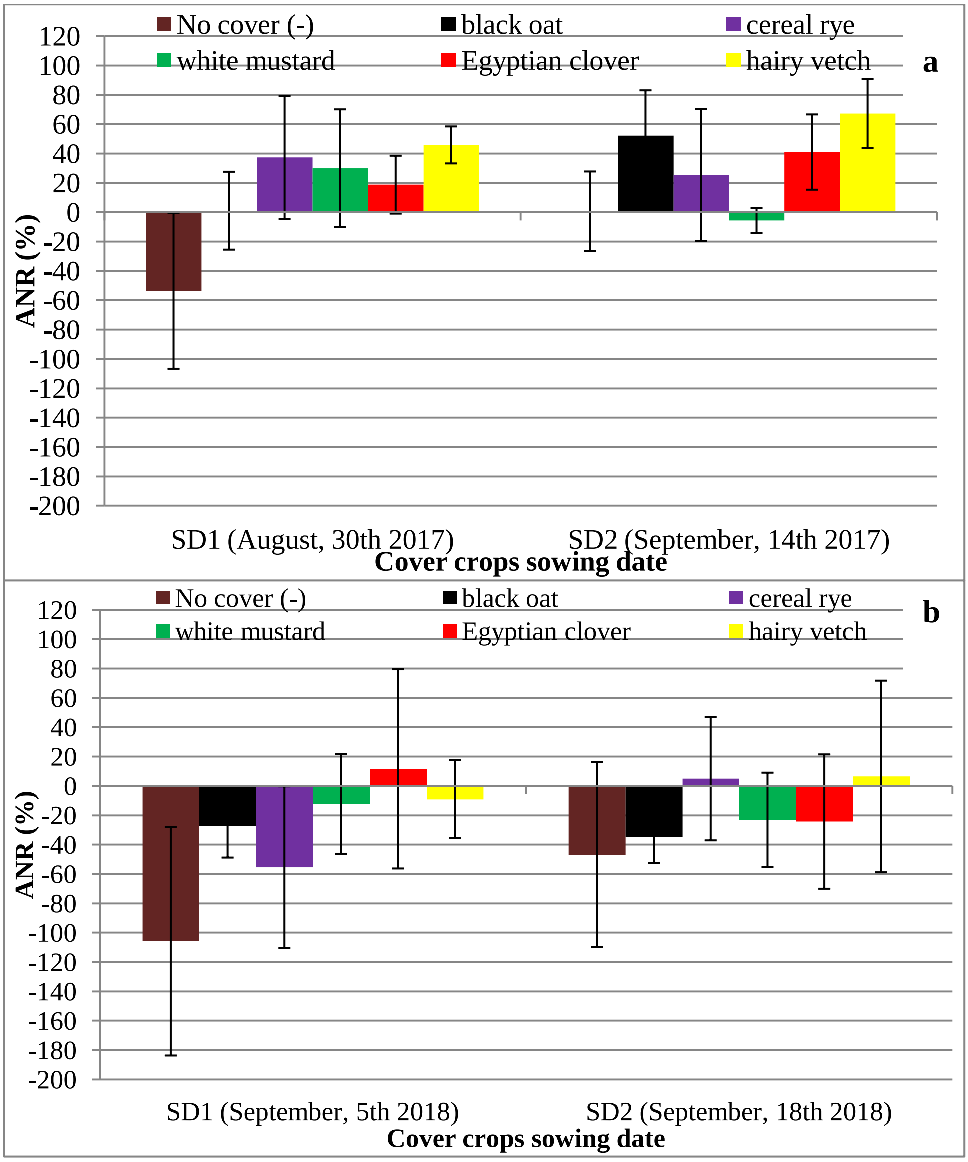


Figure 3.6. *Apparent Nitrogen Recovery for the first year (a) and the second year (b). Each value represents the mean ± standard deviation of four measurements. No cover (-): plots (bare soil) left without weed control (growth of spontaneous weed)*

3.11. Soil nitrogen balance during the cover crop-maize cropping

Table 3.6 presents a summary of the soil N balance during two years of five cover crop species (sown at two dates: end August vs. mid-September)-maize cropping. Values of N input from cover crops in the first year are values determined in November 2017 (due to that we did not collect samples in March 2018). In general, these values are over estimated for black oat, white mustard and Egyptian clover (winter-killed cover crops). However, the N input of hairy vetch is under estimated due to that it is a winter-hardy cover crop. The N balance was higher in the second year compared to the first year. It was due to the low N input (after cover crops incorporation) and uptake (after maize cultivation). In general, the cumulative N balance (of both years) was higher when cover crops were sown (i) in SD1 compared to SD2 and (ii) sown compared to keeping the soil bare.

Table 3.6. *Summary of the Nitrogen balance (kg N ha^{-1}) of the soil-crop system during two years of five cover crop species-maize cropping. No cover (-): plots (bare soil) left without weed control (growth of spontaneous weed) and no cover (+): plots (bare soil) with weed control (application of herbicides).*

		Year 1				Year 2				
		March	May	August		March	June	August		
Cover crop		N input		N output	Nitrogen balance	N input		N output	Nitrogen balance	Cumulative N balance
Sowing date	Species	from cover crops	from fertilizers	after Maize		from cover crops	from fertilizers	after Maize		
1	black oat	53.7	100.0	-193.6	-39.9	40.4	100.0	-161.7	-21.3	-61.2
	cereal rye	46.2	100.0	-215.5	-69.3	8.1	100.0	-164.5	-56.4	-125.7
	white mustard	88.9	100.0	-224.6	-35.7	29.1	100.0	-180.8	-51.7	-87.4
	Egyptian clover	31.5	100.0	-212.1	-80.6	20.8	100.0	-205.2	-84.4	-165.0
	hairy vetch	114.1	100.0	-267.0	-52.9	74.5	100.0	-177.3	-2.8	-55.7
	no cover (-)	0.0	100.0	-153.2	-53.2	0.0	100.0	-151.1	-51.1	-104.3
	no cover (+)	0.0	100.0	-198.1	-98.1	0.0	100.0	-211.7	-111.7	-209.8
2	black oat	52.5	100.0	-230.0	-77.5	15.5	100.0	-171.2	-55.7	-133.2
	cereal rye	48.3	100.0	-210.0	-61.7	11.3	100.0	-198.8	-87.5	-149.2
	white mustard	77.5	100.0	-185.2	-7.7	12.5	100.0	-179.8	-67.3	-75.0
	Egyptian clover	28.0	100.0	-217.4	-89.4	2.5	100.0	-173.3	-70.8	-160.2
	hairy vetch	56.7	100.0	-253.3	-96.6	36.1	100.0	-201.9	-65.8	-162.4
	no cover (-)	0.0	100.0	-193.2	-93.2	0.0	100.0	-170.3	-70.3	-163.5
	no cover (+)	0.0	100.0	-187.5	-87.5	0.0	100.0	-177.6	-77.6	-165.1

4. Discussion

The low yield of Maize registered in the second year compared to the first year can be related to temperature. In fact, despite that the sowing date of maize in the second year was 7 days earlier than the first year, low temperature and in consequence low cumulative GDD (18 and 10% less than the first year, respectively for maize at V6 growth stage and harvest) partially explains differences in biomass between the two years. We observed differences in the slope of cumulative GDD. Low temperatures observed in the second year have probably slowed down the leaf growth and can explain the direct effect on low maize AGB at V6 growth stage, maize nitrogen uptake and later on the maize yield at harvest. In addition, less cumulative precipitation was registered in the second year compared to the first year and irrigations started at the end of May (At V9 growth stage). It seems that maize biomass at V6 growth stage and yield in the second year were narrowly affected by low temperatures from sowing to V6 growth stage and then by water shortage from V6 growth stage to harvest. In fact, 60% of total precipitations in the first year were registered from V6 growth stage to harvest while only 41% of total precipitations were registered in the same period. Irrigations occurred in both years but were not able to cover the lack of water. Our results confirms those of Cupina et al. (2017) who indicated in a study conducted in temperate region (similar to our conditions) that ability of cover crops to provide benefit for a subsequent crop is highly related to weather conditions, mainly precipitation, while Valkama et al. (2015) indicated in a meta-analysis conducted from studies in Nordic countries, that the amount of annual precipitation did not change the effect of catch crops on grain yield. According to Cupina et al. (2017), the low soil moisture after cover crops incorporation is one of unfavorable conditions for mineralization of organic matter after incorporation of cover crops.

Maize N concentration at V6 growth stage in the first year showed a difference by 42% between maize following different cover crops sown at two different dates (end-August and mid-September). This variability can be due to the C/N ratio of the cover crops at termination but it is not possible to confirm it due to that we did not took samples at cover crops termination in March 2018. However, for the second year, we did not find significant differences in maize N concentrations between cover crop species and two sowing dates. It seems that, in this year, nitrogen was not the limiting factor for maize growth and boosts the probability that temperature is the limiting factor as mentioned above. Also, it explains the high N concentration of maize at V6 growth stage in the second year compared to the first year. Compared to the N dilution curve for C4 crops proposed by Greenwood et al. (1990), for both years and at both sampling dates (V6 and R5 growth stages), we notice that our results had a lower a and b coefficients.

Apart the first year SD1, our results indicated a neutral effect of delaying sowing date of cover crops by 15 days (from end-August to mid-September) on maize biomass at V6 growth stage and at harvest in August. Van Eerd (2018) mentioned, in a similar research conducted for four years in Canada, that cover crop planting date (August (10th to 30th) or September (8th to 24th) variable from year to year) did not affect sweet corn yield nor was there a cover crop by planting date interaction (P>0.05). The lack of planting date effect and interaction for maize was surprising because there were considerable differences in cover crop biomass and N accumulation among planting dates in both the fall and following spring (Results of cover crops N uptake are reported in Chapter 2).The absence of effect of cover crops sowing date could be explained by the availability of nitrogen from cover crops. From our results, nitrogen amounts provided from cover crops are different but as indicated by Parr et al. (2014), cover crop C/N ratios can determine net mineralization or immobilization as ratios >25 to 30:1 limit plant available nitrogen due to microbial immobilisation. Legume cover crop species are known to impact N availability for up to 8 weeks following termination.

In a meta-analysis prepared by Marcillo and Miguez (2017), a neutral to positive contribution of winter cover crops to corn yields was detected, while Komainda et al. (2018) reported that cover crop presence had no effect on maize dry matter and N yields, but the N uptake efficiency of maize responded significantly to the N accumulation of cover crops. In our experiment, maize yield following legume winter cover crops (hairy vetch SD1 in the first year) was in accordance with results summarized in an updated meta-analysis by Marcillo and Miguez (2017) who found that corn that followed a legume winter cover crop yielded 21% (17-29%) more than without a cover. The lack of effect of hairy vetch on maize yield in the second year (SD1, lower yield than no cover + and SD2, +11% than no cover (+)) can be explained by the low N uptake of this cover crop compared to the first year. In fact, the N uptake of hairy vetch in November of the first year (2017) was higher (114 and 56 kg N ha^{-1} for SD1 and SD2, respectively) than the N uptake in March of the second year (2019) (74 and 36 kg N ha^{-1} for SD1 and SD2, respectively). We expect that the N uptake of hairy vetch in the first year at termination (March) was higher (we did not take samples) because hairy vetch is a winter-hardy cover crop that continue growing even at low temperature. Also, a prediction of N uptake in March, based on results of this cover crop in the second year, shows an increase by at least 50% from November to March for both sowing dates and C/N ratio was similar for both years. Miguez and Bollero (2006) analyzed corn response to hairy vetch, finding higher yields relative to no cover at low and high corn N rates, suggesting that legume winter cover crops benefits result from improved soil N availability but can also extend beyond N supply. Also, our results were not consistent with those found by Salmerón et al. (2011) and Cupina et al. (2017). Salmerón et al. (2011) indicated a maize yield reduction by 4 Mg ha^{-1} after barley and by 1 Mg ha^{-1} after mixture of barley and oilseed rape. They explained these yield reduction by deficiency caused by insufficient N mineralization from the cover crops due to a high C/N ratio (barley) or low biomass N content (oilseed rape) and/or lack of synchronization with maize N uptake. Cupina et al. (2017) indicated different effects of cover crops on silage maize yield in two years. In the first year, cover crops had a negative effect on silage maize yield while in the second year, a higher yield of maize was recorded after cover crops. Marcillo and Miguez (2017) explained legume winter cover crops contribution to higher yields at low N fertilizer rate by higher N mineralization. Hairy vetch, as a legume cover crop, can contribute nitrogen to following crops through N_2 fixation, which may increase crop yields compared with other cover crops (Clark, 2007; Etemadi et al., 2017).

For grasses, rye and oat in our experiment, maize AGB following rye SD1 at V6 growth stage in the first year was low than maize following other cover crops of the same sowing date. It can be explained by the allelopathic effect of cereal rye on maize seeds. At harvest, compared to maize following no cover crop +, maize following rye and oat was in general within the ranges (-2 to +2% for the ratio maize following cover crop compared to maize following no cover crop) found by Marcillo and Miguez (2017) in an updated meta-analysis. Low yield of maize following rye compared to yield of maize following no cover crop in the second year can be explained by the C/N ratio of rye at termination (17 for rye SD1 and 20 for rye SD2, data shown in chapter 2) and in consequence the N immobilization. One other factor that can explain the low yield of maize following rye is the possible presence of phytotoxic compounds exuded by the roots. Removal or retention of the aboveground cereal rye residue had no consistent effect on yield as reported by Raimbault and Tollenaar (1990). These authors recommended harvest or termination of cereal rye early and cultivation followed by a two to three weeks fallow period before planting corn to overcome any allelopathic effects of the cereal rye. This recommendation was not respected in the second year of our experiment and maize was sown 12 days after rye termination. In another field experiment realized in Iowa State, Acharya et al. (2017) indicated that shorter intervals increased seedling disease and reduced corn emergence, shoot growth and grain yield of corn following winter rye compared to corn planted 10 or more days after rye termination or without rye. According to the same author, incidence of *Pythium* spp. increased with shorter intervals (less than 8 days before planting corn) while incidence of *Fusarium* spp was not different between rye and no rye treatment. Krueger et al. (2011) and Pantoja et al. (2015) found rye biomass production to have a direct relationship to maize yield penalty, while Malone et al. (2014) found, in a modeling study, that rye N uptake had a strong relationship with NO_3-N losses. Also, Snapp and Surapur (2018) indicated that, in Michigan (USA) over 8 years, corn yields were not reduced in plots with a winter rye cover, under widely variable weather conditions. Overall, a rye cover crop had minimal impact on soil C but was an effective N management tool with no corn yield penalty. Vyn et al. (2000), O'Reilly et al. (2012) and Thilakarathna et al. (2015) explained neutral effects of grasses and non-legume cover crops in general by desynchronization of N released by cover crops with the N requirements of a following crop and therefore non-legume cover crops do not provide an N credit. In general, Martinez-Feria et al. (2016) explained abiotic effects on maize can arise from changes in the soil via: (i) the addition of organic C and N (shoot and root); (ii) changes in soil surface cover that alter soil temperature and water dynamics; and (iii) changes in the state variables such as inorganic N and soil water at the time of cover crop termination. For some cover crops, the mechanism of cover crop-induced crop yield responses is not known but may be due to changes in microbial communities (e.g., radish is non-mycorrhizal) or lack of synchronization with N mineralization of cover crop residues and main crop N demand (Van Eerd, 2018). The net release or temporal immobilization of formerly accumulated N after termination of the cover crop depends on the cover crop species N and C content and quantity, as well as the tillage intensity and climatic conditions (Rosecrance et al., 2000; Kuo and Jellum, 2002; Radicetti et al., 2016; White et al., 2016). The dynamic and transient natures of soil N pools make it difficult to predict synchrony between soil N mineralization and crop N demand. Further, year-to-year variability in soil moisture, microbial activity and interactions with historical management add complexity, particularly under rain-fed conditions (Snapp and Surapur, 2018). According to Olesen et al. (2007), the use of catch crops increased grain N concentration more than the application of manure did, even though the effect of the catch crops on grain yield was small. For efficient green manuring, an appropriate amount of N accumulation is required as well as synchrony between maize N uptake and N release (Schipanski et al., 2014; Finney et al., 2016).

All the factors discussed above (temperature, water, cover crop sowing dates and species, N mineralization, ...) can explain the differences found in apparent nitrogen recovery (Figure 3.6) and the importance of using cover crops instead of keeping the soil bare was assessed by the high nitrogen recovery of maize following a cover crop compared to maize following no cover crops to reduce the environmental and economic impact of fertilizers.

5. Conclusions

In this experiment, we demonstrated the absence of interaction between cover crop species and sowing date for. maize AGB at six leaves growth stage and maize yield at dent maturity growth stage during the two years of experiment. ANR of maize was higher for the first year than the second year with maize following hairy vetch SD2 had the highest recovery (+67%). Also, in all cases, the importance of sowing cover crops instead of keeping the soil bare was demonstrated by the higher recovery of maize following cover crop species compared to maize following no cover crop (-) treatment.

Chapter 4. Nitrogen mineralization of five pure cover crops shoots in two soils

1. Introduction

The application of organic amendments, i.e. plant residues, compost green and animal manure into the soil is a standard practice sustaining soil organic matter, enhancing soil microbial activity, improving soil physical properties and increasing nutrients availability (Wang et al., 2014). Well-grown cover crops produce large amounts of residue and retain or add nutrients by scavenging inorganic N, reducing erosion, and fixing atmospheric N (Tonitto et al., 2006; Hoorman et al., 2009; Liu et al., 2015). Cover crops influence subsequent crop yield primarily via their effect on N availability (Torbert et al., 1996; Vaughan and Evanylo, 1998). When a green manure crop is terminated and incorporated into the soil, it undergoes decomposition, mineralization and nitrification, and as a consequence can release plant-available N (mineral N; NH_4-N plus NO_3-N) for the subsequent cash crop. This can potentially help farmers to reduce the rate of fertilizer N for the next cropping season (Woli et al., 2016). Nitrogen mineralization is controlled by many factors, including residue composition (Ranells and Wagger, 1996; Jensen et al., 2005) and environmental factors such as temperature and soil moisture (Ruffo and Bolero, 2003; Wang et al., 2006). Mineralization of soil organic C and N is an indicator of microbial activity, and C and N cycling in the soil (Gregorich et al., 1997; Filip, 2002). Residues biomass decomposition have a significant effects on soil inorganic N in terms of N mineralization or N immobilization, which complicates cover crop effects on potential plant available N. Cover crops C/N ratio is one of the principal determinants of cover crop residue effects on soil N availability (Hargrove 1986; Pantoja et al. 2016; Ranells and Wagger 1996; Smith et al., 1987). It is a predictor of green manure N fertilizer value (Wagger, 1989; Diallo et al., 2006) because it is correlated significantly ($r^2 = 0.88$) to soil NH_4-N and NO_3-N concentration (Frankenberger and Abdelmagid Jr., 1985). Also, total N content (kg ha^{-1}) is one of the primary drivers of N supply to the subsequent crop (Finney et al., 2016; Tonitto et al., 2006; Vigil and Kissel, 1991; White et al., 2014). Reported N fertilizer replacement values for winter-grown cover crops to subsequent cash crops vary greatly (Lawson et al., 2012). Legume cover crops can maintain a low tissue C:N ratio and accumulate a high biomass N content, leading to a high N supply from decomposing residues. On the other hand, grass cover crops tend to have a tissue C:N ratio that increases with plant maturity (Greenwood et al., 1990), so N supply becomes dependent on the timing of cover crop termination (Clark et al., 2007, 1994; Vaughan and Evanylo, 1998), and is usually lower than that from legumes (Miguez and Bollero, 2005). Laboratory incubations that are conducted at optimum moisture and temperature provide a method for comparing N availability across a range of residue types and compositions, which improves our capacity to predict cover crop N availability (Robertson et al., 1999). In laboratory conditions, few studies have explored N mineralization of mixtures rye-vetch (Lawson et al., 2012) or pure cover crops such as rye (Woli et al., 2016), fodder radish (Hu et al., 2018, Thomsen et al., 2016), white mustard and perennial ryegrass (Thomsen et al., 2016). Not all cover crop species were studied in the published studies. Also, Jensen et al. (2005) have reported results of incubation of different parts of the cover crops (whole plant, green leaves, stem, mature straw and pods and spikes) but the cover crop shoots that we have had a C/N ratio, ranging between 10 and 25, close to the equilibrium between mineralization and immobilization. According to literature data, it is not so easy to predict the net N mineralization of residues with C/N ratio between 10 and 25 so we decided to make our own incubation experiment to obtain a specific response related to the two soils. In this study, our objective was to establish the course of N mineralization from five pure cover crop species and weed shoots, grown in field, incubated in laboratory conditions (constant temperature and water content) for 84 days in two soils by measuring the net accumulation of ammonium and nitrate in soil (one, two, three, six and twelve weeks after incorporation) under the impact of differences in cover crops botanical families (Brassicaceae, Fabaceae and Poaceae), and two dates of collection for winter-killed cover crops (autumn and spring).

2. Material and Methods

This incubation experiment was carried out in the framework of the CoCrop project (http://sites.unimi.it/cocrop/).

2.1. Soil sampling

The soils of our study were sampled from two sites that are "Orzinuovi" site located in Brescia, Italy (45°23'55.2"N, 9°54'30.2"E) and "Sant'Angelo Lodigiano" located in Lodi, Italy (45°13'57.6''N, 9°25'36.7''E). At both sites, soil was taken at 0-30 cm depth from four plots of the control treatment (plots kept without cover crops; no added amendments; weed managed by applying herbicide), homogenized to make a composite sample then sieved through a 2-mm mesh screen to remove large plant material and stone fragments. The soil of Orzinuovi is sandy loam (54% sand, 15% clay and 31% silt) with high presence of skeleton (54%), neutral pH (pH in soil: water 6.8) and 3.9% of organic matter (C/N 9.1), while the soil of Sant'Angelo is loam (45% sand, 14% clay and 41% silt) with absence of skeleton, a sub-acid pH (6.0) and 1.5% of organic matter (C/N ratio 8.8). Average annual temperature and precipitation in Orzinuovi is 14.4 °C and 957 mm, respectively, while in Sant'Angelo is 13.2 °C and 830 mm. We determined the gravimetric field capacity of both soils in the laboratory and it was 18 and 16% for Orzinuovi and Sant'Angelo, respectively.

2.2. Shoot material for incubation

The incubation treatments were designed to follow the field study (Chapters 2 and 3). Cover crops were sown on September, 6^{th} 2018 in the field of Sant'Angelo. As described in Table 4.1, the material for incubation of white mustard and black oat cover crops was collected at two different dates. Material for incubation was the above-ground biomass of:

- black oat (*Avena strigosa* Schreb. cv. "Saia 6") collected in November;
- cereal rye (*Secale cereale* L. cv. "Stanko") collected in March;
- white mustard (*Sinapis alba* L. cv. "Architect") collected in November;
- Egyptian clover (*Trifolium alexandrinum* L. cv. "Mario") collected in November;
- hairy vetch (*Viccia villosa* Roth cv. "Villana") collected in March;
- weed grown in control plots collected in March;
- white mustard collected in March;
- black oat collected in March.

In addition to these treatments, a control treatment was added (soil without cover crop). For each treatment, cover crops shoot material samples were taken from four plots that were fertilized in pre-sowing at 50 kg N ha^{-1}. Samples were dried in the stove at 105 °C, sieved at 0.2 mm using Ultra Centrifugal Mill ZM 200 (Retsch, Haan, Germany) then unified in a unique sample in an equal proportion (25% of the total sample was taken from one of the four plots).

Table 4.1. *Treatments (cover crops shoot material), date of collection, C and N contents, C/N ratio and shoot material weight added in soil.*

Treatment (Cover crop shoot material)	Date of collection	C g/g	N g/g	C/N ratio	Shoot material weight added in soil (mg)
Control (CO)	-	0	0	-	-
Weed in control plots (WE)	March 2019	0.4082	0.0215	19.0	230
Black oat (BO)	November 2018	0.4096	0.0298	13.6	160
Black oat (BO+)	March 2019	0.4235	0.0214	19.8	150
White mustard (WM)	November 2018	0.3926	0.0222	17.7	150
White mustard (WM+)	March 2019	0.4150	0.0195	21.3	215
Egyptian clover (EC)	November 2018	0.3737	0.0327	11.4	110
Cereal rye (CR)	March 2019	0.4166	0.0164	25.4	115
Hairy vetch (HV)	March 2019	0.3958	0.0392	10.1	80

2.3. Incubation and analyses

Soil was pre-incubated for 7 days. All treatments were replicated four times in an individual plastic bottle of 50 ml and maintained at 100% of soil field capacity (-50 kPa) with moisture controlled by weighing and distilled water addition with a pipette at least twice a week throughout the incubation period (30 g of soil at 18 and 16% moisture equivalent to 100% of field capacity, respectively for Orzinuovi and Sant'Angelo soil). We have calculated the weight of shoot material for incubation, based on the C/N ratio determined using an elemental analyzer Carlo Erba NC Thermoquest, model NA 1500 series 2 (Carlo Erba, Milano, Italy).The shoot material was mixed into the soil by hand with a spatula. The caps of the plastic bottles were not tightly sealed, which allowed gas exchange, but slowed drying. Plastic bottles were arranged in a complete randomized design and placed in a dark room under controlled temperature at 20 °C. At 7, 14, 21, 42 and 84 days after start of incubation, concentrations of NO_3-N and NH_4-N were determined for each plastic bottle. Potassium chloride (KCl; 100 ml 1 M) was added in 250 ml polyethylene bottles that were shaken on a reciprocal shaker for 2 hours (Cavalli et al., 2016). The extract was filtered through No. 2 Whatman filters (Whatman International Ltd, Maidstone, England) and kept at −20 °C until analysis. Ammonium and nitrate concentrations in the soil extracts were determined by flow injection analysis and spectrometric detection (FIAstar 5000 Analyzer, Foss Tecator, Denmark).

2.4. Data analysis

Inorganic-N was determined by summing NH_4-N and NO_3-N concentrations.

$$\textbf{Inorganic-N}_{(t)} = \textbf{NH}_4\textbf{-N}_{(t)} + \textbf{NO}_3\textbf{-N}_{(t)}$$

Where NH_4-N (t) is the ammonium concentration (mg N/kg of dry soil) at time t and NO_3-N (t) is the nitrate concentration (mg N/kg of dry soil) at time t.

Net N mineralization was calculated according to the equation:

Net N mineralization $_{(t)}$ =

[Inorganic-N $_{\text{Treatment (t)}}$ – Inorganic–N $_{\text{Control (t)}}$] * [kg of dry soil / applied N $_{(t=0)}$] * 100

Where kg of dry soil is the total of soil in the plastic bottle (30 g) and weight of added shoot material (Table 4.1) and applied N $_{(t=0)}$ is the product of weight and N concentration of added shoot material at the day zero of start of incubation.

3. Results

3.1. Ammonium concentration

Ammonium concentration at the beginning of the incubation experiment was different between the Orzinuovi and Sant'Angelo soils (1.24 and 0.48 mg N/kg of dry soil, respectively; Figure 4.1 a and b).

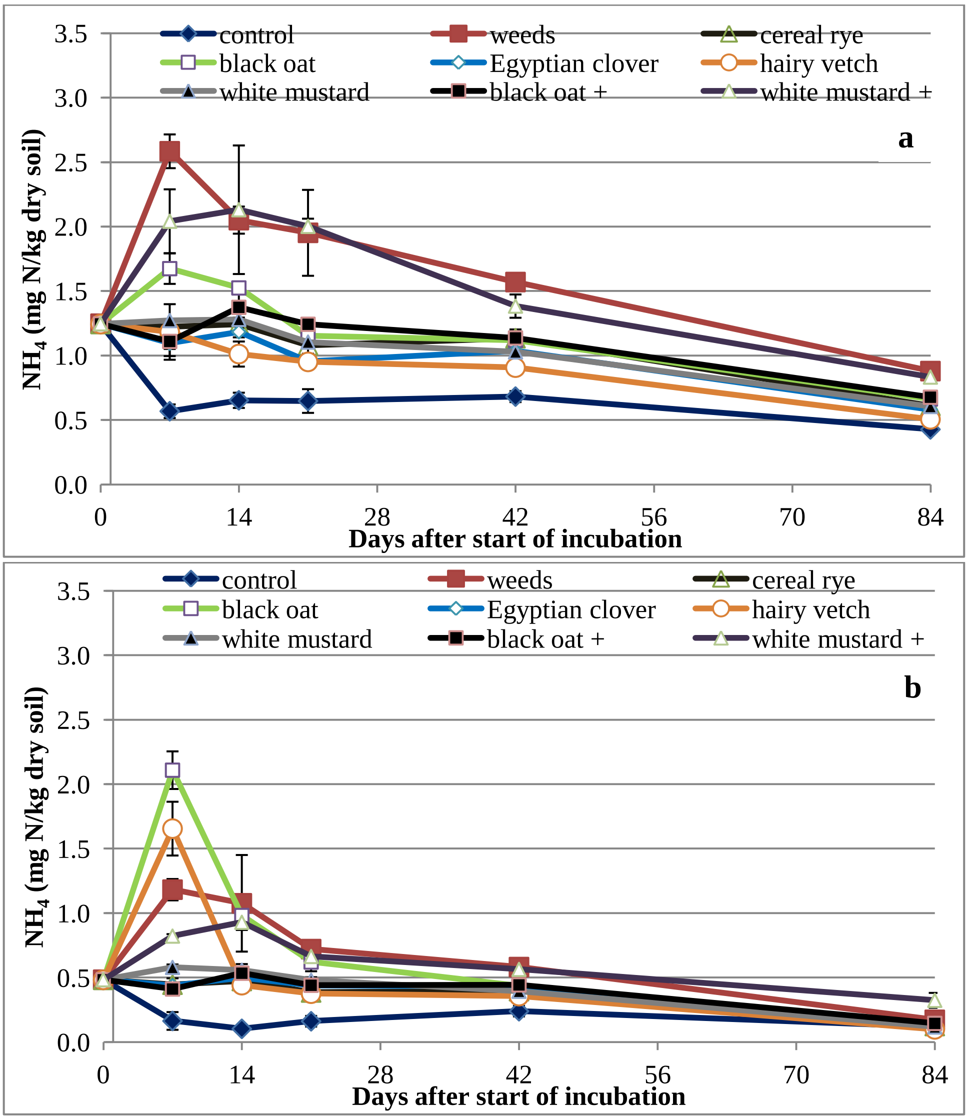


Figure 4.1. *Evolution of the NH_4-N concentration of soils amended with cover crop and weed shoots during 84 days after start of incubation. Orzinuovi (a) and Sant'Angelo (b) soils. Vertical bars represent standard deviation of four replicates. Black oat and white mustard shoots were collected in two dates: November and March ("+" sign).*

3.2. Nitrate concentration

As for the ammonium concentration, nitrate concentration was higher at Orzinuovi soil (62.23 mg N/kg of dry soil) than Sant'Angelo soil (47.15 mg N/kg of dry soil; Figure 4.2 a and b). For both soils, treatments had different trends during the whole period of incubation but quite similar for both soils. In fact, for both soils, hairy vetch and black oat had the highest nitrate concentration during the whole incubation period and it was increasing within time (118.1 and 105.5 mg N/kg of dry soil for hairy vetch and 113.1 and 112.2 mg N/kg of dry soil for black oat collected in November, respectively for Orzinuovi and Sant'Angelo soils 84 days after start of incubation). Egyptian clover and white mustard, both collected in November, had a lower rate of nitrate accumulation than hairy vetch and black oat, but higher than other treatments (94.5 and 77.9 mg N/kg of dry soil for Egyptian clover and 80.5 and 75.9 mg N/kg of dry soil for white mustard, respectively for Orzinuovi and Sant'Angelo soils 84 days after start of incubation). For control treatment, nitrate concentration increased slightly (+9.28 and +10.95 mg N/kg of dry soil for Orzinuovi and Sant'Angelo soils, respectively) at the end of the incubation (84 days after start of incubation) compared to the beginning (day of incubation). For other treatments (weed, cereal rye, black oat + and white mustard +), all of them collected in March, nitrate concentration was very low 7 days after start of incubation, then it increased and reached the highest value 84 days after start of incubation. The rate of accumulation of nitrate concentration was variable for the four treatments (weed, cereal rye, black oat + and white mustard +).

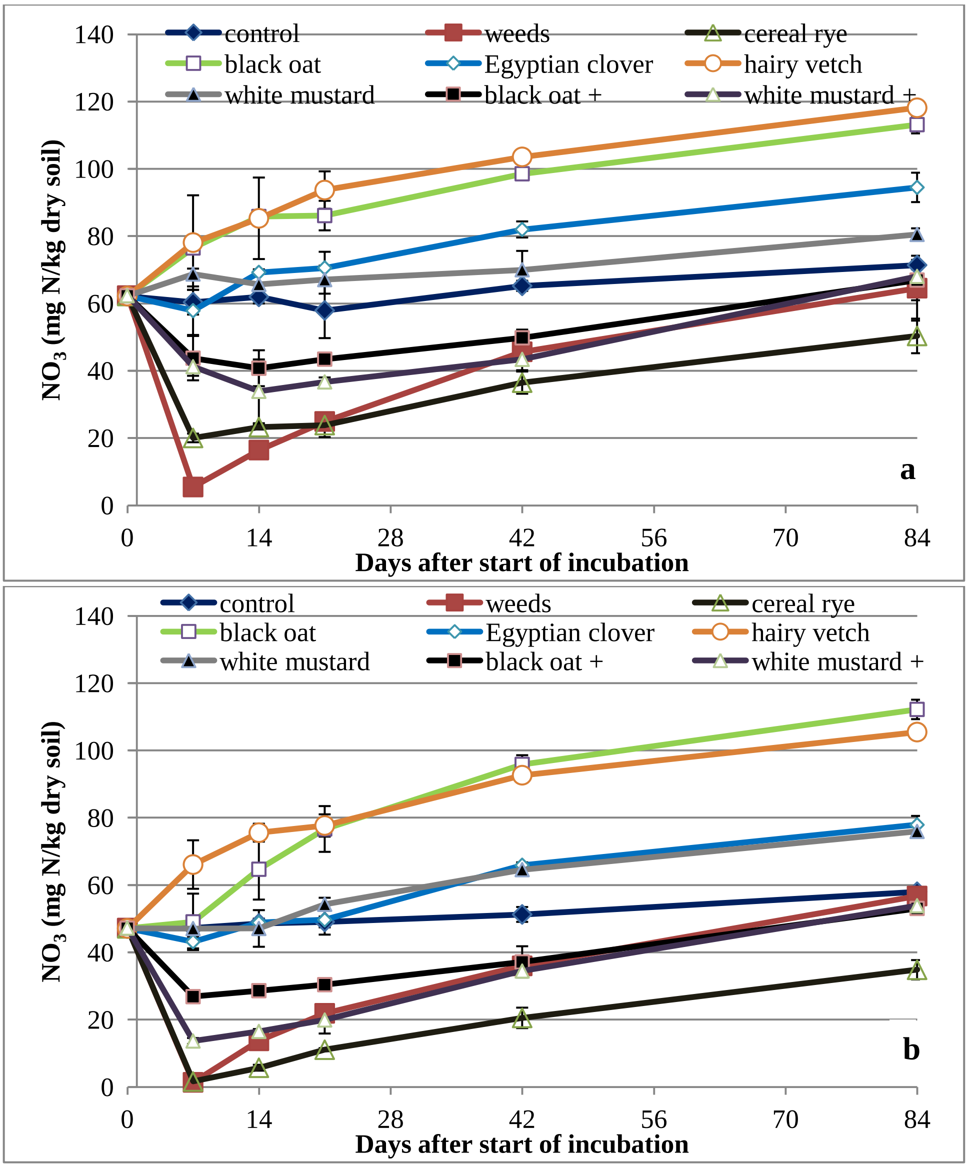


Figure 4.2. *Evolution of the NO_3-N concentration of soils amended with cover crop and weed shoots during 84 days after start of incubation. Orzinuovi (a) and Sant'Angelo (b) soils. Vertical bars represent standard deviation of four replicates. Black oat and white mustard shoots were collected in two dates: November and March ("+" sign).*

3.3. Inorganic N

Inorganic N content in both soils is presented in Figure 4.3 a and b. As it is the sum of ammonium and nitrate concentrations, inorganic N of hairy vetch and black oat was the highest compared to other treatments for both soils during the 84 days of incubation. For Orzinuovi soil, the inorganic N of Egyptian clover was higher than white mustard (95.06 vs. 81.11 mg N/kg of dry soil; respectively 84 days after start of incubation) while for Sant'Angelo soil, inorganic N concentration of both treatments was similar. Black oat + and white mustard + (both collected in March) had a low inorganic N concentration, compared to the initial inorganic N concentration at the day of incubation, from the 7th to the 70th day after start of incubation. After 84 days of incubation, inorganic N concentration was slightly higher than the initial inorganic N concentration, for both soils (Orzinuovi and Sant'Angelo). Cereal rye and weed, both of them collected in March, had a very low inorganic N concentration

(24.5 and 2.07 mg N/kg of dry soil for cereal rye and 8.0 and 2.56 mg N/kg of dry soil for weed , respectively for Orzinuovi and Sant'Angelo soils, 7 days after start of incubation). Then, inorganic N concentration of both treatments increased gradually and reached 51.0 and 34.9 mg N/kg of dry soil for cereal rye and 65.4 and 56.9 mg N/kg of dry soil for weed, respectively for Orzinuovi and Sant'Angelo soils, 84 days after start of incubation.

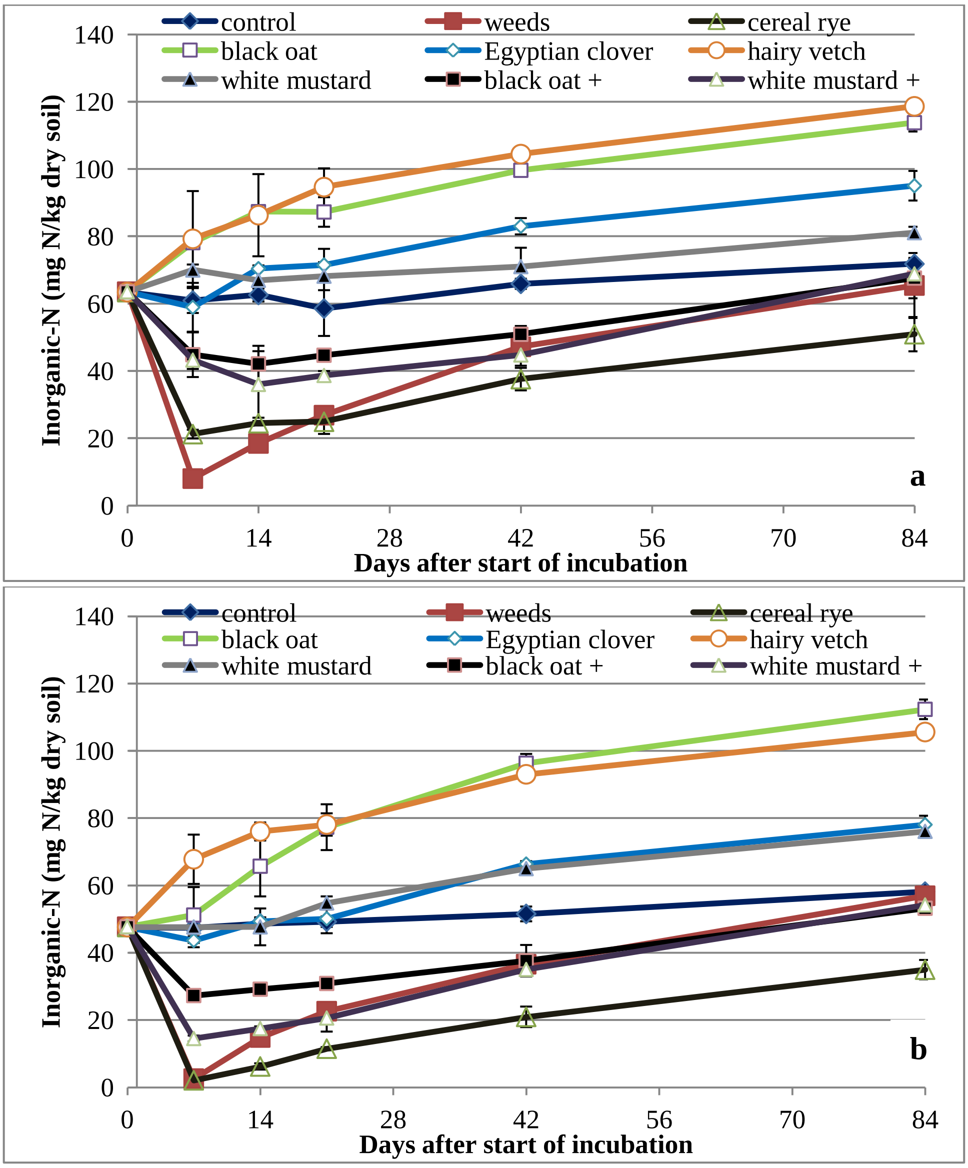


Figure 4.3. *Evolution of the soil inorganic-N concentration of soils amended with cover crop and weed shoots during 84 days after start of incubation. Orzinuovi (a) and Sant'Angelo (b) soils. Vertical bars represent standard deviation of four replicates. Black oat and white mustard shoots were collected in two dates: November and March ("+" sign).*

3.4. Net N mineralization

After 84 days of incubation, net N mineralization ranged between -33 and +45% for Orzinuovi soil (Figure 4.4 a) and -37 and +46% for Sant'Angelo soil (Figure 4.4 b). For Orzinuovi soil, hairy vetch, collected in March, had a progressive increase of net N mineralization during the whole incubation period (84 days) and reached 45% at the end of the incubation period. Similarly to hairy vetch, black oat had also a progressive increase of net N mineralization during the whole incubation period (84 days) but at a lower rate compared to hairy vetch (net N mineralization was 27% 84 days after start of incubation). The difference between hairy vetch and black oat in net N mineralization was due to higher rate of mineralization of hairy vetch in the first and the third week of incubation, while the rate of mineralization was similar in other dates. Egyptian clover did not mineralize N during the first week after incubation and started mineralization in the second week at a higher rate than black oat but 84 days after incubation, it reached 19%. White mustard collected in November, had a similar rate of mineralization to black oat during the first week of incubation then the rate of mineralization was almost close to zero and from the 7^{th} (8%) to the 84^{th} day after start of incubation (8%), net N mineralization of white mustard did not increase. White mustard +, black oat +, weed and cereal rye had a negative rate of N mineralization (i.e. N immobilization) during the first week after start of incubation. The rate of N immobilization of cereal rye was the highest, followed by weed and then black oat + and white mustard +, 7 days after start of incubation. After 84 days of incubation, the net N mineralization was close to zero for weed (-4%), black oat + (-4%) and white mustard + (-2%) while for cereal rye it was -33%.

For Sant'Angelo soil (Figure 4.4 b), treatments had a similar trend of N mineralization or immobilization to Orzinuovi soil but with slightly different rates. Hairy vetch had the highest rate of N mineralization among all the treatments and reached 46% of net N mineralization (84 days after start of incubation). Also, as for Orzinuovi soil, the difference in the final N mineralization (84 days after start of incubation) between hairy vetch and black oat is due to the low rate of N mineralization of black oat compared to hairy vetch in the first week of incubation. Then, rates of mineralization of both cover crops were similar, except the third week after start of incubation where rate of N mineralization of black oat was higher than hairy vetch (close to zero). White mustard and Egyptian clover, both collected in November, had an identical trend of N mineralization. In fact, rate of N mineralization was close to zero during the first three weeks after start of incubation then it increased and reached 16 and 17%, respectively for white mustard and Egyptian clover, 84 days after start of incubation. Cereal rye had the highest rate of N immobilization than weed, black oat + and white mustard +, 7 days after start of incubation. It was higher than the rate of N immobilization in Orzinuovi soil. Weed, black oat + and white mustard + had a similar trend during the whole incubation period and 84 days after start of incubation, N mineralization was close to zero. It was -1% for weed, -5% for black oat + and -3% for white mustard +. For cereal rye, Net N mineralization was -37%, 84 days after start of incubation.

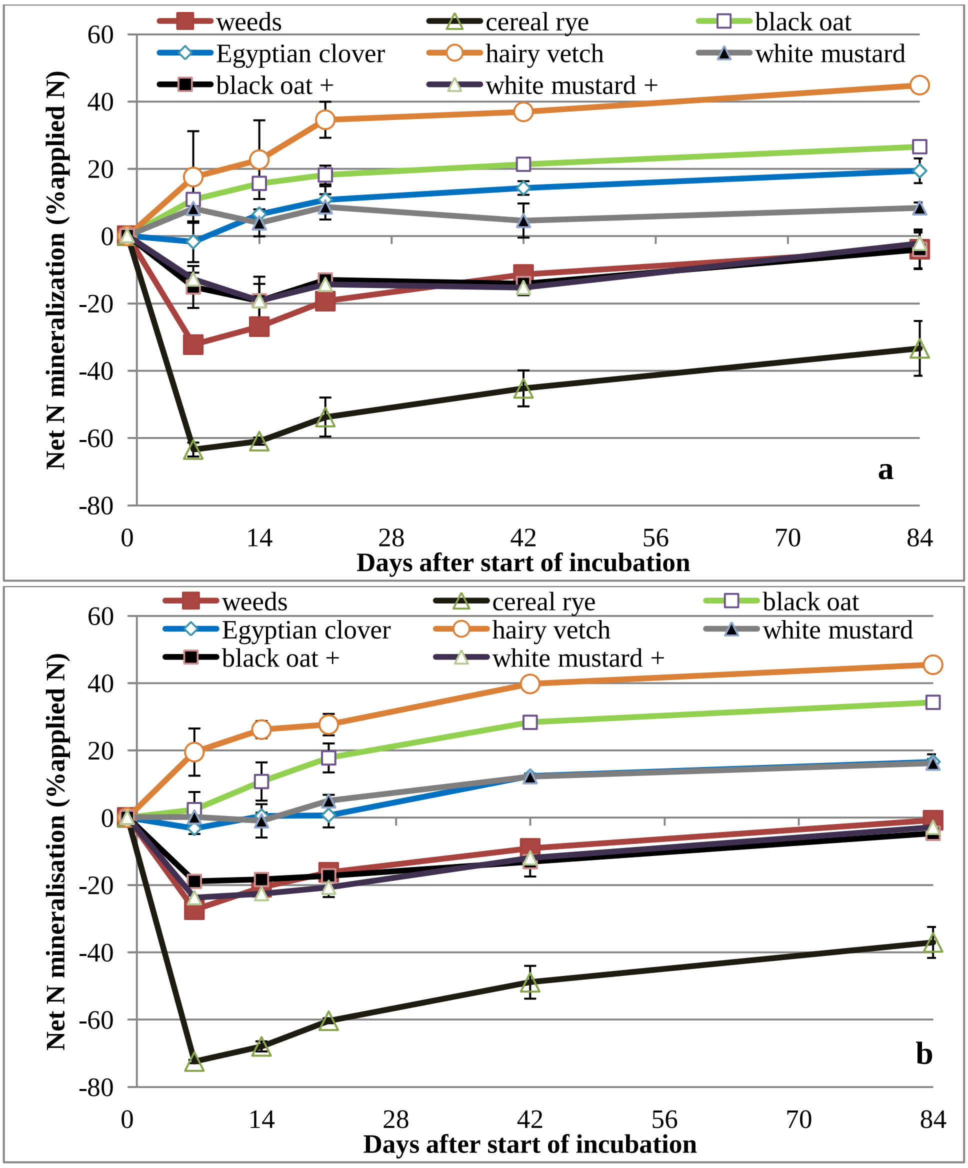


Figure 4.4. *Net N mineralization of soils amended with cover crop and weed shoots during 84 days after start of incubation. Orzinuovi (a) and Sant'Angelo (b) soils. Vertical bars represent standard deviation of four replicates. Black oat and white mustard shoots were collected in two dates: November and March ("+" sign).*

3.5. Relationship between C/N ratio and Net N mineralization of cover crops and weed shoots

Figure 4.5 shows the relationship between C/N ratio of the added materials and net N mineralization of cover crops and weed in Orzinuovi and Sant'Angelo soils, 84 days after start of incubation. For both soils, we had a good correlation between C/N ratio and net N mineralization. It was 0.91 and 0.86 for Orzinuovi and Sant'Angelo soils, respectively. This means that as the C/N ratio increases the net N mineralization decreases.

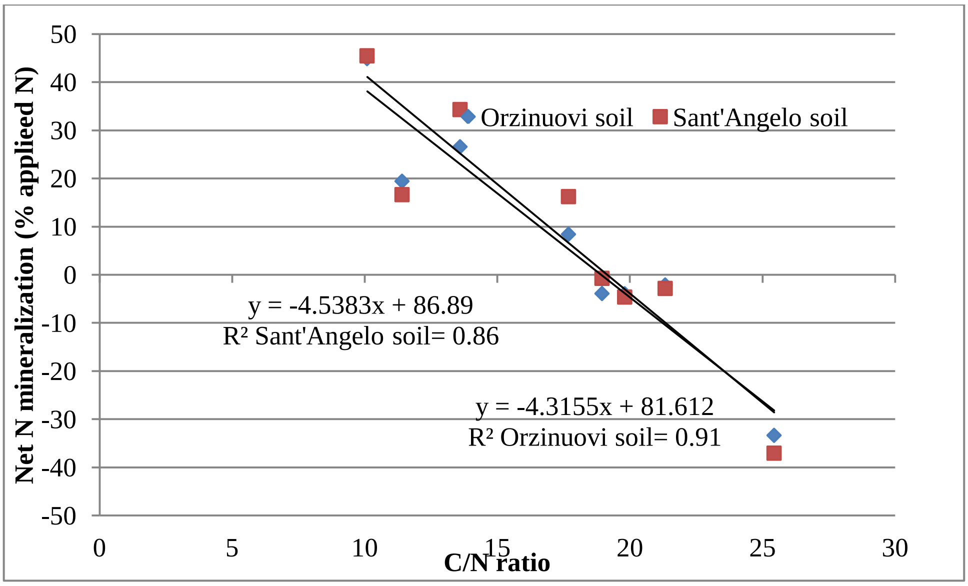


Figure 4.5. *Relationship between C/N ratio and net N mineralization of cover crops and weed in Orzinuovi and Sant'Angelo soils, 84 days after start of incubation.*

4. Discussion

Predicting C and N mineralization of plant residues returned to soil is very important for soil N availability (Hassan, 2013). Our results confirmed those of Nakhone and Tabatabai (2008), Li et al. (2013) and Perdigão et al. (2017) who reported that legumes mineralized more rapidly than non-legumes. In fact, the high net N mineralization of hairy vetch (Figure 4.4) obtained in Orzinuovi and Sant'Angelo soils is related to its high N content and low C/N ratio (Table 4.1). Black oat shoots collected in November had also a relatively high net N mineralization, compared to other shoots but lower than hairy vetch. This difference in net N mineralization could be explained by the differences in C/N ratio of both shoots, where black oat had a C/N ratio of 13.6 and hairy vetch had a C/N ratio of 10.1.

The second legume cover crop (Egyptian clover) had a different trend in N mineralization in the two soils. Nitrogen mineralization started 21 days after start incubation for Sant'Angelo soil, while for Orzinuovi soil it started 7 days after start of incubation. The same observation was valid for white mustard. This difference could be related to soil texture and organic matter. White mustard shoots, collected in November, having a C/N ratio of 17.7, were able to start N mineralization 7 days after start of incubation in a sandy loam soil having 3.9% of organic matter (Orzinuovi soil; Figure 4.4 a) while, for a loam soil having 1.5% of organic matter (Sant'Angelo soil; Figure 4.4 b), net N mineralization of white mustard residues started 21 days after start of incubation.

Our results have not confirmed those of Paul and Clark (1989) and Trinsoutrot et al. (2000) who indicated that net N mineralization occurs when C/N ratios of crop residues are < 25. In fact, for our incubation experiment, weed, black oat + and white mustard + had a C/N ratio of 19, 19.8 and 21.3, respectively (Table 4.1). These values were not able to start mineralization of N even 84 days after start of incubation. From our results, it seems that the threshold of C/N ratio to start N mineralization is 19 (Figure 4.5). In this case, the threshold of C/N ratio to start N mineralization may vary slightly (18.9 to 19.1) within the soil texture and organic matter as it was explained for white mustard shoots above. Other studies have attempted to give critical C/N ratios for N mineralization of vegetable crop residues, i.e. the C/N ratio at the break point between net N mineralization and net N immobilization. Critical C/N ratios depend on the duration of the incubation considered. Values in literature range from C/N = 20 for 4 week incubations (Iritani and Arnold, 1960), to C/N = 30 (Fox et al., 1990) or C/N = 40 for long term incubations of 11 to 44 weeks (Vigil and Kissel, 1991). Das et al. (1993) observed a critical C/N ratio of 46 after 30 days. This value increased to 95 after 120 days of incubation. Janzen and Kucey (1988) and De Neve and Hofman (1996) found the critical C/N ratio increase from 24 to 44 after 14 and 84 days of incubation, respectively.

Cereal rye had the lowest N mineralization among all the treatments during the 84 days of incubation for both soils. It was immobilizing N rather than mineralizing. Our results confirmed those of Redin et al. (2014) who indicated that leaves and stems of the Poaceae family exhibited gradual decomposition, resulting in more gradual and stronger N immobilization than which exhibited by Fabaceae species. These results can be attributed to the presence of higher cellulose and hemicellulose contents but also to low N content (total and soluble). The quality of crop residue mixtures, and particularly their N content, will more influence the net availability of N with higher availability of nitrogen from crop residues having higher N content (mainly legume cover crops compared to non-legume cover crops). Higher availability of nitrogen can be increased in situations where plant residues are not incorporated into soil by plowing due to higher contact between soil and crop residues. In addition to its very high C/N ratio (25.4), compared to other shoots, other typical factors had favored the N immobilization of cereal rye such as lignin, polyphenols, proteins and soluble carbohydrates and could explain differences on C and N mineralization among the green manures (Nakhone and Tabatabai, 2008). Soluble polyphenols slow the mineralization of residue N by forming complexes with proteins, thus making them inaccessible to the microorganisms (Mafongoya et al., 1998).

For winter-hardy cover crops (hairy vetch and cereal rye in this experiment), it depends on the botanical family of the cover crop and the cellulose and hemicellulose content of the residue as explained by Redin et al. (2014).

The effect of soil was smaller than the effect of cover crop residues and date of collection. In fact, the trend of nitrogen mineralization of the cover crop residues was similar for both soils, with an exception of Egyptian clover and white mustard collected in November. These two treatments started nitrogen mineralization two weeks later (21^{st} day after start of incubation) in Sant'Angelo soil compared to Orzinuovi soil (7^{th} day after start of incubation). The soil texture of the two soils was similar (Sandy and sandy loam soils for Orzinuovi and Sant'Angelo, respectively) and can explain the lower effect on nitrogen mineralization compared to the effect of cover crop residues and date of collection.

5. Conclusions

In this laboratory incubation experiment conducted under constant temperature of 20 °C and soil moisture of 100% field capacity, we have found differences in N dynamics (mineralization or immobilization) among the cover crop shoot materials incubated in two soils with different texture. Shoots of Fabaceae (hairy vetch) started N mineralization immediately after start of incubation with hairy vetch having the highest N mineralization 84 days after start of incubation, while Poaceae such as cereal rye immobilized N during the whole incubation period. White mustard and Egyptian clover collected in November had a similar trend in N mineralization. Black oat, white mustard and weed (all of them collected in March) were affected by their high C/N ratio (19.8, 21.3 and 19.0, respectively) and were immobilizing N during 84 days of incubation.

Chapter 5. Maize growth as affected by winter-hardy cover crop species, termination method and weed management

1. Introduction

Cover crops provide weed suppression either through competition (Mirsky et al., 2013), smothering (Hutchinson and McGiffen, 2000), or allelopathic activity (Barnes et al., 1987; Kunz et al., 2016). The weed suppressive potential of cover crops may depend on the species (or mixture of species) chosen, and the method of cover crop termination and residue management (Wortman et al., 2013). The timing and method of cover crop termination have both been shown to affect yield-influencing factors including: soil moisture availability, weed communities, cover crop and soil N content, and crop N uptake (Daniel et al., 1999; Mirsky et al., 2009; Parr et al., 2011; Wortman, 2012). Termination methods resulting in maximum surface residue and minimal soil disturbance have the greatest potential to inhibit weed germination and growth (Teasdale et al., 1991, 2007). The cover crop residues remain on the soil surface and act as a mulch that suppresses weed, also protecting the soil from rapid desiccation and keeping the soil moisture at good levels for cash crop seed germination or plant establishment (Bavougian et al., 2019). Cover crops can be terminated climatically (i.e., winterkill), chemically, or through various mechanical measures (e.g., plowing, disking, mowing, roller-crimping, or undercutting). The most appropriate termination method will depend on the farm management objective (Wortman et al., 2013). Herbicides have become the dominant tool for weed management in most modern agricultural systems (Weisberger et al., 2019) because they provide an easy and cost-effective way of controlling weed in crops and result in increased crop vigor and yield. Weed control still relies on the use of synthetic herbicides with 128 t of herbicides sold in EU-28 in 2014, i.e., 33% of pesticide sold (Eurostats, 2016—http://ec.europa.eu/eurostat/), despite widely acknowledged detrimental environmental and ecological impacts (Stoate et al., 2009) and major issues of herbicide resistance (Heap, 2014). In a survey conducted by Sustainable Agriculture Research and Education (SARE) in 2014, out of 1691 cover crop users, 48% have indicated that they terminate cover crops using herbicides, 21% have used tillage and 20% of cover crops users have selected winter-killed cover crops. There is, therefore, an urgent need to move towards more sustainable weed management strategies that are much less reliant on herbicide use. The use of winter-hardy cover crops has the inconvenient of the hardness of termination before sowing the following cash crop. Mechanical management of cover crops at termination and mechanical control of weed in the cash crop could be an alternative for a more sustainable management. Also, the choice of a total or partial mechanical management was not assessed in previous studies. The objectives of this study were to assess three managements of winter-hardy cover crops and weed in the following cash crop (maize). Managements are related to cover crops termination method and weed control (chemical vs. mechanical).

2. Material and Methods

2.1. Field presentation

The experiment was carried out in the framework of the CoCrop project (http://sites.unimi.it/cocrop/). The field was located in Orzinuovi, Brescia, Italy

(45°23’55.2”N, 9°54’30.2”E). The soil texture was sandy loam. Organic matter is 4%, carbon to nitrogen ratio was 9.1 and pH in soil: water was 6.8.

2.2. Experimental design

The trial compared two winter-hardy cover crop species that are cereal rye (*Secale cereale* L. cv. “Stanko”) and hairy vetch (*Vicia villosa* Roth cv. “Villana”) replicated four times and receiving the following treatments:

- “Business-as-usual” management: chemical termination of cover crops + chemical control of weed in the cash crop (maize);
- “Post-glyphosate” scenario: mechanical termination of cover crops + chemical control of weed in the cash crop (maize);
- “Organic” management: mechanical termination of cover crops + mechanical control of weed in the cash crop (maize).

Chemicals application consisted of glyphosate application (1.78 kg ha^{-1}) and mechanical operation was disking. In total, there were 24 plots arranged in a split-plot design (main plot: termination method; sub-plot: cover crop species). Each elementary plot was 6 × 8 m. Table 5.1 lists the main activities of crop and soil management and above-ground biomass sampling dates of cover crops and maize. Cover crops were sown on September, 5^{th} 2018. At mid-March, cover crops were terminated and ten days later soil was tilled as a seedbed preparation for maize sowing in spring. Cover crops above-ground biomass (AGB) samples were taken in March at cover crop termination. Then, maize cv. Pioneer 2105 (FAO class 600) was sown in the last week of March and maize samples were taken at V6 growth stage and harvest (Dent maturity growth stage). Nitrogen fertilizer was applied at 200 kg N ha^{-1} after V6 growth stage. Maize was irrigated from end of May to harvest with a frequency of 15 days.

Table 5.1. *Crop and soil management and above-ground biomass sampling dates of cover crops and maize.*

Date	Activity	Notes
05/09/2018	Cover crop sowing	
12/03/2019	Biomass sampling + cover crop termination	
22/03/2019	Soil tillage (seedbed preparation for maize)	
25/03/2019	Maize sowing	
22/05/2019	Biomass sampling	Six leaves (V6 growth stage)
30/05/2019	Maize irrigation	231.4 mm
03/06/2019	Maize top dress fertilization	200 kg N ha^{-1}
13/06/2019	Maize irrigation	231.4 mm
28/06/2019		231.4 mm
12/07/2019		231.4 mm
27/07/2019		231.4 mm
05/08/2019	Maize harvest	Dent maturity (R5 growth stage)

Cover crops sowing rates are the same to those presented in Chapter 2.

2.3. Measurements

Samples of cover crops and weed above-ground biomass were taken in March 2019 at cover crops termination. Samples of 1 m^2 were taken from each plot and then dried in the stove at 105 °C. For maize sampling at 6^{th} unfolded leaf (V6 growth stage), 15 plants were taken from each plot and then dried in the stove at 105 °C. At maize harvest, 20 plants were taken to determine the above-ground biomass (AGB). At each sampling date, fresh and dry weights were taken and then the above-ground biomass was calculated. All biomass data are reported as t ha^{-1}.

2.4. Precipitation and growing degree days

Precipitation and growing degree days of cover crops are the same to those presented in Chapter 2 as second year data because cover crops of this trial were sown and terminated at the same dates. Also, for maize sown and harvested in the same dates, precipitation and growing degree days are the same to those presented in Chapter 3.

2.5. Statistical analyses

Analysis of variance was conducted to determine the effect of factors (cover crop species and cover crops termination method/weed in maize control) on cover crops and weed AGB and maize growth using SPSS 25. Homogeneity of variance and normality of distribution of data were checked before analysis of variance. Post-hoc comparisons were made using SIDAK tests.

3. Results

3.1. Cover crops above-ground biomass at termination

There was a significant difference between hairy vetch and cereal rye AGB at termination in March (Figure 5.1) with hairy vetch having a higher AGB (2.4 t ha^{-1}) compared to cereal rye (0.4 t ha^{-1}), while for weed, no significant differences of biomass were identified between cover crop treatments.

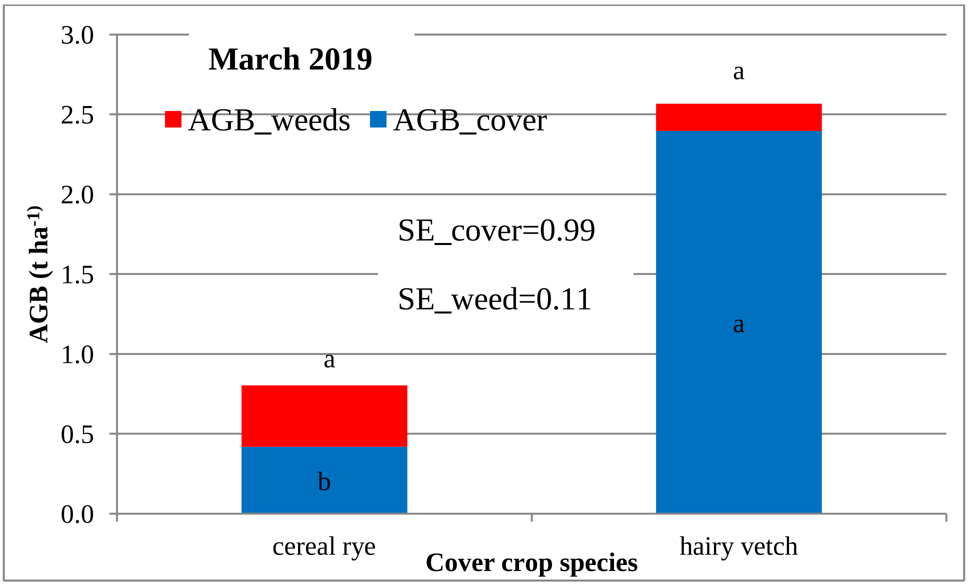


Figure 5.1. *Cover crops and weed Above-Ground Biomass (AGB) at termination. Each value represents the mean of four measurements. SE: Standard Error. Averages with the same letters are not significantly different at $P < 0.05$.*

3.2. Maize above-ground biomass at V6 growth stage

Maize above-ground biomass at V6 growth stage ranged between 0.16 and 0.27 t ha^{-1} (Figure 5.2). Significant differences were identified between cover crops. In fact, AGB of maize following hairy vetch was significantly higher than following cereal rye for the "post-glyphosate" and "organic" treatments (cover crops were terminated mechanically and control of weed in maize was chemical or mechanical): 0.27 and 0.22 vs. 0.21 and 0.16 t ha^{-1}, respectively. No significant differences were identified for both cover crops between the three managements: The highest maize AGB, for both cover crops, was for "post-glyphosate" scenario (cover crops terminated mechanically and weed in maize controlled chemically).

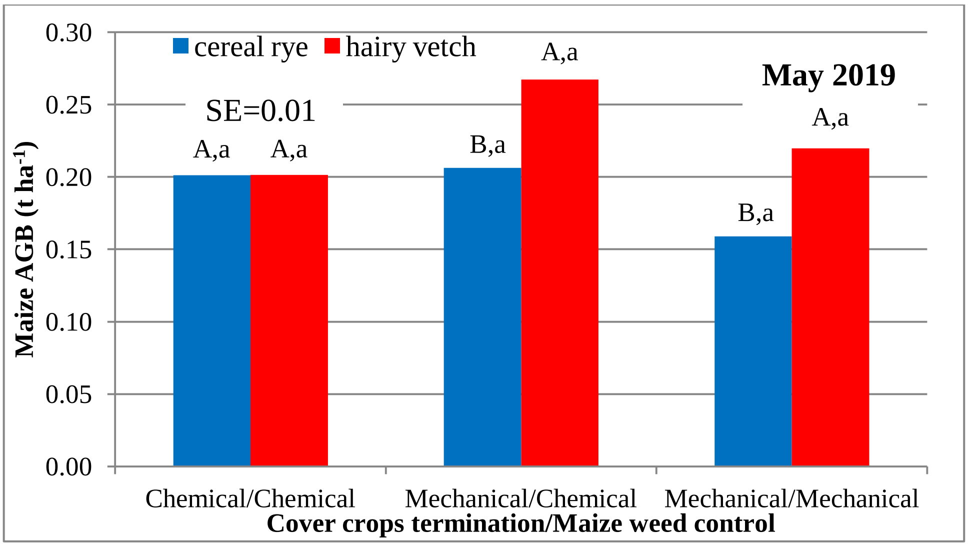


Figure 5.2. *Maize Above-Ground Biomass (AGB) at V6 growth stage. Each value represents the mean of four measurements. SE: Standard Error. Different uppercase letters denote different maize biomass among cover crop species (P<0.05). Different lowercase letters denote different maize biomass among termination/weed control methods (P<0.05). Averages were separated through LSD post hoc test. .*

3.3. Maize yield at harvest

Maize yield at harvest ranged between 15.4 and 27.3 t ha^{-1} (Figure 5.3). As in the V6 growth stage, maize in the "post-glyphosate" scenario and "organic" management following hairy vetch had a significantly (*P*<0.05) higher yield (27.3 and 19.8 t ha^{-1}, respectively) than maize following cereal rye (23.6 and 15.4 t ha^{-1}, respectively). Maize following hairy vetch in "organic" management had a significantly lower yield than maize following hairy vetch in "business-as-usual" management (*P*<0.05) and "post-glyphosate" scenario (*P*<0.01). The same conclusion is valid for cereal rye with *P*<0.01 for both managements.

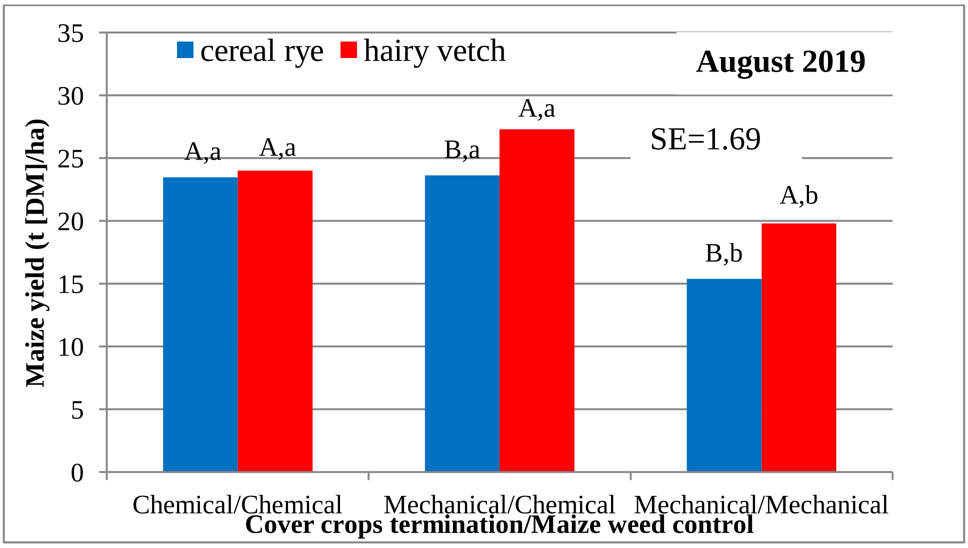


Figure 5.3. *Maize yield at harvest. Each value represents the mean of four measurements. SE: Standard Error. Different uppercase letters denote different maize biomass among cover crop species (P<0.05). Different lowercase letters denote different maize biomass among termination/weed control methods (P<0.05). Averages were separated through LSD post hoc test.*

4. Discussion

Our results indicate that, at cover crops termination (Figure 5.1), cereal rye above-ground biomass was significantly lower than hairy vetch above-ground biomass. It could be explained by the attack of *Duponchelia fovealis* Zeller larvae in the few days after sowing. Weed AGB of both cover crops was not significantly different between the two cover crops. In our experiment, we confirmed the findings of Osipitian et al. (2019) who indicated, in a meta-analysis, that cover crop termination methods (herbicide application, disking, mowing, rolling and undercutting) had no differential impact on weed suppression (10 studies, 79 observations) by cover crops for observations made from 2 to 5 weeks after termination. Also, in a two years experiment, for no-till soybean following barley, Rosario-Lebron et al. (2019) demonstrated that method and timing of termination had no significant effect on soil moisture or yield. In fact, the effect of mechanical termination of cover crops compared to chemical termination was assessed at V6 and R5 growth stages where no significant differences were identified between the "business-as-usual" management and the "post-glyphosate" scenario. However, maize AGB and yield were higher (+35% and +14% for V6 and R5 growth stages, respectively for hairy vetch and +5% and +0.6% for V6 and R5 growth stages, respectively for cereal rye) under a mechanical compared to a chemical termination of cover crops. These results show that the effect of mechanical termination is major in the beginning (V6 growth stage) and decreases over time. It can be explained by the decomposition of the cover crops residues incorporated in the soil so a higher soil-cover crop residue contact and an immediate enhancement of soil fertility (synchronization between maize demand and nitrogen availability) while for chemical termination, residues are kept on the soil surface (less contact between soil and cover crop residue) and need more time to decompose. The absence of significant difference in our experiment between the two termination methods can be explained by the effect of the high organic matter of the soil (4%) in decomposing the residues resulting from the chemical termination of the cover crops while under a mechanical termination, residues are disked in small fragments and improve immediately the soil fertility.

Differences in maize AGB (at V6 growth stage) and yield (at harvest) between the two cover crops were identified only under a mechanical termination of both cover crops ("post-glyphosate" scenario and "organic" management) where hairy vetch had a significant higher AGB (+29 and +38% for "post-glyphosate" scenario and "organic" management, respectively) and yield (+16 and +29% "post-glyphosate" scenario and "organic" management, respectively) compared to cereal rye. It can be related to the soil-cover residue contact that is greater in case of mechanical termination of cover crops as discussed above and in consequence it promotes the process of nitrogen mineralization. Also, the cover crop species factor is important especially for hairy vetch, as mentioned by Clark (2007) and Etemadi et al. (2017), a legume cover crop that can contribute nitrogen to following crops through N_2 fixation, which may increase crop yields compared with other cover crops. In our experiment, hairy vetch had more biomass (2.4 vs. 0.4 t ha^{-1}) at termination with higher nitrogen concentration and a lower C/N ratio (based on results mentioned in Chapter 2) than cereal rye. All this factors can contribute to a significant interaction between cover crop species and management method but it was not the case in this experiment neither at V6 growth stage nor at harvest.

The significantly lower maize yield following both cover crops in the total mechanical management ("organic" management) can be explained by the low effectiveness of mechanical weed control in maize compared to the chemical control. In fact, compared to the "post-glyphosate" scenario, the maize yield decreased by 28 and 35% for hairy vetch and cereal rye, respectively in the "organic" management. It seems that using chemical weed control has eliminated the competition between the maize plant and weed for resources such as light, water, space and nutrients while in a mechanical control of weed, the resource competition was not eliminated totally and weed were disked but were able to disturb the maize growth and compete especially for water that was a limiting factor for growth during this period as indicated in Chapter 3.

The results provided by this study are promising and push towards reducing the use of herbicides only for weed control in the cash crop but this experiment has the limitation that it was carried out for one year and need to be repeated for several years under different soil textures and climatic conditions using other winter-hardy cover crops.

5. Conclusions

Maize following the two winter-hardy cover crops under a "post-glyphosate" scenario" (mechanical termination of cover crops and chemical control of weed in maize) or a totally chemical management had a significantly higher yield than maize under an "organic management", while at V6 growth stage no significant differences were identified between the three managements. Also, maize AGB at V6 growth stage and at harvest were significantly higher for hairy vetch than cereal rye for the "post-glyphosate" scenario and "organic" management.

Chapter 6. General conclusions

In this PhD thesis, we assessed pure winter cover crops, planted from end-August to mid-March, as affected by plant species belonging to three botanical families (Poaceae, Brassicaceae and Fabaceae) and sowing dates (SD1: end-August and SD2: mid-September) in a field experiment during two years under conservation agriculture. A field experiment was conducted to cover the knowledge gap of using cover crops in Northern Italy to define the most suitable cover crop species and the most appropriate sowing date for a better establishment of the cover crop and later on improving the soil fertility after incorporation. The effects were tested on the cover crop itself growth, nitrogen uptake and control of weed (with and without cover crops) and the contribution of each cover crop to the following main cash crop (maize) growth and yield in terms of nitrogen recovery and immediate availability. A laboratory incubation experiment was also conducted, under constant temperature and soil moisture, to establish the course of N mineralization from five pure cover crop species and weed shoots. In addition, we conducted a one-year field experiment to assess the effects of two winter-hardy cover crops and three managements (related to cover crops termination method and weed control – chemical vs. mechanical) on the following cash crop (maize) with the objective of limiting the use of chemicals (glyphosate) for a more sustainable development.

Results of the two year field experiment (Chapter 2) have demonstrated a significant difference between the five cover crop species in above-ground biomass (AGB) with white mustard SD1 having the highest productivity (5.3 and 3.2 t ha^{-1}, respectively for the first and the second year) and Egyptian clover having the lowest AGB (less than 1 t ha^{-1}) in November (before temperatures decrease). The AGB of all cover crops, except rye, was lower when sowing date was delayed by 15 days. Cover crop biomass was lower in the second year compared to the first year and it was inversely related to weed AGB. Cover crops confirmed their ability to reduce the biomass of weed compared to keeping the soil bare. Legume cover crops had the highest N concentration compared to non-legume cover crops. Therefore, non-legume cover crops had the highest C/N ratio compared to legume cover crops. Nitrogen uptake was higher for hairy vetch than for other cover crops, except mustard, with the maximum uptake (114 kg N ha^{-1}) demonstrated in the first year at the first sowing date (November).

Then, the assessment of cover crop nitrogen contribution to the following cash crop (maize) (Chapter 3) demonstrated the absence of significant effects on maize AGB at six leaves growth stage (V6) and maize yield at dent maturity growth stage (R5). Maize was not affected neither by sowing date of cover crops (end-August or mid-September), nor by cover crop species for the two years of experiment. Apparent Nitrogen Recovery (ANR) of maize was higher for the first than the second year, with maize following hairy vetch SD2 having the highest recovery (+67%). Also, in all cases, the importance of sowing cover crops instead of keeping the soil bare was demonstrated by the higher recovery of maize following cover crop species compared to maize following no cover crop treatment.

In Chapter 4, we have found differences in N dynamics (mineralization or immobilization) among cover crop shoots incubated in two soils under controlled conditions of soil moisture and temperature in laboratory. Shoots of Fabaceae collected in November (hairy vetch) started N mineralization immediately after start of incubation with hairy vetch having the highest N mineralization, 84 days after start of incubation, while Poaceae such as cereal rye immobilized N during the whole incubation period. White mustard and Egyptian clover collected in November had a similar trend in N mineralization. Black oat, white mustard and weed, collected in March, were affected by their high C/N ratio (19.8, 21.3 and 19.0, respectively) and were immobilizing N during 84 days after start of incubation.

The one year field experiment (Chapter 5) conducted to assess the effect of three managements of winter-hardy cover crops at termination and control of weed in maize (chemical vs. mechanical) indicated that a mechanical termination of cover crops did not have significant difference on maize biomass at V6 growth stage and maize yield at R5 growth stage, compared to a chemical termination, for both cover crops (hairy vetch and cereal rye). Also, maize yield following hairy vetch was significantly higher than maize yield following cereal rye only when cover crops were terminated mechanically. In general, the “post-glyphosate” scenario (mechanical termination of cover crops and chemical control of weed in the main cash crop) was the best management but our experiment had the inconvenient that it was conducted only for one year.

In general, in this PhD thesis, through the field and laboratory incubation experiments we highlighted (i) the importance of using cover crops during winter instead of keeping the soil bare in reducing weed infestation and improving maize nitrogen recovery, (ii) the importance of sowing cover crops at the end of August instead of mid-September, for a better establishment and higher biomass of the cover crop and in consequence a higher nitrogen uptake, (iii) the absence of significant differences between maize yield affected by the five cover crop species and sowing dates tested in this work, (iv) the differences in nitrogen availability between the five cover crops after incorporation and (v) the alternative of mechanical termination of winter-hardy cover crops instead of chemical termination and the limitation of the application of herbicides to the control of weed in maize.

Considering all studied factors in this research project and based on results obtained under field conditions, I suggest choosing white mustard for the study area of Northern Italy as the best cover crop. This cover crop demonstrated, during the two years of experiment, the highest AGB, the best weed suppression, an acceptable N concentration and N uptake (compared to hairy vetch), and a relatively high C/N ratio (compared to legume cover crops). After incorporation of white mustard in the soil, maize following white mustard had a comparable biomass and yield to maize following legume cover crops without significant difference. Also, maize following the same cover crop had a slightly negative to positive N recovery (-12 and +30% for the first and the second year of experiment, respectively). In a laboratory incubation experiment, white mustard had a positive net N mineralization rate when it was collected in November and a slightly negative net N mineralization when it was collected in March. The second best cover crop is hairy vetch sown in SD1 due to its high N uptake and net mineralization of N after incorporation. In consequence, maize following hairy vetch recorded the highest yield and a high N recovery (-9 and +46%, respectively for the first and the second year of experiment). As it is a winter-hardy cover crop, hairy vetch should be terminated mechanically and a chemical weed control in maize should be applied to obtain the highest maize yield. The choice of the cover crop species can be managed according to the soil/farm case. In fact, a poor soil will need a leguminous cover crop (basically hairy vetch) to enhance the soil fertility. However, a soil with a large weed seed bank will need a cover crop more effective in weed suppression (such as white mustard as demonstrated in this work) rather than supporting soil with N.

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