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LONG-TERM EFFECT OF TILLAGE AND CROP SEQUENCE ON SOIL MICROBIAL COMMUNITY AND NITROGEN EMISSIONS IN MEDITERRANEAN ENVIRONMENT

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1. Introduction

Nitrogen (N) is the most abundant compound in the atmosphere (78%), as elemental nitrogen N_2, and the atmosphere is the reservoir pool of this element. However, N is the nutrient element more deficient in the agricultural soil (Godwin and Singh, 1998) and it is a limiting factor for the growth and the plant productivity. In fact, nitrogen is a constituent of numerous essential plant compounds including chlorophyll, nucleic acid, vitamins, and it's the basic constituent of proteins. This mismatch between the atmosphere richness and the soil shortage, is due because only a small fraction of atmospheric N is available for plants. For this reason, in order to overcome this deficiency and support production systems more and more intensive, nitrogen fertilizers were introduced. In this contest, the global fertilizers consumption, from the start of its production in the early 1900, grew rapidly from the 11.5 Mt of 1961 to the 60.7 Mt of 1980 until the 99.5 Mt of 2013 (FAOSTAT). Moreover, FAO estimated for 2018 an annual growth of 1.4 percent of the nitrogen fertilizers demand (FAO, 2015).

The high N inputs in the agroecosystem, adopted to support and stimulate agricultural production, can result in a spatial or temporal, wide N surplus because frequently the doses distributed exceed the amount of N removed from the plant and agricultural products (Velthof et al., 2009). Indeed, nitrogen fertilizer uptake from plant rarely exceed 50% of the nitrogen applied (Mosier et al., 2002). The N excesses can be subject to high losses through volatilization, denitrification and leaching (Di and Cameron, 2002). The dispersion of nitrogen in the environment, besides of representing an economic loss, can negatively affect the water quality of rivers, lakes and seas and the atmosphere and increase the greenhouse effect in the atmosphere, along with CO_2.

Nitrogen losses in agroecosystem can occur by water transport (leaching or surface runoff), or by gaseous emissions (ammonia volatilization and nitrous oxide emissions). In the recent

past, great attention was focused on leaching and runoff N losses to ground and surface waters due to the potential harmful effect to human health. In particular, consume water with a concentration in excess of 50 mg NO_3-N/l is noxious for infants and small children and has also been linked to cancer and heart disease (Grizzetti et al., 2011; Di and Cameron, 2002). Furthermore, nitrate contamination of rivers and lakes can contribute to eutrophication causing uncontrolled proliferation of algae and fish deaths (Smith & Schindler, 2009). Nitrogen losses by ammonia (NH_3) volatilization can occur when fertilizers containing ammonium (NH_4^+) or animal urine and feces are broadcasted in the fields. This substances dissolving in the soil solution react with water in a hydrolysis process. During this process, according to the soil conditions, a fraction of nitrogen up to 50% can be lost as volatile NH_3 in the atmosphere. After volatilization, ammonia can return to the soil through wet deposition by rainfall or dry deposition, attached to particulate, contributing to acid rains, occult depositions and eutrophication of natural ecosystems (Cameron and Moir, 2013). Nitrous oxide emission is the results of soil microbial activity and can occur during the nitrification and the denitrification process (NRC, 2003). This nitrogen loss can be significant, contributing also, to climate change and ozone layer depletion (Cameron and Moir, 2013). Nitrogen losses by ammonia volatilization and nitrous oxide emissions from soils is a main target of the present dissertation and will be treated with more details below.

Nitrogen cycle

Nitrogen (N) is the most abundant element in the Earth's atmosphere, hydrosphere, and biosphere, but unfortunately, it is the least readily element available to sustain life (Galloway et al., 2003). N compounds in nature can be divided into two groups: nonreactive and reactive. Non reactive nitrogen is in the form N_2, whereas the reactive include the nitrogen

contained in all biologically, photochemically, and radiatively active compounds. This second form of nitrogen includes reduced relatively reduce forms, like ammonia (NH3) and ammonium (NH_4^+), inorganic oxidized forms, like nitrogen oxide (NO_x), nitric acid (HNO_3), nitrous oxide (N_2O) and nitrate (NO_3^-), and organic compounds like urea, amines, proteins and nucleic acid. The biggest pool of nitrogen is in non reactive form, not directly available for plants, whereas reactive nitrogen is a small quantity. The nitrogen cycle is the complex of physical and biochemical transformation and transfers leading nitrogen from the no reactive form to organic, and finally, to inorganic form available for plant (Stevenson and Cole, 1999) (Fig 1).

In agricultural soils the main process responsible of the transformation from non reactive to organic form, and principal natural source of nitrogen, is the biological nitrogen fixation, whereas the transformation from organic to mineral nitrogen is due to the nitrification process. Nitrogen can be lost from the soil, and subtracted from the plant and animal uptake, through: ammonia volatilization, leaching, and denitrification (Fig 1.1).

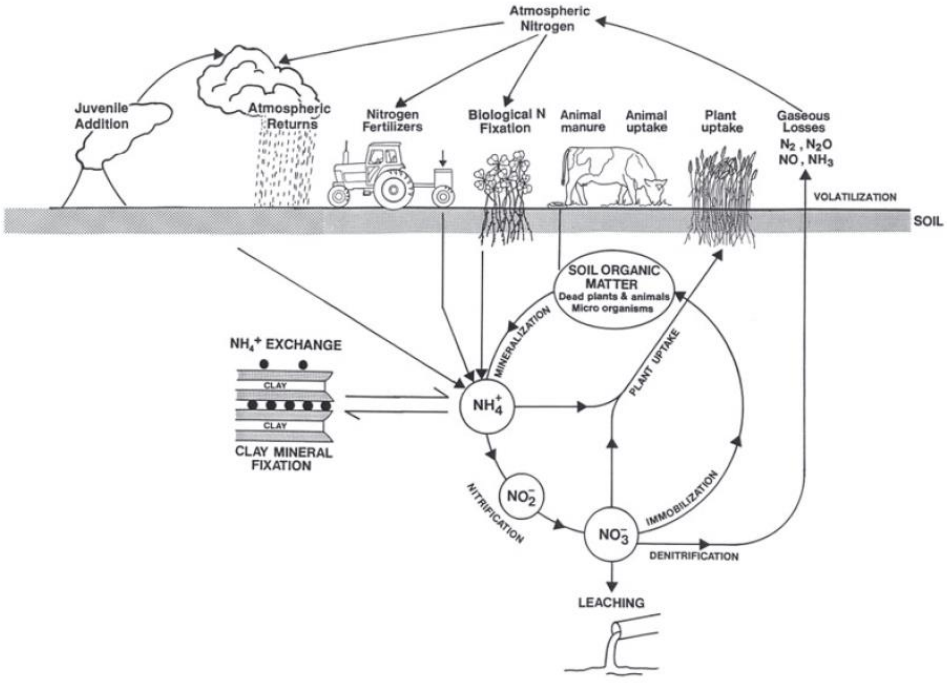

Fig 1.1 The nitrogen cycle. Source: Cameron and Moir 2012.

Ammonia volatilization and nitrous oxide emissions from agricultural soils

Ammonia volatilization

Globally, it was estimated that 54 Tg N-NH_3 are emitted each year in the atmosphere and of these 9 Tg N-NH_3 are due to the use of synthetic fertilizers (Bouwman et al., 1997). Europe agriculture, on average, emit 3.6 Tg N-NH_3 per year and is responsible of 93.3% of the total European ammonia emissions (EAA). Of total European nitrogen volatilized in agriculture, 58.9% derives from the manure management and 34.4% from agricultural soils, including the losses from nitrogenous fertilizers and from fertilized crops (EAA). Historical trend showed how in the past 50 years ammonia emissions have increased following the agricultural intensification and the increased number of livestock bred (Sutton et al., 1993). As mentioned previously, ammonia emission can have a strong impact on the environment causing occult N deposition influencing the N cycle and producing N saturation (Aber et al., 1989; Matson et al., 2002; Adams, 2003). The effects of these phenomena directly translate in eutrophication, acidification and loss of biodiversity (Pearson and Stewart, 1993; Fangmeier et al., 1994; Krupa, 2003). Eutrophication may occur in the surface waters causing algae proliferation, oxygen shortage and fish deaths. In the atmosphere ammonia can react and produce HNO_3 causing acid rain and acidification of the deposition surface. Moreover, when ammonia reacts with sulfur, nitrogen, and other acidic species can form sulfate and ammonium nitrate particulate matter witch reduce the visibility and have an impact on human health. Finally ammonia emission, determining N abundance can bring to a rapid decline in species richness (Sala et al., 2000) and can act as a secondary source of nitric oxide and nitrous oxide (Bouwman, 1990).

From the chemical point of view ammonia (NH_3) is the most reduced form of reactive, inorganic nitrogen (Aneja et al., 2008). Ammonia emission from soil mainly occurs when urea ($CO(NH_2)_2$), and less when ammonium fertilizers (NH_4^+) are broadcast into the soil. Urea is the most used nitrogen fertilizer in the word, with 76 Mt consumed in the 2011 (FAOSTAT) and represent more than 50% of the total nitrogen fertilizers consumed in the world (Glibert et al., 2006). Its diffusion is due to its low production cost and high nitrogen content, which results in low costs of transport (Roy and Hammond, 2004).

Ammonia volatilization process

Ammonia volatilization is a complex process involving physical, chemical and biological factors. This process has been described by Sommer et al. (2004). Urea in presence of water and a microbial enzyme, urease, is rapidly hydrolyzed following the reaction below:

$$CO(NH_2)_2 + 3H_2O \rightarrow urease \rightarrow 2NH_4^+ + HCO_3^- + OH^-$$

In a subsequent step, ammonium (NH_4^+), into acqueous phase, is dissociate according to temperature and pH:

$$NH_4^+ + OH^- \leftrightarrow NH_3 + H_2O$$

In this phase the relative concentration of NH_4^+ and NH_3 is determined by soil pH. At a pH above 7.0, where fewer hydrogen ions are available in soil solution to react with ammonia to form ammonium, the transformation of ammonium in ammonia is favored. Ammonia

produced is solubilized in soil water and at the boundary between air and liquid an equilibrium is established among the acqueous and gas phase (Bolan et al., 2004).

$$NH_{3(aq)} \leftrightarrow NH_{3(gas)} \uparrow$$

Ammonia (NH_3) from the acqueus phase volatilize into ambient air, following the Henry's Law, depending from air temperature and wind velocity above the solution. Ammonia solubilization in the soil water produce an increase of pH around the urea granules enhancing the losses and determining a lower use efficiency. Some authors reported that the losses of N due to NH_3 volatilization can up to the 50% of N applied (Black et al., 1985; Gioacchini et al., 2002; Sommer et al., 2004; Rochette et al., 2009; Zubigalla et al., 2002; Holokomb 2011). Urea hydrolysis usually takes place in a short time ranging from a few days until a two, three weeks (Kiss and Simhiaian, 2010; Singh et al., 2013).

The principal driver of ammonia volatilization process is the urease enzyme. The urease enzyme into the soil can derive from micro-organisms or from plant tissue in decomposition and can be present inside a ureolytic micro-organisms (bacteria, actionomycetes and fungi). When the enzyme is free into the soil, it is adsorbed on soil colloids and protected from the disruption by microbes, maintaining its function for long time (LlOyd and Shaffe, 1973; Burns et al., 1972). The accumulation of urease into the soil is the primary source of ureolysis in soils (Kiss et al., 1975).

Factor influencing ammonia volatilization

The quantity of urease enzyme in the soil has a direct effect on the magnitude and rate of ammonia volatilization. Urease in soil is correlated with the soil organic matter content and,

as mentioned above, to the capacity of soil to absorb it. Crop residues are a rich source of urease and their placement on soil surface in contact with urea, as in untilled surfaces, can stimulate ammonia volatilization (McInness et al., 1986; Palma et al., 1998).

Soil temperature is an important factor that can play a role in different phases of ammonia volatilization process. In particular high temperature, stimulating urease activity, cause a rapid rising of soil pH enhancing ammonia emission, whereas low soil temperature permit low hydrolysis rate with a smaller soil pH increase and allows urea to diffuse into the soil (Ernst and Massey, 1960). Temperature, as described above, can affect the ammonia dissociation in to the soil solution and the transition of the ammonia from the liquid phase to the gas phase (diffusion). For these reasons, lower ammonia losses are observed when urea is distributed with lower temperature (Ball & Keeney, 1983).

Water is an essential factor of the hydrolysis process and soil water content can have a great influence on ammonia volatilization, influencing the dissolution of urea granules and the diffusion of hydrolysis product into the soil. If soil water content is reduced drastically, the hydrolysis of urea can be reduced up to the stop (Vlek and Carter,1983). Water addition to the soil, by irrigation or precipitation, can have a major influence on ammonia emission. In this regard, some authors observed a massive reduction of ammonia emission after a rainfall event (>10mm), recording ammonia losses lower than 5% of the nitrogen applied (Black et al., 1987; Ferguson et al., 1984; Dawar et al., 2011; Holcomb et al., 2011).

Soil cation exchange capacity (CEC), retaining ammonium ions to the surface of soil colloids, such as clay and organic matter, help to reduce the ammonium in the soil solution, available for conversion to NH_3, and contrast the rapid soil pH increasing after the urea hydrolysis. For this reasons, in clay soil the ammonia losses are lower than in sandy soils (Cameron and Moir, 2013; Whitehead and Raistrick, 1993; Ryan et al., 1981; Ferguson et al., 1984). Moreover, soils with high CEC are usually well saturated with calcium ions witch

can contrast the pH increase caused by the urea hydrolysis and making available sites on colloids for ammonium ions (Jones et al., 2013).

Overall, condition that can favor ammonia losses are the abundance of crop residues, high temperature (>13°C), wind, drying of soil surface, neutral or basic soil pH and low cation exchange capacity (Clay et al., 1990; Ferguson and Kissel, 1986; Bouwmeester et al., 1985; Sommer and Christensen, 1992). On the contrary, combination of low temperatures, high rainfall after fertilization, low wind velocity, low soil pH and high cation exchange capacity can reduce the ammonia volatilization flow (Sommer et al., 1991, 2004; McGarry et al., 1987).

Management practices affecting ammonia emissions

Among the management practices, tillage system adopted can have a decisive role in determining the quantity of nitrogen lost through volatilization. In no tillage systems, the conjunction of surface placement and direct contact with crop residue, rich in urease, can lead to losing high amounts of nitrogen. Different authors around the word reported higher ammonia emissions in no tilled surfaces than in tilled (Keller and Mengel, 1986; Palma et al. 1998; Bacon and Freney, 1989; Rochette et al., 2008). Urea distribution in soil surface without any operation of mixing to the soil can expose the fertilizers to a rapid hydrolysis causing higher ammonia losses (Rochette et al., 2013; Rodrigues Soares, 2012). With regard to ammonia volatilization, crop residue may have a triple effect: as a source of urease enzyme, protecting the soil water from evaporation and making it available for hydrolysis, and finally, can reduce the diffusion of fertilizers into the soil (McInnes et al., 1986; Al-Kanani and MacKenzie, 1992; Jones et al., 2013; Bergostrom et al., 1998).

According to above, in order to contrast ammonia losses, fertilizer incorporation into the soil is on of most effective practice increasing urea N-use efficiency (Rochette et al., 2001; Sommer et al., 2004; Grant et al, 2001). Different author report a reduction by 86-95% of the total ammonia losses incorporating urea after distribution (Rochette et al., 2013; Fenn & Miyamoto, 1981; Prasertsak et al., 2001; Sommer et al., 2004).

As reported above, water can have a dual function in the volatilization process, activating urea hydrolysis and transporting urea hydrolysis product into the soil. A study by Turner et al. (2012) showed the importance of wheatear condition and soil water content when the urea is distributed on the soil on ammonia emissions. As mentioned above, the urea distribution before a rainfall event or irrigation, favoring the diffusion of ammonium, from the surface to the deeper soil layer, contribute to its adsorption to soil particles (Clay et al., 1990; Rodrigues & Kiehl, 1986), determining a massive reduction of nitrogen volatilization losses (Black et al., 1987; Ferguson et al., 1984; Dawar et al., 2011; Holcomb et al., 2011). In particular, research form Black et al. (1987) and Bowman et al. (1987) showed how a precipitation above 10 mm within 8 hours after the urea distribution can reduce the ammonia losses up to 93%.

A management practice applied in the recent past, in order to reduce nitrogen losses due to ammonia volatilization, is the use of urease inhibitors. These substances added to urea reduce the rate of urease activity on the soil, slowing down its action, and allowing the fertilizer to diffuse into the deep layer of soil, causing a reduction of ammonia emissions. One of most used and efficient urease inhibitor is the is the N-(n-butyl) thiophosphoric triamide (NBPT) (Bremner ,1995; Gioacchini et al., 2002; Zaman et al., 2009; Sanz-Cobena et al., 2011; Singh et al., 2013). Watson et al. (1994) reported a reduction up to 95% of ammonia volatilization using NBTP, whereas Sanz-Cobena et al. (2008) measured a 42% reduction in ammonia emission by using NBPT-coated urea in comparison to uncoated urea under Mediterranean

conditions. Finally, Sanz-Cobena et al. (2011), Damney et al. (2004) and Gioacchini et al (2002) found a reduction of ammonia emissions of 77%, 65% and 68%, respectively, using this inhibitor.

Nitrous oxide

Nitrous oxide (N_2O), along with carbon dioxide (CO_2) and methane (CH_4), is a greenhouse gases (GHG), responsible of trapping solar radiation inside the atmosphere, causing the increasing of the mean planet temperature and the climate changes. According to the Intergovernmental Panel on Climate Change of the United Nations, the concentration of N2O in the atmosphere from the year 1750 (before industrial era) increased by 16%. Although, carbon dioxide is considered the most important greenhouse gas, due to its concentration in the atmosphere higher than other GHG gases, it must not be taken into account that both the warming potential, and the average lifetime of each gas are important factors that should be taken into account when evaluating the radiative force of the emission of a system. From this point of view, nitrous oxide can be particularly dangerous for the environment with a warming potential of 280-310 times higher than CO2 and a lifetime in the atmosphere of 118-131 years (IPCC, 2001; Fleming et al., 2011). Further, N2O, in addition of being a GHG, participates directly to the destruction of ozone layer in the atmosphere, that protects the earth from sun's ultraviolet radiation. In the atmosphere an aliquot of nitrous oxide can be involved in a photolysis reaction producing nitric oxide (NO), which in turn reacts with the ozone (O3) transforming it in O2 (Chapman et al., 1930; Warneck, 1988; Portmann et al., 2012). N2O is the most important anthropogenic emission responsible of O3 destruction e the control of its release in atmosphere can be have a relevant effect on contrast O3 depletion (Portmann et al., 2012).

Nitrous oxide in atmosphere, comparing to the other GHG, represent the 6% of total emissions and its concentration is steadily rising (IPCC, 2001).

Different studies calculated that more than 80% of anthropogenic nitrous oxide emission derive from agriculture and more than 75% is emitted from agricultural soils (e.g. Isermann 1994; Duxbury et al. 1993); According to data from EPA (2015), in the United States the 74% of the human emission is caused by the agricultural soil management. In particular, agricultural soils emitted 3.5 Tg N2O- N per year (IPCC 2006).

Other authors suggested emission slightly higher (4 Tg N2O-N per year; Bouwman et al. 2009), or lower (2.8 Tg of N per year; Denman et al. 2007). One of the main reasons for the high emission of nitrous oxide in agricultural sector is the high nitrogen inputs through N-fertilizers. Matthews (1994) estimated that from 1 to 3%, of the total N applied with fertilizers is emitted in the atmosphere as nitrous oxide, equal to approximately 2.0 Tg N_2O-N per year. Mosier et al. (1996) suggested that the use of fertilizers and manure overall cause the emission of 3.0 Tg N_2O-N yr^{-1}.

The principal biochemical processes that cause the emission of nitrous oxide in agricultural systems are nitrification and denitrification.

Nitrous oxide emissions processes

Nitrification

Nitrification is the oxidation process of ammonium ion (NH_4^+) in order to obtain nitrate (NO_3^-). There are two type of nitrification, autotrophic and heterotrophic. The autotrophic nitrification is carried out by ammonia-oxydizing bacteria (AOB) and nitrite-oxidizing bacteria (NOB). this two group of bacteria had different function in the nitrification process, the first transform the ammonium in nitrite whereas the second convert the nitrite to nitrate. Those reactions permit them to get the energy useful for the growth (Hooper et al., 1997).

The first group of bacteria (AOB) includes two genera, Nitrosospira and Nitrosomonas (Head et al., 1993) whereas to the second group (NOB) belong the Nitrobacter genera, the most widespread in agricultural soils, and less common genera including Nitrococcus, Nitrospina, Nitrospira.

Heterotrophic nitrification is a process performed from a wide range of bacteria and fungi. if comparing to the autotrophic nitrificator, those micro-organisms don't link their growth to the nitrification process but it is one of many processes that they perform in their vital functions. This type of bacteria had the same enzyme of NOB bacteria and most of them combine the nitrifying activity with aerobic denitrification (Kuenen and Robertson, 1994) to regulate their growth. Fungi have their own nitrification process that take place during the process of decomposition of the organic substance (De Boer and Kowalchuk, 2001).

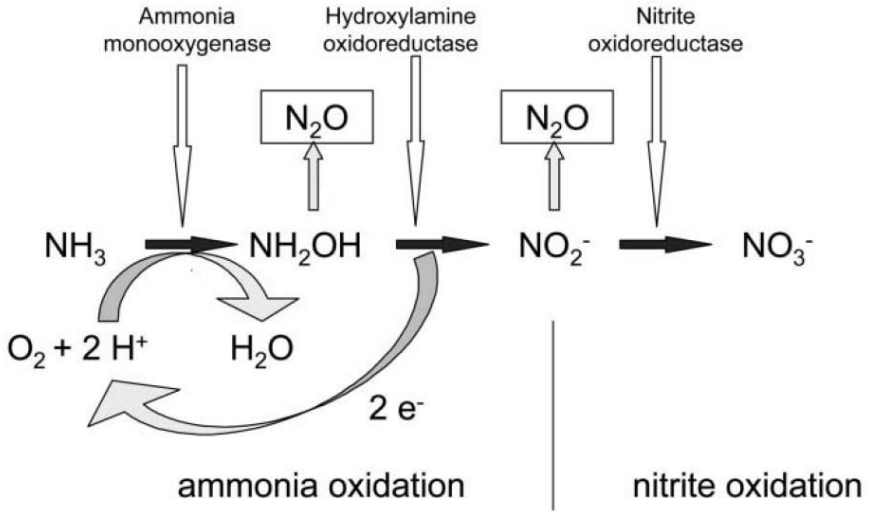

Fig 1.2 Nitrification pathway and enzymes involved. Source: Wrage et al., 2001.

During the process of nitrification, nitrous oxide emission can occur in particular conditions, when nitrite is used as terminal electron acceptor in order to limit the accumulation of nitrite under the toxic level (Ritchie and Nicholas, 1972; Wrage et al., 2005) or during the

decomposition of hydroxylamine (NH_2OH) in autotrophic and heterotrophic nitrification (Butterbach-Bahl et al., 2013; Wrage et al., 2001) (Fig. 2). With regards to heterotrophic nitrifier, they can produce N_2O also as intermediate in the conversion from NO_2^- to N_2 as with denitrifires (Wrage et al., 2001) (Fig 1.2).

Denitrification

According to Philippot et al. (2009) "denitrification is the main biological process responsible for returning fixed nitrogen to the atmosphere, thus closing the nitrogen cycle". Denitrification is the process that provides the reduction of nitrate (NO_3^-) to N_2O and N_2, by NO_2^- as intermediate product. It's an anaerobic or micro-anaerobic process of dissimilation and is performed from a wide range of bacteria like Pseudomonas, Bacillus, Thiobacillus and Propionibacterium (Firestone, 1982) but also from fungi (Liu et al 2005). During the process of denitrification bacteria use nitrate (NO_3^-) as an electron acceptor. This process it's possible thanks to several enzymes: nitrate reductase, nitrite reductase, nitric oxide reductase and nitrous oxide reductase (Hochstein and Tomlinson, 1988) (Fig 1.3).

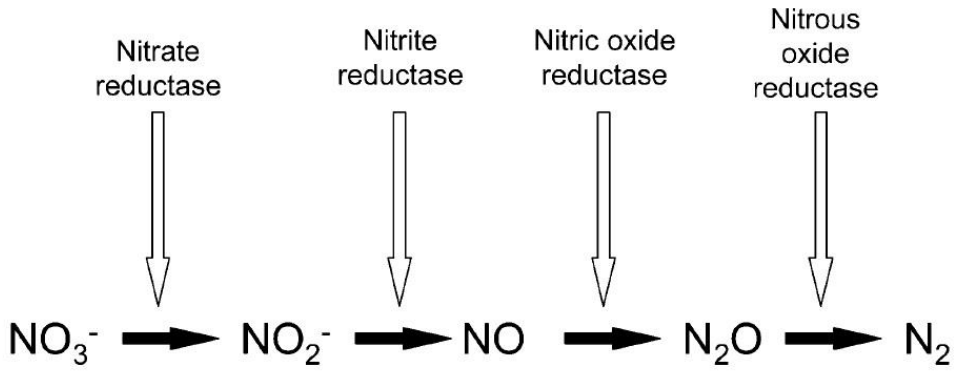

Fig 1.3. Denitrification pathway and enzymes involved. Source: Wrage et al., 2001.

The ratio N_2O/N_2 is very important in determining the impact of the denitrification process on the environment. This ratio can be affected by several factor such as the carbon availability, the nitrate abundance, and the ratio between nitrate and carbon content (Weier et al., 1993; Van Cleemput, 1998; Vinther, 1984). In particular, low soil NH_4^+ and NO_3^- concentration, high soil organic carbon content, such as increase of soil pH and aeration can reduce the N_2O/N_2 ratio, mitigating the denitrification impact on the environment (Zaman et al., 2012).

According with Graf et al. (2014) the denitrification process is a modular process where if some group of microorganisms have all enzymes required for each step of the process, others, may not have the nitrous oxide reductase and produce only N_2O as end product (Philippot et al., 2011). Moreover, fungal denitrification produces as a final product exclusively N_2O instead of N_2, probably due to lack of N_2O reductase (Kubota et al., 1999)

Factor influencing nitrous oxide emissions

Nitrous oxide emission from soil is a complex process caused from different process and conditioned by several soil characteristics as nitrogen availability and form, pH, carbon and organic matter content, texture and structure, water content and, finally, by management practices.

Nitrogen availability is a key component of the denitrification process, as substrate. A higher content of nitrogen in the soil result in higher nitrous oxide emissions (Saggar et al., 2009). For this reason, soil nitrogen supply by fertilizers is a main cause of nitrous oxide emissions in agricultural soils. The amount of NH_4^+ available for the nitrification control the emissions from this process, whereas NO_3^- can affect directly the emissions due to a denitrification.

Soil organic carbon and ready available carbon, sustaining the microbial growth and providing a carbon source for the denitrification process, stimulate the biological soil activity and thus also the denitrification rate. This applies above all for the heterotrophic denitrifiers (Groffman et al., 1987).

Soil pH can also have an effect on the soil microbiota and thus can influence the nitrous oxide emission. If for nitrification the optimal conditions range from pH 7 to 5, for denitrification the optimum is slightly larger, ranging from 5 to 8 (Flessa et al., 1998).

Soil temperature can have a major impact on the life of microorganisms determining the velocity of the biochemical processes and the microbial growth. The denitrification rate, such as other process, increases with the raising of temperature (Ryden, 1986).

Soil water and oxygen content is a factor that can have a great impact on denitrification process and on the nitrous oxide emission. In particular, condition of high soil water content, even occasional, may cause an increase of denitrification rate of soil (Mosier et al., 1986; Saggar et al., 2009). The relationship between the liquid and gas phase in the soil are mediate by the soil texture due to its influence on soil drainage. For this reason, soil with a good drainage like sandy soils have a lower rate of denitrification than a heavy clay soils (Tan et al. 2009). Besides, the enzyme of the first part of denitrification process are lower sensitive to the oxygen soil content than the other enzyme in the following parts of the denitrification process; this results in a shifting of N_2O/N_2 ratio to the N_2O when the O_2 concentration is high, whereas and to N_2, with a lower soil oxygen content (Cameron and Moir et al., 2012) (Fig 1.4). The alternation of aerobic and anaerobic conditions or the presence of aerobic zone adjacent to anaerobic site promotes the denitrification process leading to peaks of N_2O emission (Davidson, 1992; Hütsch et al., 1999; Ruser et al., 2006). Nitrification and denitrification processes are both affected by soil water content, and a strong correlation was observed between N_2O emission and water-filled pore space (WFPS). This parameter,

relating the soil bulk density with the water content, represent how the water in the soil contrast the oxygen diffusion (Heincke e Kaupenjohann, 1999).

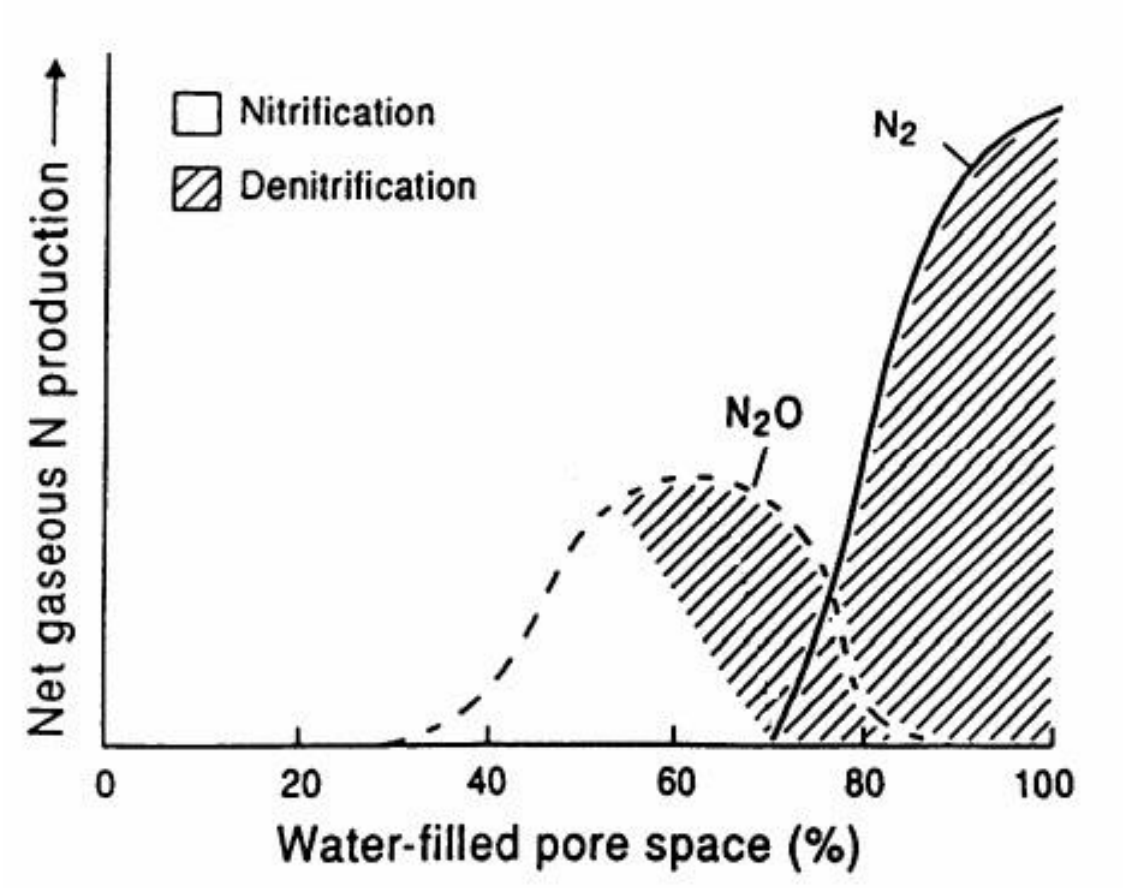

Fig 1.4 Relation between soil water-filled pore space and relative fluxes of N-gases. Source: Davidson, 1991

The two process, nitrification and denitrification, have a different optimal value of WFPS: for the aerobic process (nitrification), it is 60%, for the anaerobic process (denitrification) it should be higher than 80% (Ruser et al., 2006; Skopp et al., 1990) (Fig 1.4).

As previously mentioned, soil oxygen availability can affect N_2O/N_2 ratio, as well as also WFPS have an effect on it. Yoshinari (1993) studied the effect of WFPS on nitrogen emission due to nitrification and denitrification. In the denitrification process until the 80% there are greater emission of N_2O, whereas above, N_2 prevails (Fig 1.4).

Management practices affecting nitrous oxide emissions

Agronomical practices can play a determinant role to limit nitrous oxide emissions in the atmosphere and contrast climate change.

The conversion from conventional tillage to no tillage system, although it may bring many environmental and agronomical benefits (Amato et al., 2013), can result in an increase of nitrous oxide emissions (Almaraz et al., 2009; Lijun et al., 2007; Rochette et al., 2008). Other studies reported higher nitrous oxide rate in no tilled soil characterized by higher denitrifiers bacteria population, higher organic carbon content and higher bulk density (Rice and Smith, 1982; Ball et al., 1999). Tellez-Rio et al. (2014) in a study in Mediterranean environment report higher emissions in not tilled than tilled soils and such difference leads to doubt about the benefits due to carbon sequestration in the soil, in particular when the change in tillage system can result in a shift from one GHG emissions (CO_2) to another (N_2O). The higher N_2O emission in no tilled than in tilled soils can be the outcome of several changes in soil proprieties as WFPS, carbon and nitrogen availability and temperature. However, some authors (Kessavalou et al., 1998; Lemke et al., 1999; Chaudhary et al., 2002; Elmi et al., 2009; Parkin and Kaspar, 2006; Pelster et al., 2001 and Plaza Bonilla et al., 2014) reported lower or similar total emission between tilled and no tilled plots. The results of the studies about tillage systems can be affected by the time of application.

Some studies were conducted to assess the crop effect on nitrous oxide emissions and some of them were focused on the differences between leguminous crops and non-leguminous crops. In particular, with regards to the leguminous crops, they can contribute to N_2O emissions in two ways: during the nitrogen biological fixation and when their crop residue, rich of nitrogen, return to the soil.

The emissions from nitrogen fixation are limited and represent a minimum fraction compared to the gas emitted due to root exudates or crop residue decomposition (Rochette

and Janzen, 2005; Zhong et al., 2009). Nitrous oxide emissions from leguminous crops residues was studied from some authors. This type of residues, rich in nitrogen with a low C/N ratio, can decompose in short time releasing high quantity of nitrogen susceptible to be lost in N_2O during the nitrification and denitrification processes. (Baggs et al., 2000; Yang et al., 2002; Huang et al., 2004; Rochette et al., 2004). However, legumes providing nitrogen to the soil may decrease the nitrogen fertilizers supply promoting sustainable cropping systems (Pappa et al., 2012; Jensen and Hauggaard-Nielsen, 2003).

Nitrous oxide emissions due to crop residue decomposition depends from both the quantity of residue returned into the soil and its chemical composition (Garcia-Ruiz and Baggs, 2007; Aulakh et al., 2001). In particular, beyond the C/N ratio of the biomass, other properties as lignin content, lignin/N ratio (Millar and Baggs, 2004; Huang et al., 2004), polyphenol content (Millar and Baggs, 2004; Chaves et al., 2005) and the proportion of botanical fractions (Chen et al., 2015) can play an important role on the decomposition process influencing microbial activity and the nitrous oxide emission.

Studies regarding crop sequences showed different and contrasting results. Studies focusing on the Corn-soybean rotation from different authors leaded contrasting results including no difference between the crops (Bavin et al., 2009; Parkin and Kaspar, 2006; Sehy et al., 2008). In annual vetch, Tellez-Rio et al. (2014) observed lower nitrous oxide emissions than in wheat and barley whereas Bayer et al. (2015) observed higher emissions in vetch-maize than in oat-maize rotation. Finally, Mosier et al. (2006) report an increase of emission when soybean were inserted in rotation with corn.

The effect of tillage system and crop rotation on nitrous oxide emissions have been studied extensively in the past, but very few information is available about the effect of their interaction on soil quality and N losses. As reported by Baggs et al. (2003), nitrous oxide emissions were comparable between tillage and no tillage system after a leguminous crop,

whereas after a cereal, the emissions of no tilled plots were higher than tilled plots. On the contrary, Bavin et al. (2009) observed higher emissions after a leguminous crop in conventional tillage. In a comparison between oat-maize and vetch-maize rotations, Bayer et al. (2015) found similar emissions under conventional tillage and higher emission in the vetch-maize rotation under no tillage system.

Nitrogen supply to the soil by fertilizers or organic manure is recognized as the main agronomical practices that can cause the rise of nitrous oxide emissions. In order to control nitrogen surplus, responsible of nitrous oxide emissions and other environmental problems, and sustain the actual productivity levels is necessary to apply several measures aiming at synchronizing the availability of nutrients, especially N, from fertilizers and soil with the crop demand. The techniques used to achieve this goal are different and include the splitting of nitrogen fertilizers and the use of specials fertilizers with a controlled release of nutrient or with the addiction of urease or nitrification inhibitors (Burton et al., 2008; Maharjan et al., 2014; Mosier et al., 1996; Abalos et al., 2012; Ding et al., 2011).

2. Objective of the research

In the past, several studies were carried out to investigate the effects of tillage system and crop rotation on soil proprieties in different environment. As described before, environment and soil proprieties can have a great effect on soil biological activity and on nitrogen emissions and few studies were performed in Mediterranean area characterized by specific conditions. The understanding of the microbial soil activity during the cropping cycle can help to have a better comprehension of biochemical process on the soil. In addition, it is crucial to apply the best cropping management practices in order to limit their impact on the environment without reducing yield. In order to achieve this goal, long-term experiment give the opportunity to do an accurate study of the effects of each management practice. To understand the effect of tillage system and crop sequence on soil microbial activity nitrogen losses the present research has been structured in four parts. Chapters 2 and 3 are focused on the characterization of the soil microbial dynamics and community through biochemical and molecular techniques whereas the chapters 4 and 5 study the soil nitrogen emissions, under field condition and in the laboratory. In particular, the experiment in the chapter 4 studied the effect of tillage system and crop sequence on the ammonia and nitrous oxide emission under field condition, whereas the experiment in the chapter 5 investigate in depth the effect of crop residues on soil proprieties, CO_2 and N_2O emissions.

3. Effect of long-term tillage system and crop sequence on microbial dynamics

The aim of this experiment is to characterize the soil microbial activity and dynamics under a tillage system and crop sequence in a long-term experiment.

Material and Methods

Experimental site

The trial was conducted under rainfed conditions at the Pietranera farm, which is located about 30 km north of Agrigento, Sicily, Italy (37°30' N, 13°31' E; 178 m asl), on a deep, well-structured soil classified as a Chromic Haploxerert (Vertisol), with a slope of about 7%. Soil characteristics (measured at the beginning of the experiment and referring to the 0–0.40-m layer) were as follows: 525 g kg^{-1} clay, 216 g kg^{-1} silt, 259 g kg^{-1} sand, pH 8.1 (1:2.5 H_2O), 14 g kg^{-1} total C (Walkley–Black), 1.29 g kg^{-1} total N (Kjeldahl), 36 mg kg^{-1} available P (Olsen), 340 mg kg^{-1} K_2O (exchangeable K), 35 cmol kg^{-1} cation exchange capacity, 0.38 cm^3 cm^{-3} water content at field capacity (matric potential = –0.01 MPa), and 0.16 cm^3 cm^{-3} at the permanent wilting point (matric potential = –1.5 MPa). The climate of the experimental site is semiarid Mediterranean, with a mean annual rainfall of 572 mm, concentrated mostly during the autumn–winter period (September–February; 76%), and spring (March–May; 19%). A dry period occurs from May to September. Mean air temperatures are 15.9°C in fall, 9.7°C in winter, and 16.5°C in spring.

Experimental Design and Crop Management

The long-term field experiment, which began in fall 1991, was set up as a strip-plot design with two replications. Treatments were soil tillage systems (no tillage, NT; reduced tillage, RT; and conventional tillage, CT) and crop sequences (continuous wheat, W–W; wheat–faba

bean, W–FB; and wheat–berseem clover, W–BC). A detailed description of the experiment can be found in Giambalvo et al. (2012) and Amato et al. (2013).

In this study, the experimental factors tested were tillage (conventional tillage and no tillage) and crop (WW, continuous wheat; WF, faba bean after wheat; FW, wheat after faba bean). Conventional tillage (CT) consisted of one mouldboard ploughing to a depth of 30 cm in the summer, followed by one or two shallow harrowing (0–15 cm) operations before planting. No tillage (NT) consisted of sowing by direct drilling. Plot size was 370 m^2 (18.5 × 20.0 m). In NT plots, weeds were controlled before planting with glyphosate at a dose of 533 to 1066 g a.e. ha^{-1}, depending on the development of weeds. Every year, WW and FW plots were broadcast fertilized with 69 kg ha^{-1} of P_2O_5 before planting. Nitrogen fertilizer was broadcast on the soil surface at 120 kg N ha^{-1} in WW plots and 80 kg N ha^{-1} in FW plots. The total amount of N fertilizer was split with 50% applied immediately before planting (as diammonium phosphate and urea) and 50% applied at mid-tillering (end of March; during this experiment, it was before the 2nd soil sampling) as ammonium nitrate. WF plots were broadcast fertilized with 46 kg ha^{-1} P_2O_5 before planting and received no N fertilizer. Crop planting was always in December using a no-till seed drill with hoe openers under both CT and NT, making the appropriate sowing depth adjustments to ensure a homogeneous planting depth (3–5 cm). Faba bean cv. Gemini was sown at 40 viable seeds m^{-2} with an inter-row spacing of 75 cm. No rhizobial inocula were applied before planting because soil has a native rhizobial population. Durum wheat, cv. Anco Marzio, was planted in rows spaced 16 cm apart at 350 viable seeds m^{-2}. In WW and FW plots, weeds were controlled by applying post-emergence at the early growth stage of the crop. In WF plots, weeds were controlled mechanically by shallow hoeing (with minimum soil disturbance) when plants were at the third-leaf stage; if necessary, the operation was repeated at the seventh-leaf stage. Faba bean were harvested in late June or beginning of July, leaving standing straw and uniformly

spreading crop residues. Wheat was harvested also in late June or beginning of July and stubble (about 20–25 cm from the soil surface) was left standing. Wheat straw was baled and removed from the field. The soil surface covered by mulch in the NT treatments was always >30%.

Soil sampling and analysis

Inside the framework of the long-term experiment, previously described, during the cropping season 2013/2014, two soil samples (each composed of 3 mixed subsamples) per plot were collected separately from the 0–15 cm and 15–30 cm soil layers in December 2013 (before sowing), April 2014 (wheat heading), and July 2014 (wheat harvest) (144 soil sample in total). Soil samples were air-dried and then gently sieved to pass through a 4 mm mesh sieve. Visible pieces of crop residues and roots were removed by hand before sieving and then samples were stored in plastic bags at room temperature until analysis.

Microbial biomass C (MBC) and N (MBN) were determined by the fumigation– extraction method (Brookes et al. 1985; Vance et al. 1987). Moist (50 % WHC) soil aliquots (equivalent to 25 g oven-dry soil) were fumigated with alcohol-free chloroform in vacuum desiccators for 24 h in the dark. After removing the chloroform by repeated evacuations, the soil samples were extracted with 0.5 M K_2SO_4 (K_2SO_4 4:1 soil, v/w) for 45 min on a horizontal shaker (70 rpm). Unfumigated soil samples were similarly extracted and used as controls. All soil extracts were filtered through Whatman 42 paper and then analyzed for organic C by acid dichromate oxidation and for total N by the Kjeldhal method. Organic C and total N held in unfumigated soil extracts constituted the extractable C (C_{extr}) and the extractable N (N_{extr}), respectively. MBC and MBN were estimated as the differences between the organic C and

total N extracted from fumigated and unfumigated samples, respectively, multiplied by a conversion factor (kEC) of 2.64 for MBC and by 2.22 (kEN) for MBN.

Total organic carbon (TOC) was obtained by the Walkley–Black procedure (Nelson and Sommers 1996). Total nitrogen content was retrieved Kjeldhal method (Kjeldhal, 1883).

Basal respiration was determined by incubating 10 g of remoistened soil samples to 50% of WHC for a 24 h in 125 cm^3 air-tight glass bottles at 20°C. The CO_2 evolved after 24 h of incubation was measured by sampling an aliquot of gas from the bottles by using a syringe and injecting it into a gas-chromatograph (TRACE GC-Thermo Scientific, Milano, Italia) equipped with a thermal conductivity detector (TCD). Metabolic quotient (qCO2; Anderson and Domsch, 1993) was calculated and expressed as mg CO_2-C g^{-1} MBC h^{-1}, whereas the microbial quotient as the ratio between MBC and TOC (MBC/TOC) and expressed in percentage.

Soil denitrifying enzyme activity (DEA) was determinated according with Steenwerth et al. (2008) and Šimek et al. (2004). Briefly, in a 125 ml flask were added 20 g of soil, sieved at 4 mm and previously humidified to 50% of the WHC, and 20 ml of solution constituted by 1mM of glucose, 1mM of KNO_3 and 1 g l^{-1} of chloramphenicol. Then, flask were sealed with a rubber cap, vacuated using a vacuum pump, and the internal atmosphere was replaced with 99.999% He. After that, ten milliliters of internal atmosphere have been replaced with pure acetylene using a syringe, in order to block the conversion of N_2O to N_2, and the internal pressure was equilibrated to atmospheric pressure. The flask were then shake on a horizontal shaker at 75 shots per minute. After the addition of acetylene in 30 and 60 min, 1 ml samples of headspace atmosphere were taken with a gas-tight syringe and N_2O was measured on the TRACE-GC (Thermo Scientific, Milano, Italia) gas chromatograph equipped with a 80-100 mesh stainless-steel column packed with Poropak Q column and a ^{63}Ni ECD detector operating at column and injector, base and detector temperature of 40,

300 and 350°C, respectively. DEA was calculated from the N_2O increase during a half an hour incubation (30 – 60 min).

Changes in microbial population were assessed by analysing the ester-linked phospholipid fatty acids (PLFA) composition of the soil, a simple, fast and effective method for assessing community structure in soil, based on the different fatty acid composition of groups of microorganisms (Schutter and Dick, 2000; Tunlid and White, 1992). Lipids were extracted from soil using the procedure described by Bardgett et al. (1996) and (Frostegard et al., 1991), based on the method of (Bligh and Dyer, 1959) and modified by White et al. (1979). Briefly, the soil sample is added to a single-phase mixture of chloroform:methanol:buffer solution (1:2:0.8, v/v/v) for total lipid extraction, lipids are then separated into neutral-, glyco- and phospho-lipids (PLFAs) in a silicic acid column. PLFAs are converted, by alkaline methanolysis (Dowling et al., 1986), to fatty acid methyl esters that are transferred to 2-mL vials and analyzed by gas chromatography to determine the types and quantities of each one. All solvents used were of high purity, glass distilled and filtered. Chloroform was stabilized with amylenes. Samples were analysed by a gas chromatograph (FOCUS GC-Thermo Scientific, Milano, Italia) equipped with a flame ionization detector and a fused-silica capillary column Mega-10 (50 m×0.32 mm I.D.; film thickness 0.25 μm; stationary phase 100 % cyanopropyl polysiloxane). The GC temperature progression was 115 °C for 5 min, increase at a rate of 1.5 °C per minute from 115 to 230 °C, and at 230 °C for 2 min. Both injection port and detector were set up at 250 °C, respectively, and He (grade 5.5) at 1 mL min^{-1} in a constant flow mode was used as carrier. The injected volume was 1 μL in a splitless mode. Peak areas were quantified by adding nonadecanoic acid methyl ester (19:0; N-5377, Sigma Chemical Inc.) as internal standard. Identification of peaks was based on comparison of retention times to known standards (Supelco Bacterial Acid Methyl Esters mix cat no. 47080-U and Supelco 37 Component FAME mix cat no. 47885-U) confirmed

by gas chromatography mass spectrometry (GC±MS). The abundance of individual fatty acid methyl-esters was expressed on a dry weight basis per unit soil (g). Fatty acid nomenclature used was that described by (Frostegård et al. 1993). PLFAs with retention times less than C14:0, which were few in number, and greater than C20:0, generally not of microbial origin (Zelles et al., 1995), were deleted from the data set.

Total PLFA concentration was used for the microbial biomass quantification according to Federle et al. (1986) and Bailey et al. (2002). Following Laudicina et al. (2012), i15:0, a15:0, 15:0, i16:0, cis16:1Ω7, a17:0, i17:0, 17:0, cyl7:0, cis18:1Ω7 and cyl9:0 were used to represent bacterial phospholipid fatty acids (Frostegard and Baath 1996; Bailey et al. 2002); C18:2Ω6,9c, was used as an indicator of fungal biomass (Frostegard and Baath 1996). Furthermore, Gram-positive bacteria and Gram-negative were distinguished, respectively, by i15:0, a15:0, i16:0, i17:0, a17:0 (O'Leary and Wilkinson 1988; Zogg et al., 1997) and 16:1Ω7, 18:1 Ω7, cy17:0, cy19:0 (Zelles 1999; Waldrop et al., 2000). The ratio Gram-positive bacteria:Gram-negative was calculated. The ratio of C18:2Ω6,9c:bactPLFAs was taken to represent the ratio of fungal:bacterial biomass in soil (Frostegard and Baath 1996). Finally, microfauna biomass was calculated using C20:1w9c, C20:4W6, C20:5W3c fatty acids.

The weather data were collected from a weather station located within 500 m of the experimental site.

Before performing parametric statistical analyses, normal distribution and variance homogeneity of the data were checked by Kolmogorov–Smirnoff goodness-of-fit and Levene's tests, respectively. Two-way ANOVA with repeated measures was performed with tillage (CT, conventional tillage and NT, no tillage) and crop (WW, continuous wheat; FW wheat after faba-bean and WF, faba bean after wheat) as factors. Fisher

values (F) were used to individuate the different degree of variance, explained by single or interacting experimental factors. Statistical analyses were carried out with SAS statistical package (SAS 2008). LSD ($P<0.05$) post-hoc test to compare tillage management within the same cropping system was performed when significant differences were found. Pairwise mean comparison between the two cropping systems at the same tillage management was performed by the Student's t-test. Significant differences were considered at a $P<0.05$. Data reported are the arithmetic means of four samples and are expressed on an oven-dry basis (105°C) of soil ± SE.

Results

Weather conditions

The weather conditions during the experimental period are shown in Fig 3.1. Total rainfall in 2013-2014 was 603 mm, 12% higher than the long-term average (1980-2014) but with a similar rain distribution during the crop cycle. Mean year temperature was of 15.2°C slightly lower than the long-term annual average of 15.9°C. Temperature trend was similar to the long-term average with more marked differences observed in the last period of the cropping cycle.

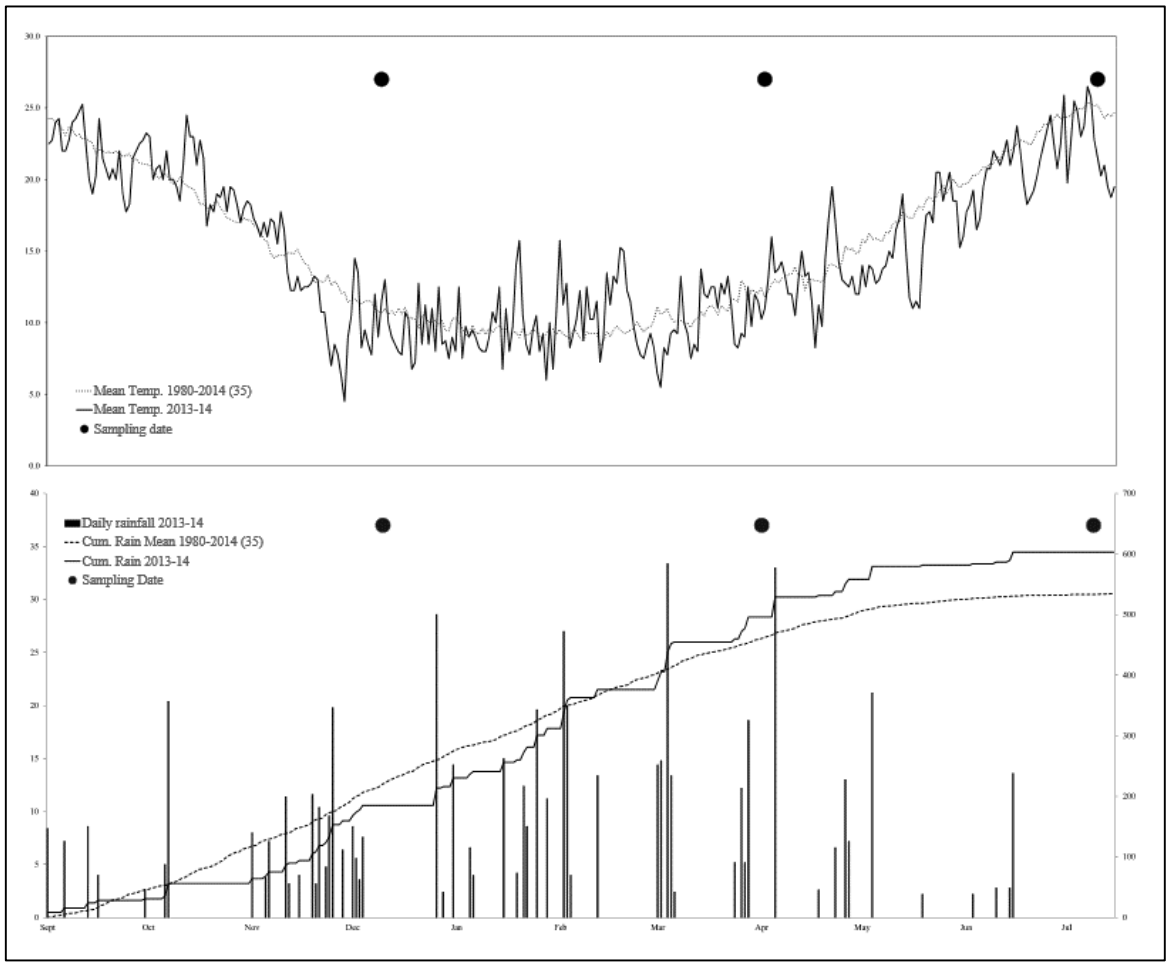

Fig 3.1 Daily air temperature and daily and accumulated rainfall at the experimental site during the growing season (2013-2014); the 35-yr average daily temperatures and accumulated rainfall are also included. The points indicates the sampling time.

TOC, TON and TOC/TON ratio

In the 0-15 cm soil layer, Total organic carbon (TOC) ranged from 13.1 g kg^{-1} in WF-CT plots to 18.9 g kg^{-1} in FW-NT (Tab 1). Both, tillage system and crop sequence affected TOC concentration in the soil. Tilled plots always showed a lower content of carbon than untilled plot (on average, 4.33 g C kg^{-1} soil less in tilled than untilled plots). Regarding the crop sequence, FW plots showed 1.0 g C kg^{-1} soil and 1.5 g C kg^{-1} soil more TOC content than WW and WF in both tillage system with more, on average,. In the deep soil layer (15-30 cm), TOC content was affected by crop sequence but not from tillage system. In particular, TOC ranged from 13.8 g kg^{-1} to 14.3 g kg^{-1}, with the highest values (14.2 g kg^{-1} and 14.3 g kg^{-1}) in both tilled and untilled FW. In NT plots, FW and WF did not show significant difference for TOC (Tab 3.1).

Total organic nitrogen (TON) content in the 0-15 cm soil layer was affected only by the tillage system. TON ranged from 1.31 g kg^{-1} in WW-CT to 1.90 g kg^{-1} WW-NT and, on average, TON was 0.55 g kg^{-1} higher in NT than CT. In the 15-30 soil layer, experimental treatment didn't affect the total organic nitrogen (Tab 3.1).

The TOC/TON ratio in the 0-15 soil layer ranged from 9.2 to 10.5 and wasn't affected by the experimental treatment (Tab 3.1).

		0-15 cm soil layer						15-30 cm soil layer					
		TOC g kg^{-1}		TON g kg^{-1}		TOC/TON %		TOC g kg^{-1}		TON g kg^{-1}		TOC/TON %	
Tillage	Crop												
CT	WW	13.4		1.31		10.3		13.8		1.34		10.3	
	FW	14.2		1.35		10.5		14.2		1.46		9.8	
	WF	13.1		1.34		9.7		13.2		1.49		8.8	
NT	WW	17.5		1.90		9.2		13.7		1.41		9.7	
	FW	18.9		1.88		10.0		14.3		1.53		9.4	
	WF	17.3		1.87		9.3		14.0		1.32		10.6	
Test of Between-Subjects Effects		F	Pr > F	F	Pr > F	F	Pr > F	F	Pr > F	F	Pr > F	F	Pr > F
Tillage		184.12	<.0001	236.68	0.0042	7.50	0.1115	3.35	0.0943	0.06	0.8359	0.39	0.5946
Crop		8.21	0.0022	0.03	0.0967	1.66	0.2988	5.21	0.0140	2.55	0.1935	0.52	0.6291
Tillage * Crop		0.27	0.7658	0.25	0.7883	0.19	0.8363	1.96	0.1640	3.83	0.1175	10.90	0.0240

Tab 3.1 Effect of tillage system and crop sequence on Total organic carbon (TOC), Total nitrogen (TON) and the ratio TOC/TON.

MBC, Cextr, MBN, Nextr and MBC/MBC ratio

Microbial biomass carbon (MBC), on the 0-15 soil layer, ranged from 254.0 mg kg^{-1} to 661.1 mg kg^{-1}. MBC was affected by both the experimental factor among the sampling time. In particular, NT showed on average 211.0 mg kg^{-1} more MBC than CT. With regard to the crop sequence both wheat plots, WW and FW showed on average, 122.1 mg kg^{-1} more MBC than WF. In the 15-30 cm layer, Tillage x Crop interaction for MBC was significant. In particular, differences between crop sequences were more evident in CT than NT, with conventional tilled wheat plots (either under continuous wheat or after fababean) showing a higher MBC than fababean (Tab 3.2).

The evolution of MBC of the 0-15 cm soil layer between the sampling times varied by both Tillage and Crop. MBC increased slightly during the cropping season in CT plots cultivated with wheat (WW and FW), whereas in NT plots, wide fluctuation of MBC content were observed. MBC did not show significant differences among sampling times in the 15-30 cm soil layer (Tab 3.2).

Extractable carbon (Cextr) ranged, from 34.8 mg kg^{-1} to 94 mg kg^{-1} in 0-15 cm layer, and from 29.8 mg kg^{-1} to 82.5 mg kg^{-1} in 15-30 cm soil layer. In each soil layers, the effect of tillage on Cextr was not constant between the soil layers: in 0-15 cm soil layer, Cextr was higher in NT than CT, whereas an opposite result was found in the 15-30 cm soil layer (Tab 2). During the season, Cextr in CT plots varied but not constantly, whereas in NT plot, Cextr was constant (in WW) or slightly reduced (FW and WF) from the first to the third sampling time. In the 15-30 cm soil layer, the variation during time of Cextr in all CT plots was similar to that observed for the 0-15 cm soil layer, especially in wheat. In NT plots, variation of Cextr in the 15-30 cm soil layer during time was mild and few differences among sampling times were found (Tab 3.2).

0-15 cm soil layer

		MBC			Cext			MBN			Next			MBC/MBN		
		mg kg^{-1}			mg kg^{-1}			mg kg^{-1}			mg kg^{-1}			%		
Tillage	Crop	Dec	Apr	Jul	Dec	Apr	Jul	Dec	Apr	Jul	Dec	Apr	Jul	Dec	Apr	Jul
CT	WW	302.7	396.0	364.4	59.0	32.3	83.0	38.5	52.8	57.2	10.7	8.5	13.0	8.2	8.5	6.5
	FW	384.7	420.2	385.8	69.5	34.8	60.1	52.9	53.5	52.4	13.5	11.4	15.5	8.2	8.2	8.2
	WF	254.0	275.3	278.8	68.8	45.6	76.8	46.9	50.1	55.4	12.8	10.9	7.0	5.8	5.5	5.5
NT	WW	592.2	629.3	627.8	82.8	70.9	73.9	66.3	104.8	68.4	16.4	13.1	15.4	9.3	6.0	10.2
	FW	661.1	494.8	578.1	93.8	62.1	76.0	84.9	71.7	68.4	17.9	13.8	11.7	8.0	7.0	8.8
	WF	546.9	368.9	461.9	94.0	44.6	58.1	76.8	41.7	89.1	14.5	10.9	10.5	7.2	9.6	5.3
Test of Between-Subjects Effects		F	Pr > F		F	Pr > F		F	Pr > F		F	Pr > F		F	Pr > F	
Tillage		327.36	<,0001		20.1	0.0042		123.11	<,0001		3.34	0.1176		4.12	0.0888	
Crop		48.74	0.0002		0.19	0.8346		1.87	0.2344		1.7	0.2604		8.16	0.0194	
Tillage * Crop		4.83	0.0562		4.01	0.0784		2.77	0.1408		0.6	0.5807		2.38	0.1738	
Tests of Within-Subjects Contrasts		F	Pr > F		F	Pr > F		F	Pr > F		F	Pr > F		F	Pr > F	
Time		1.34	0.2980		24.46	<,0001		0.30	0.7449		8.74	0.0046		0.13	0.8776	
Time * Tillage		10.73	0.0021		6.23	0.0140		0.51	0.6110		2.6	0.1154		0.32	0.7300	
Time * Crop		4.01	0.0272		1.39	0.2954		4.17	0.0241		3.34	0.0466		1.06	0.4176	
Time * Tillage * Crop		1.11	0.3972		1.63	0.2301		2.75	0.0784		2.75	0.0781		2.03	0.1540	
Greenhouse-Geisser			0.9173			0.8475			0.7945			0.9103			0.7142	
Huynh-Feldt-Lecoutre			1.3014			1.1457			1.0341			1.2854			0.8749	

15-30 cm soil layer

		MBC			Cext			MBN			Next			MBC/MBN		
		mg kg^{-1}			mg kg^{-1}			mg kg^{-1}			mg kg^{-1}			%		
Tillage	Prev Crop	Dec	Apr	Jul	Dec	Apr	Jul	Dec	Apr	Jul	Dec	Apr	Jul	Dec	Apr	Jul
CT	WW	324.9	336.0	289.5	56.6	29.8	81.2	56.1	50.7	47.5	11.2	6.7	15.7	6.7	6.7	6.7
	FW	372.4	371.3	402.3	82.5	38.7	66.1	56.1	62.7	57.4	13.7	10.8	16.7	6.7	5.9	7.1
	WF	279.4	267.3	264.6	75.1	63.0	69.4	50.9	42.0	50.0	12.3	11.4	11.3	5.5	6.4	5.3
NT	WW	382.1	368.3	390.6	48.6	40.0	53.3	32.2	68.6	47.4	17.1	12.1	9.9	12.0	5.5	8.4
	FW	382.9	309.1	324.6	55.2	38.8	48.7	58.6	37.3	73.9	11.5	12.2	11.0	6.9	8.7	4.4
	WF	345.3	285.5	294.5	63.4	42.1	34.5	49.1	24.7	46.6	8.7	9.4	10.1	7.4	12.2	6.3
Test of Between-Subjects Effects		F	Pr > F		F	Pr > F		F	Pr > F		F	Pr > F		F	Pr > F	
Tillage		8.81	0.025		36.0	0.0010		3.81	0.1		0.56	0.4841		60.79	0.0002	
Crop		44.81	0.0002		2.06	0.2085		16.09	0.0039		1.21	0.3621		8.48	0.0178	
Tillage * Crop		24.02	0.0014		2.48	0.1644		0.83	0.4795		1.32	0.3340		15.64	0.0042	
Tests of Within-Subjects Contrasts		F	Pr > F		F	Pr > F		F	Pr > F		F	Pr > F		F	Pr > F	
Time		1.24	0.3243		19.34	0.0		1.74	0.2175		3.2	0.0770		2.27	0.1458	
Time * Tillage		1.04	0.3826		5.09	0.0251		2.32	0.1407		5.4	0.0212		2.35	0.1380	
Time * Crop		0.33	0.8492		5.18	0.0117		5.57	0.0090		1.34	0.3111		4.26	0.0225	
Time * Tillage * Crop		0.67	0.6232		1.81	0.1925		6.18	0.0061		3.09	0.0580		0.97	0.5181	
Greenhouse-Geisser			0.6479			0.6644			0.8689			0.5917			0.6201	
Huynh-Feldt-Lecoutre			0.7515			0.7816			1.1923			0.6522			0.7018	

Tab 3.2 Effect of tillage system and crop sequence on Microbial Biomass Carbon (MBC), Extractable Carbon (Cextr), Microbial Biomass Nitrogen (MBN), Extractable Nitrogen (Nextr) and MBC/MBC ratio.

Microbial biomass nitrogen (MBN) ranged from 38.5 mg kg^{-1} to 104.8 mg kg^{-1} in the 0-15 soil layer and from 24.7 to 73.9 mg kg^{-1} in the 15-30 cm soil layer. In the 0-15 cm soil layer MBN was affected by the tillage system with NT>CT (on average, +24.6 mg kg^{-1}), whereas in the 15-30 cm soil layer, MBN was affected by crop sequences with FW>WW>FW. Along the cropping season, in the 0-15 cm soil layer, MBC was affected by the interaction between sampling time and crop sequence. In particular, in CT plots, WW showed an increasing trend during the cropping season, whereas, in FW and WF, MBC was constant across the sampling times (Tab 3.2).

Extractable N (Nextr) ranged from 7.0 mg kg^{-1} to 17.9 mg kg^{-1} in 0-15 cm layer and from 6.7 mg kg-1 to 17.1 mg kg^{-1} in 15-30 soil layer. In both soil layers, Nextr wasn't affected by the experimental factors and mostly change by time, with a reduction from December to April (Tab 3.2).

MBC/MBN ratio (i.e. the C:N ratio of the soil bacterial biomass) in the 0-15 cm soil layer, was affected by crop. In particular, MBC/MBN of plots grown with wheat (WW and FW) was on average 24.6% higher than that of fababean . In the 15-30 soil layer, MBC/MBN ratio was affected by the interaction Tillage x Crop. Although in CT plots the MBC/MBN ratio was very similar in the different rotations and crops, in NT plots, WW and WF showed a higher ratio than FW (Tab 3.2).

MBC/TOC ratio, CO_2^{24h}, qCO_2

MBC/TOC ratio, in both soil layers investigated, was affected by the interaction between tillage and crop (Tillage x Crop). Crop effect on MBC/TOC ratio was mediated by the tillage system, in particular in the 0-15 cm soil layer, CT plots cultivated with wheat had a higher

ratio than WF plot, whereas in NT, MBC/TOC decreased as follows: WW>FW>WF. In the 15-30 cm soil layer, MBC/TOC of wheat (both in WW and FW) was higher than fababean in both CT and NT (Tab 3.3).

CO_2^{24h}, or basal respiration (BR), in the 0-15 cm soil layer ranged from 2.8 to 12.5 mg C-CO_2 kg^{-1} soil d^{-1} and was affected by the interaction tillage × crop. NT plots showed higher level of basal respiration than CT and in both tillage system WW>FW and WF. In 15-30 cm soil layer, basal respiration ranged from 2.1 to 6.5 mg C-CO_2 kg^{-1} soil d^{-1} and was affected by Crop with WW>FW and WF. With regards to the variation along the cropping cycle, in 0-15 cm soil layer, basal respiration increased during the crop cycle, with a stronger increase in WW than WF and FW and in CT (+65.0% at the end compared to the beginning of the crop cycle) than NT (+14.6%). In the 15-30 cm soil layer, the effect of the cropping system was similar, but to a lesser extent, to that observed in the 0-15 cm, whereas few effect of tillage and time were found (Tab 3.3).

Metabolic quotient, qCO_2, ranged from 0.32 to 0.85 mg CO_2–C g^{-1} MBC h^{-1}, in 0-15 cm soil layer and from 0.24 to 0.84 mg CO_2–C g^{-1} MBC h^{-1}in the 15-30 cm soil layer. In the superficial soil layer, metabolic quotient was affected by the interaction Tillage x Crop. If in the CT plots data showed a marked difference between treatments, with WW and WF>FW, the difference between crop were slightly evident in NT plots. In the 15-30 cm soil layer, metabolic quotient was affected only by crop with a FW plots with a lower value of the metabolic quotient than WW and WF. Sampling time had a significant effect on the metabolic quotient in the 0-15 cm soil layer wheres no effect was observed in the deep layer of soil (Tab 3.3).

0-15 cm soil layer

		MBC/TOC			BR			qCO_2		
		%			mg C-CO_2 kg^{-1} d^{-1}			mg CO_2–C g^{-1} MBC h^{-1}		
Tillage	Crop	Dec	Apr	Jul	Dec	Apr	Jul	Dec	Apr	Jul
CT	WW	2.2	3.0	2.7	2.9	7.2	6.9	0.4	0.8	0.8
	FW	2.7	2.9	2.7	3.0	3.3	3.1	0.3	0.3	0.3
	WF	1.9	2.1	2.1	2.8	4.1	4.4	0.5	0.6	0.7
NT	WW	3.4	3.5	3.7	7.2	11.8	12.5	0.5	0.8	0.9
	FW	3.3	2.7	3.1	8.9	6.2	8.3	0.6	0.5	0.6
	WF	2.8	2.4	2.7	8.9	4.3	7.9	0.7	0.5	0.7
Test of Between-Subjects Effects		F	Pr > F		F	Pr > F		F	Pr > F	
Tillage		87.76	<,0001		699.3	<,0001		22.53	0.0	
Crop		53.23	0.0002		120.9	<,0001		32.62	0.0006	
Tillage * Crop		8.26	0.0189		9.49	0.0139		6.31	0.0334	
Tests of Within-Subjects Contrasts		F	Pr > F							
time		1.43	0.2781		4.97	0.0		5.09	0.0251	
time * Tillage		9.4	0.0035		4.59	0.0331		1.02	0.3905	
time * Crop		2.97	0.0643		8.42	0.0018		2.63	0.0867	
time * Tillage * Crop		0.17	0.9510		1.62	0.2333		0.41	0.8007	
Greenhouse-Geisser			0.6962			0.6392			0.6476	
Huynh-Feldt-Lecoutre			0.8407			0.7359			0.751	

15-30 cm soil layer

		MBC/TOC			BR			qCO_2		
		%			mg C-CO_2 kg^{-1} d^{-1}			mg CO_2–C g^{-1} MBC h^{-1}		
Tillage	Crop	Dec	Apr	Jul	Dec	Apr	Jul	Dec	Apr	Jul
CT	WW	2.3	2.4	2.2	4.3	6.5	5.1	0.5	0.8	0.7
	FW	2.6	2.6	2.9	4.5	4.6	4.7	0.5	0.5	0.5
	WF	2.0	2.1	2.0	5.1	2.9	4.3	0.7	0.5	0.7
NT	WW	2.8	2.7	2.9	4.6	6.2	4.7	0.5	0.7	0.5
	FW	2.7	2.3	2.2	2.1	3.2	3.3	0.2	0.4	0.4
	WF	2.4	2.2	2.1	5.2	3.7	3.7	0.6	0.5	0.6
Test of Between-Subjects Effects		F	Pr > F		F	Pr > F		F	Pr > F	
Tillage		6.48	0.0437		2.4	0.1701		4.19	0.1	
Crop		40.74	0.0003		5.49	0.0440		4.99	0.0529	
Tillage * Crop		28.76	0.0008		2.32	0.1791		0.15	0.865	
Tests of Within-Subjects Contrasts		F	Pr > F							
time		0.37	0.6959		0.16	0.9		0.32	0.7294	
time * Tillage		0.78	0.4789		0.16	0.8525		0.4	0.6761	
time * Crop		0.14	0.9627		3.32	0.0475		1.66	0.2243	
time * Tillage * Crop		0.9	0.4934		0.3	0.8734		0.46	0.7653	
Greenhouse-Geisser			0.7207			0.7863			0.8242	
Huynh-Feldt-Lecoutre			0.8874			1.0174			1.096	

Tab 1.3 *Effect of tillage system and crop sequence on MBC/TOC ratio, CO_2^{24h}, qCO_2.*

Denitrifying Enzyme Activity (DEA)

Denitrifying Enzyme Activity (DEA) ranged from 11.5 to 196.7 µg N kg $soil^{-1}$ h^{-1}, in the top soil layer (0-15 cm depth) and from 6.2 to 29.9 µg N kg $soil^{-1}$ h^{-1} in the deep soil layer (15-30 cm depth) investigated (Fig 2). In the topsoil, DEA was influenced by the interaction Tillage x Crop. On average, in the topsoil layer NT showed a higher DEA than CT (+82.3 µg N kg $soil^{-1}$ h-1). With regard to the crop, in CT the difference between crops were restricted (on average, from 16.5 to 18.6 µg µg N kg $soil^{-1}$ h^{-1}), whereas in NT plots the effect

of the crop was more evident (on average, from 72.1 to 125.9 µg µg N kg soil^{-1} h^{-1}) with WW>FW>WF. In the 15-30 cm soil layer, DEA was affected by both Tillage and Crop. In particular, CT had a higher value than NT (+ 2.97 µg µg N kg soil^{-1} h^{-1}), although the difference between the two treatments was limited (on average, 15.6 vs 12.7 µg µg N kg soil^{-1} h^{-1}). Finally crop effect showed a trend with WW and FW>WF (Fig 3.2).

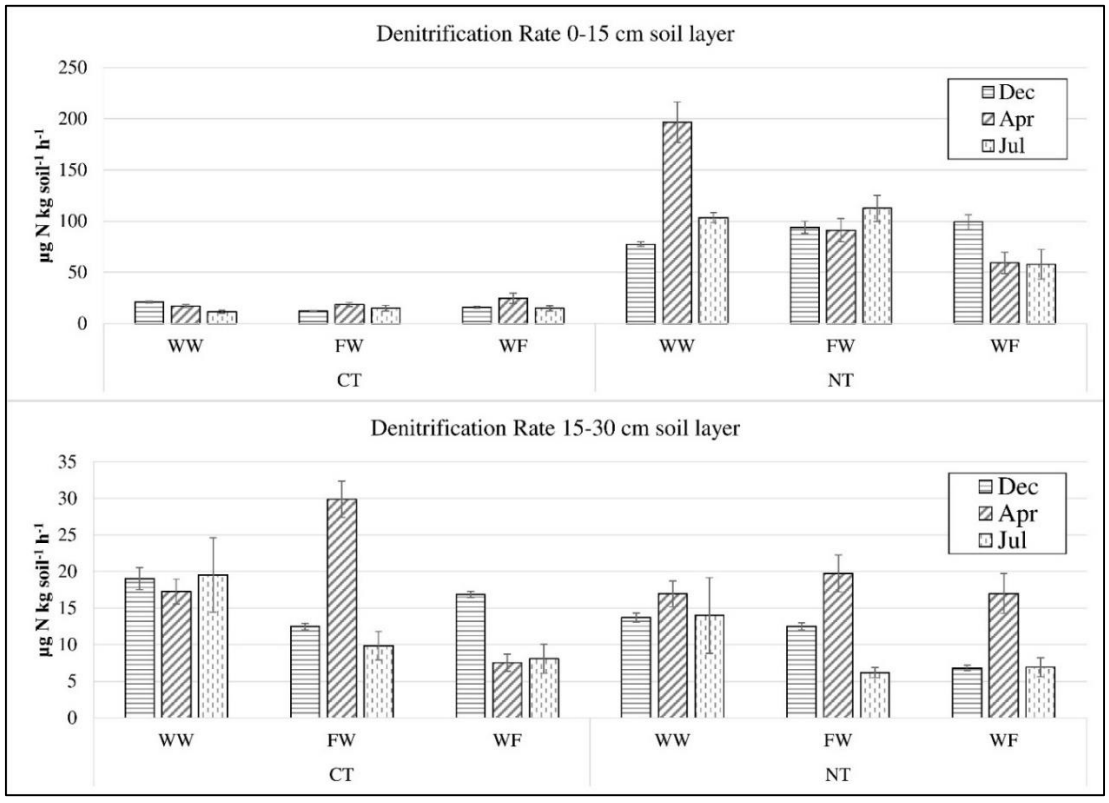

Fig 3.1 Effect of tillage system and crop sequence on Denitrifying Enzyme Activity (DEA).

PhosphoLipid-derived Fatty Acids (PLFA)

Study the PhosphoLipid-derived Fatty Acids (PLFA) is useful to characterize microbial community in soils. Total microbial biomass, ranged from 209.1 to 543.1 nmol g^{-1} of soil in the 0-15 cm soil layer and from 211.2 to 651.0 nmol g^{-1} in the 15-30 cm soil layer. Microbial biomass of both soil layers was affected by the interaction Tillage x Crop. In particular, in the 0-15 cm soil layer NT plots, on average, registered a PLFA content of 396.7 nmol g^{-}

[1]comparted to CT with 257.7 nmol g^{-1} (-139.00 nmol g^{-1}) (Tab 3.4). With regards to the Crop effect, on average, WW (333.1 nmol g^{-1}) and FW (339.5 nmol g^{-1}) showed values slightly higher than WF (308.9 nmol g^{-1}). However, in CT no differences were recorded among the crops, whereas NT showed a trend where WW and FW had higher microbial content than WF. In the 15-30 cm soil layer differences between tillage systems were similar to the topsoil, with NT>CT (on average, 342.07 vs 263.3 nmol g^{-1}). With regard to the crop both wheat plots (either sown on CT or NT) showed a higher microbial biomass content than WF plots. Also in the deeper soil layer, within the tillage system, no differences between crops were observed in CT, whereas the trend in NT was WW>FW>WF (Tab 3.4).

Soil bacteria PLFA quantity ranged from 105.4 to 301.8 nmol g^{-1}, in the 0-15 cm soil layer, and from 95.3 to 295.4 nmol g^{-1}, in the 15-30 cm soil layer. Soil bacteria PLFA in both soil layers was affected by the interaction Tillage x Crop. In the 0-15 cm soil layer, NT>CT (+83.6 nmol g^{-1}) whereas slight differences were observed, on average, among the crops. Within each tillage system, in CT, no differences between the crops were observed, whereas in NT, both wheat plots showed higher bacterial PLFA than WF plot (+29.0 nmol g-1). Also in the 15-30 cm soil layer NT>CT (+49.9 nmol g^{-1}), while with regard to the crop, WW and FW>WF (on average, +31.7 nmol g^{-1}). With regards to each tillage system, in CT, slight differences were observed with FW>WW and WF, and in NT, more definite differences were detected with WW>FW>WF. Regarding to the season variation, in the 0-15 cm soil layer, *PLFA* was affected by the interaction Time x Tillage x Crop, whereas in the 15-30 cm soil layer, it was affected only by Time x Tillage and Time x Crop interactions (Tab 3.4).

Soil fungal PLFA, in the 0-15 cm soil layer, ranged from 1.0 to 35.5 nmol g^{-1}, while in the 15-30 cm soil layer, from 0.7 to 35.6 nmol g^{-1}. In the superficial soil layer the quantity of fungal PLFA was affected from Tillage system, especially before sowing, whereas difference between the tillage systems reduced in the later samplings. Also, in the deeper

soil layer, differences between tillage system were similar to the topsoil but such differences were more evident in spring than before sowing or after harvest (Tab 4). Gram positive bacteria ranged from 40.4 to 101.5 nmol g^{-1} in the 0-15 cm soil layer, whereas in the 15-30 cm soil layer, from 40.0 to 105.2 nmol g^{-1}. In the superficial soil layer, $BacG^{+}$ PLFA content was affected from the tillage system, with NT>CT (+27.5 nmol g^{-1}). In the deeper soil layer, both tillage system and crop had an effect on the gram positive bacteria. In particular, NT (62.13 nmol g^{-1})>CT (49.04 nmol g^{-1}), with an increasing of 13.09 nmol g^{-1}, while with regards to crop, plots cultivated with wheat in both tillage system shown higher level of gram positive bacteria than WF. Seasonal trend of gram positive bacteria was affected by the interaction Tillage X Time, in the superficial soil layer, and from the interactions Time x Tillage and Time x Crop in the deeper soil layer (Tab 3.4). Gram negative bacteria PLFA content ($BacG^{-}$), in both soil layer studied ranged form 45.8 to 190.2 nmol g^{-1} and was affected by the interaction Tillage x Crop. On average, in both soil layer NT>CT, with a differences of +49.7 nmol g^{-1} in in the 0-15 cm soil layer and of +34.7 nmol g^{-1} in the 15-30 cm soil layer between the tillage system. With regard to the crop, $BacG^{-}$ content in the CT plots didn't show differences between crops, whereas in NT both plots cultivated with wheat had higher value than WF plot. $BacG^{-}$ seasonal trend, in the two soil layers, were affected by the interactions Time x Tillage x Crop and the interactions Time x Tillage and Time x Crop, respectively, for the superficial and deep soil layer investigated (Tab 3.4). Soil microfauna PLFA content, mainly constituted by protozoa and nematodes, was affected by Crop in the 0-15 cm soil layer and by the interaction Tillage x Crop in the 15-30 cm soil layer. Soil PLFA from microfauna, ranged from 2.4 to 6.3 nmol g^{-1} in the 0-15 cm soil layer and from 1.9 to 11.2 nmol g^{-1} in the 15-30 cm soil layer. In both soil layers investigated,plots cultivated with wheat (WW and FW) had higher content than plot cultivated with faba bean, irrespective to tillage system (Tab 3.4).

0-15 cm soil layer

		Microbial biomass			Bacteria			Fungi			BacG+			BacG-			Microfauna			B/F			B+/B-		
PLFA [nmol g-1]		Dec	Apr	Jul	Dec	Apr	Jul	Dec	Apr	Jul	Dec	Apr	Jul	Dec	Apr	Jul	Dec	Apr	Jul	Dec	Apr	Jul	Dec	Apr	Jul
CT	WW	237.1	281.0	222.9	112.6	120.2	116.3	13.2	15.5	1.1	41.1	49.9	45.0	61.8	60.5	60.8	3.4	6.3	2.6	8.9	7.7	106.0	0.7	0.8	0.7
	FW	243.7	289.9	275.7	117.1	123.3	143.1	12.8	15.8	1.0	44.9	51.3	53.4	62.8	61.4	77.6	3.3	5.8	2.9	9.4	7.8	149.3	0.7	0.8	0.7
	WF	240.1	243.1	285.6	114.6	105.7	144.3	14.5	12.3	1.0	40.4	45.3	56.6	64.8	50.8	74.7	2.9	4.6	2.7	8.3	8.7	150.0	0.6	0.9	0.8
NT	WW	459.4	255.2	543.1	235.7	117.0	301.8	21.3	8.1	1.3	92.8	41.3	88.6	119.3	67.0	190.2	4.2	5.0	4.6	11.3	14.5	225.5	0.8	0.6	0.5
	FW	478.4	265.4	483.9	244.4	122.5	270.0	29.3	8.7	1.1	101.5	44.6	84.4	122.6	68.5	166.6	5.0	3.7	3.6	8.3	14.1	251.6	0.8	0.7	0.5
	WF	479.7	209.1	395.7	236.0	105.4	217.3	35.5	6.3	1.0	96.8	44.6	80.7	118.2	52.1	117.5	4.4	2.4	3.6	7.5	16.7	214.3	0.8	0.9	0.7
Test of Between-Subjects Effects		F	Pr > F		F	Pr > F		F	Pr > F		F	Pr > F		F	Pr > F		F	Pr > F		F	Pr > F		F	Pr > F	
Tillage		514.06	<,0001		494.02	<,0001		7.43	0.034		425.7	<,0001		394.5	<,0001		2.6	0.155		43.99	0.001		12.39	0.013	
Crop		9.25	0.015		7.03	0.027		1	0.421		2.6	0.155		13.1	0.006		14.1	0.005		1.55	0.288		18.65	0.003	
Tillage * Crop		9.95	0.012		8.11	0.020		1.74	0.254		0.29	0.760		13.82	0.006		1.19	0.367		1.16	0.375		13.87	0.006	
Tests of Within-Subjects Contrasts		F	Pr > F		F	Pr > F		F	Pr > F		F	Pr > F		F	Pr > F		F	Pr > F		F	Pr > F		F	Pr > F	
time		50.01	<,0001		81.01	<,0001		189.42	<,0001		55.32	<,0001		93.6	<,0001		15.46	0.0005		496.95	<,0001		25.24	<,0001	
time * Tillage		71.13	<,0001		59.42	<,0001		59.97	<,0001		74.34	<,0001		53.87	<,0001		28.2	<,0001		35.28	<,0001		29.21	<,0001	
time * Crop		1.3	0.325		0.83	0.533		4.84	0.015		0.21	0.926		2.7	0.079		3.6	0.038		1.76	0.202		5.95	0.007	
time * Tillage * Crop		4.39	0.021		3.9	0.030		2.06	0.149		1.6	0.244		5.8	0.008		1.3	0.343		1.1	0.403		0.95	0.471	
Greenhouse-Geisser			0.953			0.925			0.512			1.0			0.8			1.0			0.5007			0.5721	
Huynh-Feldt-Lecoutre			1.386			1.318			0.519			1.4			1.1			1.4			0.5011			0.6187	

15-30 cm soil layer

		Microbial biomass			Bacteria			Fungi			BacG+			BacG-			Microfauna			B/F			B+/B-		
PLFA [nmol g-1]		Dec	Apr	Jul	Dec	Apr	Jul	Dec	Apr	Jul	Dec	Apr	Jul	Dec	Apr	Jul	Dec	Apr	Jul	Dec	Apr	Jul	Dec	Apr	Jul
CT	WW	260.7	288.7	224.9	123.1	128.6	119.4	15.9	14.4	1.0	44.9	54.0	47.3	68.0	62.2	61.0	3.3	6.3	2.3	7.9	9.0	114.3	0.7	0.9	0.8
	FW	278.1	295.4	287.8	130.9	127.5	146.1	18.2	16.6	1.3	51.0	54.4	53.9	68.9	62.0	80.1	4.0	6.3	2.2	7.5	8.0	124.3	0.7	0.9	0.7
	WF	267.5	217.0	249.9	127.2	95.3	131.1	17.1	10.6	1.2	44.7	40.4	50.7	71.8	45.8	68.9	2.8	4.7	2.2	7.5	9.0	113.5	0.6	0.9	0.7
NT	WW	334.5	651.0	270.4	203.2	295.4	144.3	9.2	35.6	1.1	62.8	105.2	44.3	130.1	166.3	89.2	3.0	11.2	2.2	22.0	8.7	142.6	0.5	0.6	0.5
	FW	273.4	507.1	235.5	151.5	236.0	125.8	9.0	22.1	0.9	53.2	88.6	40.0	88.4	129.2	76.2	3.0	8.9	1.9	17.0	11.1	149.7	0.6	0.7	0.5
	WF	288.9	306.5	211.2	154.4	156.5	111.3	15.9	13.1	0.7	56.5	65.2	43.4	87.3	76.4	58.1	2.8	4.0	2.0	10.3	11.9	154.6	0.7	0.9	0.7
Test of Between-Subjects Effects		F	Pr > F		F	Pr > F		F	Pr > F		F	Pr > F		F	Pr > F		F	Pr > F		F	Pr > F		F	Pr > F	
Tillage		29.29	0.0016		36.5	0.0		3.51	0.110		28.7	0.0		37.7	0.0		5.7	0.054		16.52	0.007		43.51	0.001	
Crop		9.25	0.015		7.03	0.027		1	0.421		2.6	0.155		13.1	0.006		14.1	0.005		1.55	0.288		18.65	0.003	
Tillage * Crop		9.95	0.012		8.11	0.020		1.74	0.254		0.29	0.760		13.82	0.006		1.19	0.367		1.16	0.375		13.87	0.006	
Tests of Within-Subjects Contrasts		F	Pr > F		F	Pr > F		F	Pr > F		F	Pr > F		F	Pr > F		F	Pr > F		F	Pr > F		F	Pr > F	
time		66.63	<,0001		28.45	<,0001		207.77	<,0001		42.67	<,0001		12.09	0.0013		158.04	<,0001		609.04	<,0001		27.83	<,0001	
time * Tillage		57.52	<,0001		51.84	<,0001		37.67	<,0001		44.03	<,0001		33.95	<,0001		14.4	0.0		7.36	0.0082		0.95	0.4131	
time * Crop		15.63	0.000		9.26	0.001		16.57	<,0001		6.92	0.004		7.4	0.003		12.6	0.000		0.5	0.735		2.39	0.108	
time * Tillage * Crop		4.54	0.018		1.68	0.220		8.94	0.001		1.3	0.336		1.3	0.325		5.9	0.007		0.5	0.735		0.23	0.915	
Greenhouse-Geisser			0.728			0.969			0.559			0.6			0.9			0.6			0.5101			0.7075	
Huynh-Feldt-Lecoutre			0.901			1.424			0.596			0.7			1.3			0.7			0.5162			0.8621	

Tab 3.4 Effect of tillage system and crop sequence on microbial group PhosphoLipid-derived Fatty Acids (PLFA), Bacterial/Fungal PLFA ration and Gram Positive/Gram Negative PLFA ratio.

Bacteria/fungi ratio (B/F), an important indicator to observe changes in soil microbial community structure, in both soil layer ranged from 7.5 to 154.6 and was mostly affected by the tillage system adopted. In particular, in both soil layer NT had an higher bacteria/fungi ratio than CT. The differences between the two tillage system on average were of 34.2 and of 14.1 in the 0-15 cm and in the 15-30 cm soil layers, respectively. Seasonal variations of B/F ratio were affected in both soil layer from the interaction Time x Tillage. During the cropping season B/F ratio had a considerable increase in the third sampling time, in all treatment tested, as a consequence of the decrease of fungal biomass due to a limited availability of water in the soil (Tab 4).

Gram positive/Gram negative bacteria ratio (B^+/B^-) in both soil layer investigated ranged from 0.5 to 0.9 and was affected from the interaction Tillage x Crop. In particular with regard to the tillage system in both soil layer, on average, CT>NT, while looking to the crop WF had a higher value than the both plots cultivated with wheat, WW and FW. Specifically, if in CT plots no differences were observed between all three crops, in NT distinct differences were observed between them, with WF>WW and FW. The seasonal variation of B^+/B^- ratio in the 0-15 cm soil layer was affected by the interactions Time x Tillage and Time x Crop, while in the 15-30 cm soil layer, only from the sampling Time (Tab 3.4).

Observing the percentage content of each microbial group, respect to the total microbial biomass, can be useful to analyze the presence of shift in microbial community across the treatment, irrespective of the quantitative variations. In the 0-15 cm soil layer the tillage effect on bacteria and fungal biomass was significant and contrasting; from the one hand, bacteria biomass was higher in NT than CT, from the other hand, CT had more fungal biomass than NT (Fig 3). With regards to the gram positive and gram negative bacteria, the first were affected by the interaction Tillage x Crop while the second from both experimental factor, Tillage and Crop but not from the interaction between them (Fig 3.3). Gram positive

bacteria similar higher value in all CT plots and in the NT plot cultivated with faba bean, while both wheat plots, WW and FW, in NT shown lower B^+ content (Fig 3). With regard to the B^-, the percentage content of this microbial group was higher in NT than in CT, while observing crop, plots cultivated with wheat showed higher content than faba bean plots, in both tillage system applied (Fig 3.3). Soil microfauna was affected by both treatment applied. CT plots showed higher value than NT, as well as, WW than FW and WF plots (Fig 3.3).

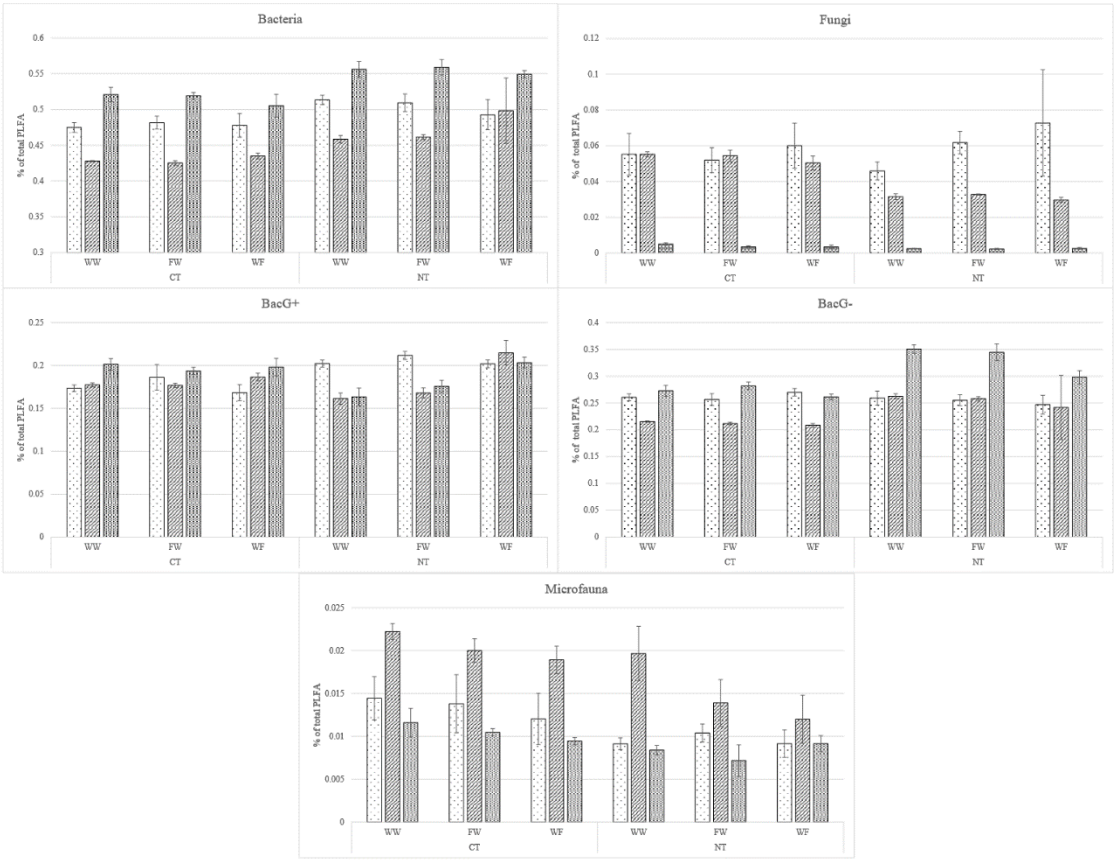

Fig 2.3 Effect of tillage system and crop sequence on microbial group PLFA percentage content on 0-15 cm soil layer

In the 15-30 cm soil layer, bacteria content, was affected by tillage system, with NT>CT. Fungal biomass percentage content, was affected by the interaction Tillage x Crop. In particular, no differences between the crops were observed in CT, with similar value in NT-

WF plot, whereas NT-WW and NT-FW plots showed a lower fungal content (Fig 3.4). B^+ and B^- percentage content were affected by the interaction Tillage x Crop (Fig 3.4). Gram positive bacteria shown higher value in all CT plots and in CT-WF plot than in both NT plots cultivated with wheat (Fig 3.4). Gram negative bacteria content, instead to what was observed for gram-positive bacteria, was higher in both NT plot cultivated with wheat than in NT plot cultivated with faba bean and all CT plots. Finally soil microfauna content, in the deeper soil layer investigated, was affected by the tillage system adopted with CT>NT (Fig 3.4).

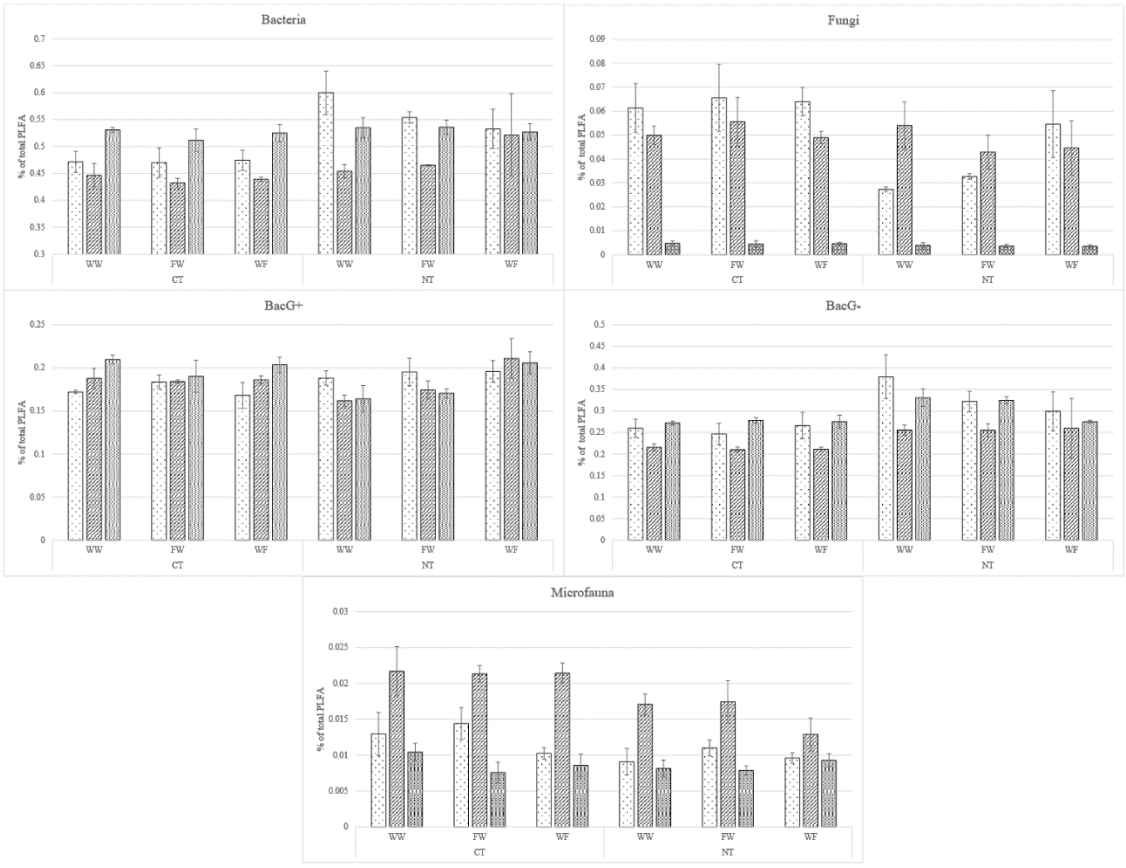

Fig 3.4 Effect of tillage system and crop sequence on microbial group PLFA percentage content on 15-30 cm soil layer

4. Effect of long-term tillage system and crop sequence on bacterial biomass (16s), amoA (nitrification), and nosZ (denitrification) genes abundance.

The aim of this experiment was to test the effect of tillage system and crop sequence on the abundance of bacterial gene 16s, nitrification gene amoA and denitrification gene nosZ in a long-term experiment.

Material and Methods

Soil sampling

During the cropping season 2013/2014, soil samples (composed by mixing 3 subsamples) were collected from the superficial layer of WW, WF and FW plots for both tillage system. In the wheat plots just one sample was collected, while in the faba bean plots, samples were separately collected at the middle of the plant rows (Bulk soil) , and close to the plant roots (Rizospheric soil). Soil samples were stored frozen and then gently sieved to pass through a 2 mm mesh sieve.

Nucleic acid extraction and amplification

A 2 g subsample of soil was used to extract DNA using the RNA PowerSoil® Total Isolation Kit (MoBio) following the manufacturer's instructions. DNA was quantified using a Nanodrop ND-1000 spectrophotometer (Thermo Fisher Scientific). DNA was amplified from purified soil DNA using specific primer for 16S, AmoA and nosZ genes. For the 16S: PCR was performed using the F341 and R907 primers in order to amplify the V3-V5 hypervariable regions (550 pb). The PCR program was initiated by a hot start of 5 min at 94

°C; after 9 min of initial denaturation at 95°C, a touchdown thermal profile protocol was used, and the annealing temperature was decreased by 1 °C per cycle from 65 °C to 55 °C; then 20 additional cycles at 55 °C were performed. Amplification was carried out with 1 min of denaturation at 94 °C, 1 min of primer annealing, and 1.5 min of primer extension at 72 °C, followed by 10 min of final primer extension.

For amoA, a nitrifying bacterial gene, PCR was performed using amoA-1F and amoA-2R primers (491 bp). PCR program was performed with an initial denaturation at 94°C for 90 s and then 20 cycles of denaturation at 94°C for 40 s, annealing at 53°C for 30 s, and extension at 72°C for 40 s.

For NosZ, a denitrifying bacterial gene, PCR was performed using nosZ-1840F and nosZ-2090R primers (267 bp). PCR conditions consisted of an initial denaturating step opf 95°C for 15 min, followed by 30 cycles of 95°C for 15 s, 60°C for 30 s, 72°C for 30° s and a final step of 72°C for 8 min.

For all the three genes, the total reaction mixture of PCR consisted of 25 µl with the following ingredients: soil DNA dilution (from 1:10 to 1:5), 1 µl of both primer, front and rear, at concentration of 10 µM for 16s primers and 2.5 µl at concentration of 30 µM for amoA and nosZ primers, 2 µl of 0.2mM dNTPs, 0.15 µl of 5 U Taq polymerase (Bioline), 2.5 µl of 10X PCR buffer, 0.75 µl of 1.5 mM $MgCl_2$ and sterile Milli-Q water to a final volume of 25 µl. Sterile water was used as a negative control to replace DNA in PCR reactions. PCR products were analyzed by electrophoresis in 2% agarose gels stained with GelRed®. The PCR results for each gene are used in order to choose the best DNA PCR concentration for qPCR.

Quantitative real-time PCR

Real time PCR (qPCR) was performed on BioRad iQ 5 QPCR. Amplification was performed in 20 µl reaction mixtures composed by 10.5 µl of SyberGreen 2x, 0.84 µl of both primers (at concentration of 10 µM and 30 µM as described above), and sterile Milli-Q water to a final volume of 20 µl. Primers and qPCR condition were the same of PCR amplification described above. For each gene, 16s, amoA and nosZ, a standard curve were constructed using plasmid relating Ct (cycle threshold) to the added mass of linearized plasmid DNA and the number of gene copies. The amount of template DNA was calculated by interpolating the cycle threshold with the standard curve, determined by the Bio-Rad iQ5 software program. All reactions were carried out in triplicate with four replication per qPCR.

Statistical analysis

One way analysis of variance (ANOVA) followed by t-test was performed to check for quantitative differences between samples. All statistical analyses were done using SAS statistical package (SAS 2008). LSD ($P<0.05$) post-hoc test to compare tillage management within the same cropping system was performed when significant differences were found. Pairwise mean comparison between the two cropping systems at the same tillage management was performed by the Student's t-test. Significant differences were considered at a $P<0.05$. Data were presented as mean values ± SE.

Results

The number of 16s gene number of copies was affected by the interaction Tillage x Crop. On average, among the crop treatment, CT > NT (+43%), whereas among crops, both wheat plots showed higher values than faba bean plots, with a trend FW>WW>WF. Within wheat plots, it was possible to observe a marked differences between the two tillage system applied, with NT>CT, while no difference was noticed in FW between the tillage systems. Faba bean plots showed, on average, higher number of copies in NT than in CT. With regard to the kind sample (bulk or rhizospferic) an opposite behaviour were observed among samples: in CT plot, rizopheric soil had a lesser number of copies of 16s gene than bulk soil, in NT WF-Rizo showed a higher value of number of copies than WF-Bulk (Fig 4.1).

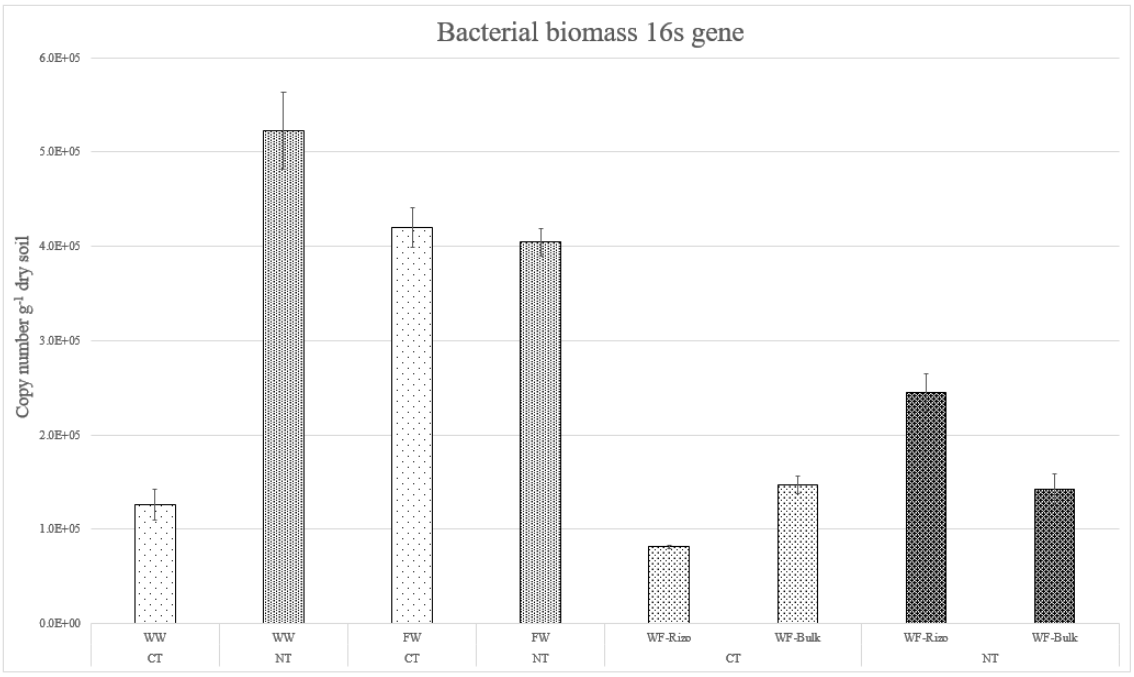

Fig 4.1 16s gene copy number across the treatments.

The amoA number of copies, on average, was higher in NT plots than CT plots (+61%) and in WF plots than WW and FW (+34%). Also the number of copies of amoA gene was affected by the interaction Tillage x Crop. In WW and WF plots a marked difference between tillage system was observed, while FW the difference among them was negligible. With regard to WF plots, in CT both type of soil sample showed a similar number of gene copies, whereas in NT, rizospheric soil sample had higher number of gene copies of amoA than bulk soil sample (Fig 4.2).

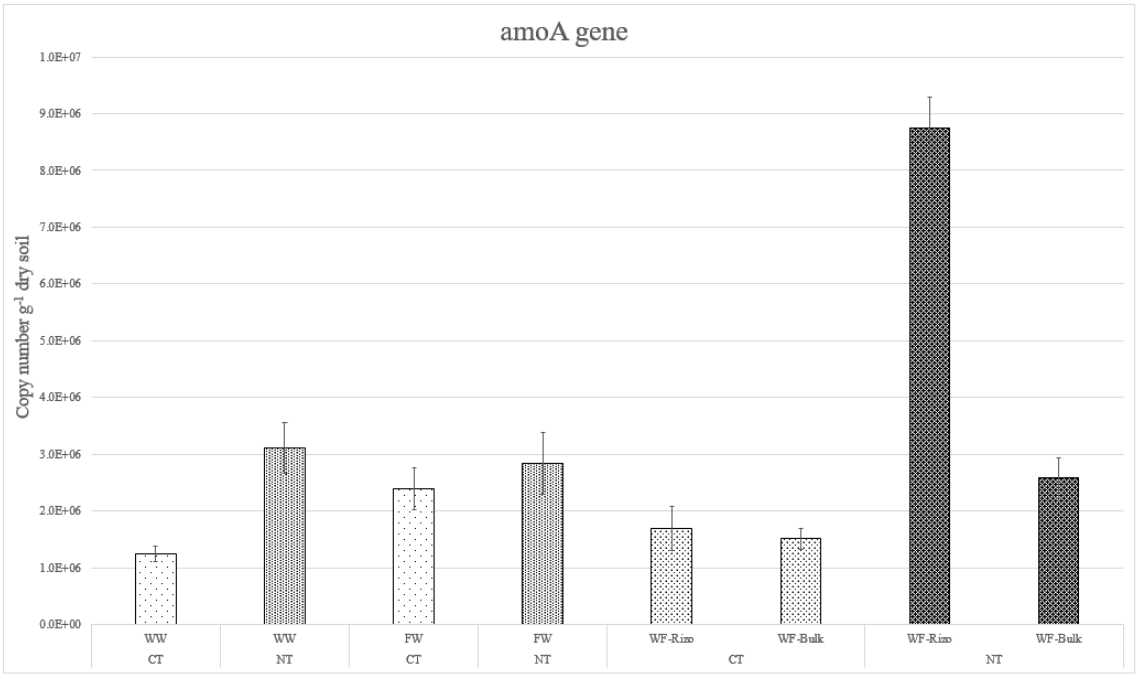

Fig 4.2 AmoA gene copy number across the treatments.

NosZ number of copies was affected by both treatments tested tillage system and crop residue, but not from their interaction. With regard to the tillage system, NT>CT (on average, +31%) wherease WF plots showed a higher number of gene copies than WW and FW (on average, +36%). Number of gene copies in WF plots showed a different trend according to the sample type and the tillage system. In particular, in CT plots, bulk soil had a higher

number of copies of nosZ than rizospheric soil, whereas NT showed an opposite trend, with WF-Rizo>WF-Bulk (Fig 4.3).

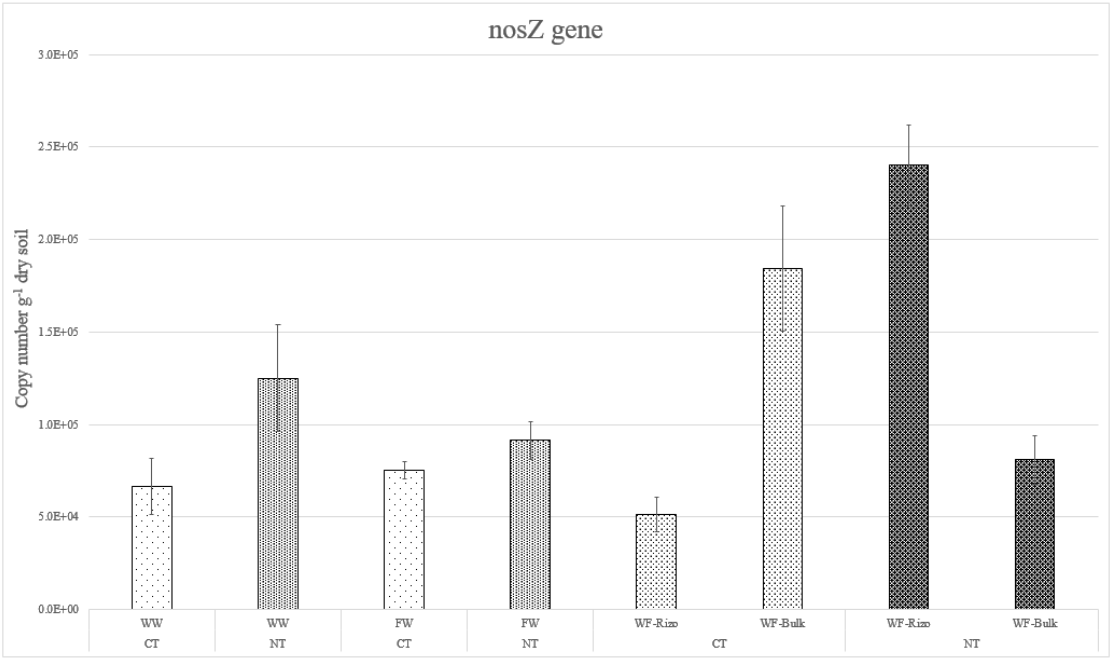

Fig 4.1 NosZ gene copy number across the treatments.

5. Effect of long-term tillage system and crop sequence on nitrogen emissions under Mediterranean climate

The aim of this experiment was to test the effect of tillage system and crop sequence on NH_3 and N_2O emissions in a long term experiment.

Materials and Methods

Experimental site

The trial was conducted under rainfed conditions at the Pietranera farm, which is located about 30 km north of Agrigento, Sicily, Italy (37°30’ N, 13°31’ E; 178 m asl), on a deep, well-structured soil classified as a Chromic Haploxerert (Vertisol), with a slope of about 7%. Soil characteristics (measured at the beginning of the experiment and referring to the 0–0.40-m layer) were as follows: 525 g kg–1 clay, 216 g kg–1 silt, 259 g kg–1 sand, pH 8.1 (1:2.5 H2O), 14 g kg–1 total C (Walkley–Black), 1.29 g kg–1 total N (Kjeldahl), 36 mg kg–1 available P (Olsen), 340 mg kg–1 K2O (exchangeable K), 35 cmol kg–1 cation exchange capacity, 0.38 cm3 cm–3 water content at field capacity (matric potential = –0.01 MPa), and 0.16 cm3 cm–3 at the permanent wilting point (matric potential = –1.5 MPa). The climate of the experimental site is semiarid Mediterranean, with a mean annual rainfall of 572 mm, concentrated mostly during the autumn–winter period (September–February; 76%), and spring (March–May; 19%). A dry period occurs from May to September. Mean air temperatures are 15.9°C in fall, 9.7°C in winter, and 16.5°C in spring.

Experimental Design and Crop Management

The long-term field experiment, which began in fall 1991, was set up as a strip-plot design with two replications. Treatments were soil tillage systems (no tillage, NT; reduced tillage, RT; and conventional tillage, CT) and crop sequences (continuous wheat, W–W; wheat–faba bean, W–FB; and wheat–berseem clover, W–BC). A detailed description of the experiment can be found in Giambalvo et al. (2012) and Amato et al. (2013).

In this study, the experimental factors tested were tillage (conventional tillage and no tillage) and crop (WW, continuous wheat; WF, faba bean after wheat; FW, wheat after faba bean). Conventional tillage (CT) consisted of one mouldboard ploughing to a depth of 30 cm in the summer, followed by one or two shallow harrowing (0–15 cm) operations before planting. No tillage (NT) consisted of sowing by direct drilling. Plot size was 370 m^2 (18.5 × 20.0 m). In NT plots, weeds were controlled before planting with glyphosate at a dose of 533 to 1066 g a.e. ha^{-1}, depending on the development of weeds. Every year, WW and FW plots were broadcast fertilized with 69 kg ha^{-1} of P_2O_5 before planting. Nitrogen fertilizer was broadcast on the soil surface at 120 kg N ha^{-1} in WW plots and 80 kg N ha^{-1} in FW plots. The total amount of N fertilizer was split with 50% applied immediately before planting (as diammonium phosphate and urea) and 50% applied at mid-tillering (end of March; during this experiment, it was before the 2nd soil sampling) as ammonium nitrate. WF plots were broadcast fertilized with 46 kg ha^{-1} P_2O_5 before planting and received no N fertilizer. Crop planting was always in December using a no-till seed drill with hoe openers under both CT and NT, making the appropriate sowing depth adjustments to ensure a homogeneous planting depth (3–5 cm). Faba bean cv. Gemini was sown at 40 viable seeds m^{-2} with an inter-row spacing of 75 cm. No rhizobial inocula were applied before planting because soil has a native rhizobial population. Durum wheat, cv. Anco Marzio, was planted in rows spaced 16 cm

apart at 350 viable seeds m^{-2}. In WW and FW plots, weeds were controlled by applying post-emergence at the early growth stage of the crop. In WF plots, weeds were controlled mechanically by shallow hoeing (with minimum soil disturbance) when plants were at the third-leaf stage; if necessary, the operation was repeated at the seventh-leaf stage. Faba bean were harvested in late June or beginning of July, leaving standing straw and uniformly spreading crop residues. Wheat was harvested also in late June or beginning of July and stubble (about 20–25 cm from the soil surface) was left standing. Wheat straw was baled and removed from the field. The soil surface covered by mulch in the NT treatments was always >30%.

Soil sampling and analysis

Ammonia emission was monitored after the application of fertilization (at sowing and tillering), in two cropping cycle (2013-14 and 2014-15). Soil ammonia volatilization was evaluated using the the method of Conway microdiffusion incubation adapted for soil by Bremner and Krogmeier (1988) according to Qi et al. (2012). Briefly, a small plastic jar (5.0 cm of diameter) containing 20 ml of 3% of boric acid solution was suspended above the ground. Than, an airproof chamber with a diameter of 15.0 cm was put on the soil surface covering the plastic jar. The camber was anchored to the ground through small wire arches in order to avoid gas leakages from the system and the chamber movement caused by wind. Three chambers per plot were placed for a total of 36. The ammonia volatilized from soil was trapped by boric acid solution and then determinate by titration with 0.01 N H_2SO_4 back to the original pH. The boric acid solution was replaced until the emission was negligible, on average, 7 times on each measurement epoch. Total ammonia volatilization was calculates as the sum of the ammonia volatilized during each day.

Soil nitrous oxide flux was measured over two cropping cycles, from sowing to harvest, in 2013-14 and 2014-15. Both greenhouse gas (GHG) fluxes were sampled using the closed chamber technique (Hutchinson and Mosier 1981; Clayton et al., 1994; Baker et al., 2003). Three polyvinyl chloride opaque chamber, with a diameter of 31.5 cm and height of 30.0 cm, where placed in each plot. The chambers were fitted in a polyvinyl chloride frame inserted into the soil to a depth of 5 cm in order to minimize the later diffusion of gases and avoid the soil disturbance. The frames were placed at the beginning of each sampling year, after the plant emergence, enclosing two plant rows in the wheat plots and one row in the faba bean plots, and were removed at the end of the crop cycle. During the cropping system, the chambers height was progressively increased to accommodate crop growth, using appropriate extension of the same diameter of the chamber, with a maximum height of 90.0 cm. At the bottom of each chamber, in the wall, a rubber stopper with a three-way stopcock was placed in order to take a gas sample. Gas samples (vol. 10 mL) were taken at 0, 30 and 60 min after the chamber closure from the headspace of each chamber using a 10 mL syringes, fitted on the three-way stopcock, connected with a needle in order to store the samples in a 7 mL pre-evacuated *Exetainer*® (Labco Limited, Uk). The air inside the chamber was manually mixed before the sampling. Air samples were taken simultaneously for each crop in both tillage system. To minimize the effect of diurnal variation in emissions the samples were taken at the same time of day (8 a.m.-13 p.m.) and reversing the starting plot every sampling epoch. After air sampling, the chambers were immediately removed from the base frames to minimize enclosure effects on soil environmental conditions and plant growth. Samples were taken 8 times for each cropping cycle/year, on average, every 20 days from sowing to harvest. Concentration of N_2O in the gas samples were determined by gas chromatography, using a TRACE-GC

(Thermo Scientific, Milano, Italia) gas chromatograph equipped with a 80-100 mesh stainless-steel column packed with Poropak Q column and a $_{63}$Ni ECD detector operating at column and injector, base and detector temperature of 40, 300 and 350°C, respectively. Flux rate was calculated from the N_2O increase of the concentration during chamber closure period of 60 minute follow equation presented by Jantalia et al. (2008):

$$f = \frac{\Delta C}{\Delta t} \times \frac{V}{A} \times \frac{m}{V_m}$$

where, $\Delta C/\Delta t$ is the change in N_2O concentration in the chamber during the closing time Δt, V and A are respectively the volume of the chamber and the area of the soil covered by the chamber. Vm is the molar volume corrected for the air temperature at the sampling time and m is the molecular weight of N_2O.

The seasonal amount of N_2O emissions were accumulated from the emission rates between every two consecutive days of the measurements by following equation according with Cai et al. (2012):

$$Cumulative\ N_2O\ emissions\ = \sum_{i=1}^{n} (F_i + F_{i+1})/2 \times (t_{i+1} - t_i) \times 24$$

The weather data were collected from a weather station located within 500 m of the experimental site. Statistical analyses were analyzed according to a strip-plot design with SAS statistical package (SAS 2008). LSD ($P<0.05$) post-hoc test to compare tillage management within the same cropping system was performed when significant differences were found. Pairwise mean comparison between the two cropping systems at

the same tillage management was performed by the Student's t-test. Significant differences were considered at a $P<0.05$. Data presented as mean values ± SE.

Results

Weather conditions

The weather conditions during the experimental period are show in Fig 5.1.

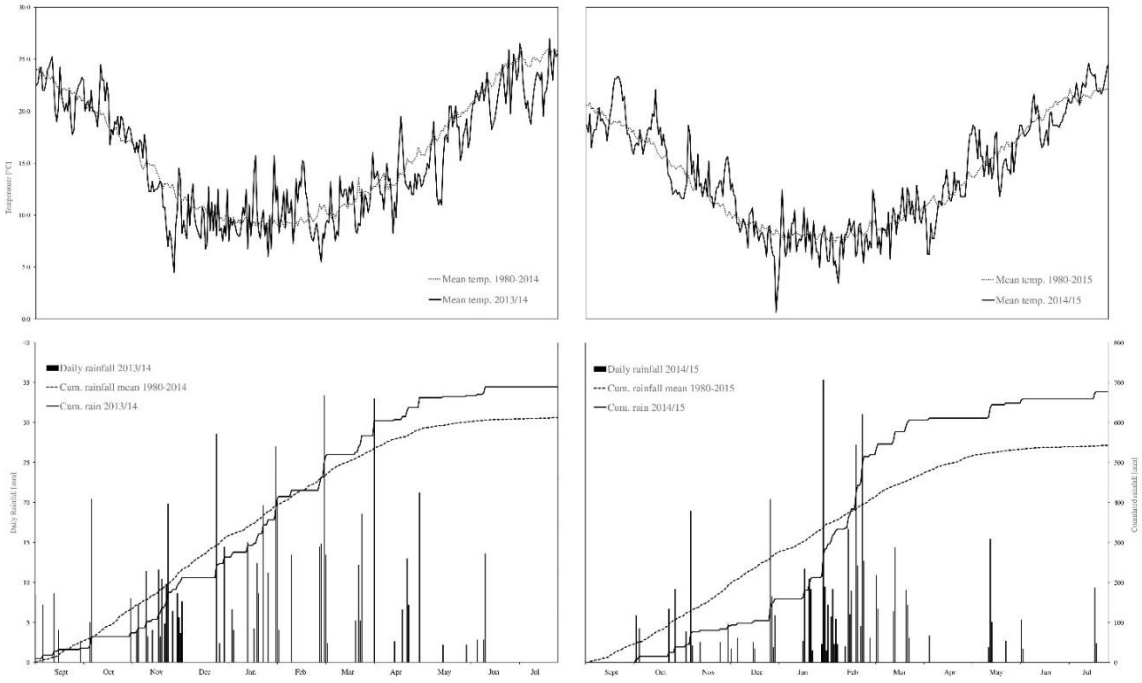

Fig 5.1 Daily air temperature and daily and accumulated rainfall at the experimental site during the growing seasons (2013-2014 and 2014-2015); the 35-yr average daily temperatures and accumulated rainfall are also included.

Total rainfall in 2013-2014 was 603 mm, 12% higher than the long term average (1980-2014) but with a similar rain distribution during the crop cycle. Mean year temperature was of 15.2°C slightly lower than the long term annual average of 15.9°C. Temperature trend was similar to the long term average with more marked differences observed in the last period of the cropping cycle. In the 2014-2015 the total rainfall was 677 mm, 24% more than the long term average (1980-2015). Rainfall distribution shown an apposite trend during the crop cycle; the period from September to January had lower rainfall than the long term average (-69.0 mm), while in the period from February to July the rainfall was much higher than the long term average (+193.7 mm). Mean year temperature was 15.8°C in accordance with the long term average.

Ammonia emissions

Soil ammonia emission at sowing and tillering, in both year, were strongly affected by the crop and, in particular, from the fertilization regimes adopted. No differences between the tillage systems were observed (Fig 5.2 and Fig 5.3). Ammonia emission after sowing ranged from 0.35 mg/m^2 (in fababean) to 4.32 mg/m^2 (in wheat), in 2013-14, and from 0.13 mg/m^2 to 2.31 mg/m^2 in the 2014-15 cropping cycle. A definite trend was observed in both year was as follows: WW>FW>WF; the emission of faba bean plot were identified as a basal level and relative to the measurement technique. Moreover, pronounced differences were found between the experimental years with the 2013-14 showing 42% higher emission than 2014-15, mainly due to the different seasonal rainfall trends. On average, the ammonia emission of WW plots were 33% and 24% higher than the FW plots, in the 2013-14 and in the 2014-15 cropping season, respectively (Fig 5.2).

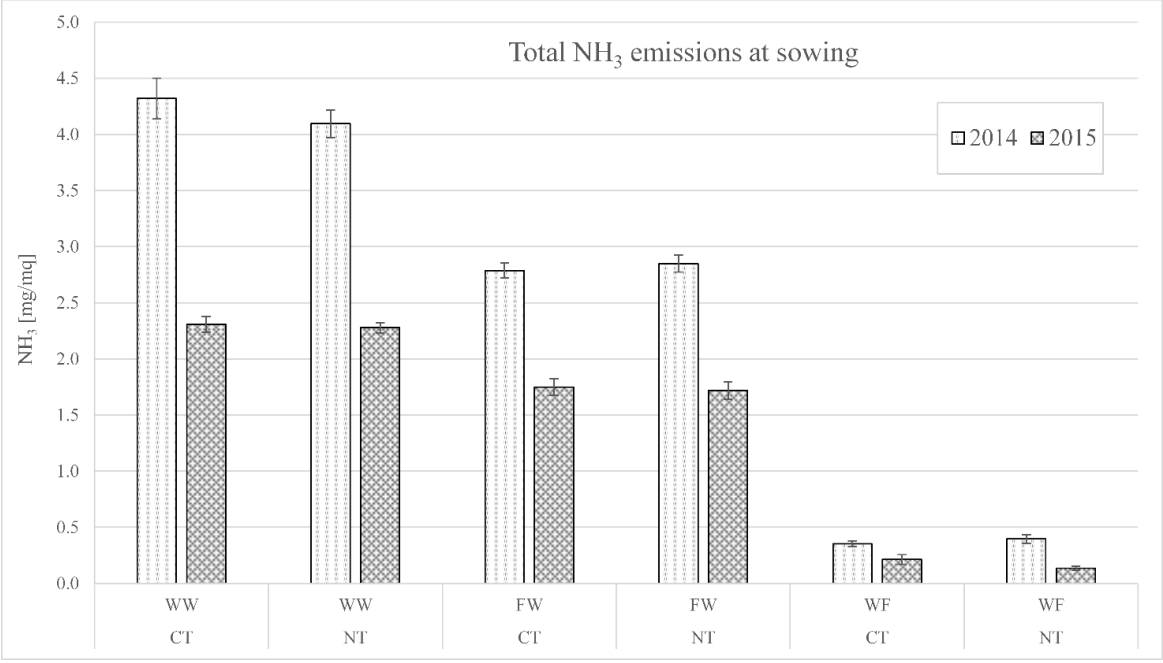

Fig 5.2 Total NH_3 emission at sowing in the 2013-2014 and in 2014-2015 cropping seasons.

After the fertilization at tillering, ammonia emission were conditioned from the crop type and it's management. Ammonia emission in wheat plots ranged from 6.9 mg/m^2 to 12.2

mg/m^2 and from 6.2 mg/m^2 to 10.2 mg/m^2, in the 2013-14 and 2014-15, respectively. In the 2013-14 the ammonia emission was 17% higher than 2014-15, with marked differences especially in WW rather than FW. In particular, on average between both tillage systems adopted, WW had 41% and 38% more emission than FW. The magnitude of ammonia emissions was higher after the fertilization at tillering than after sowing. This can be explained taking into account the different location of the fertilizer in the soil: at tillering the fertilizer was placed on the soil surface, whereas at sowing it was partially buried into the soil (Fig 5.3).

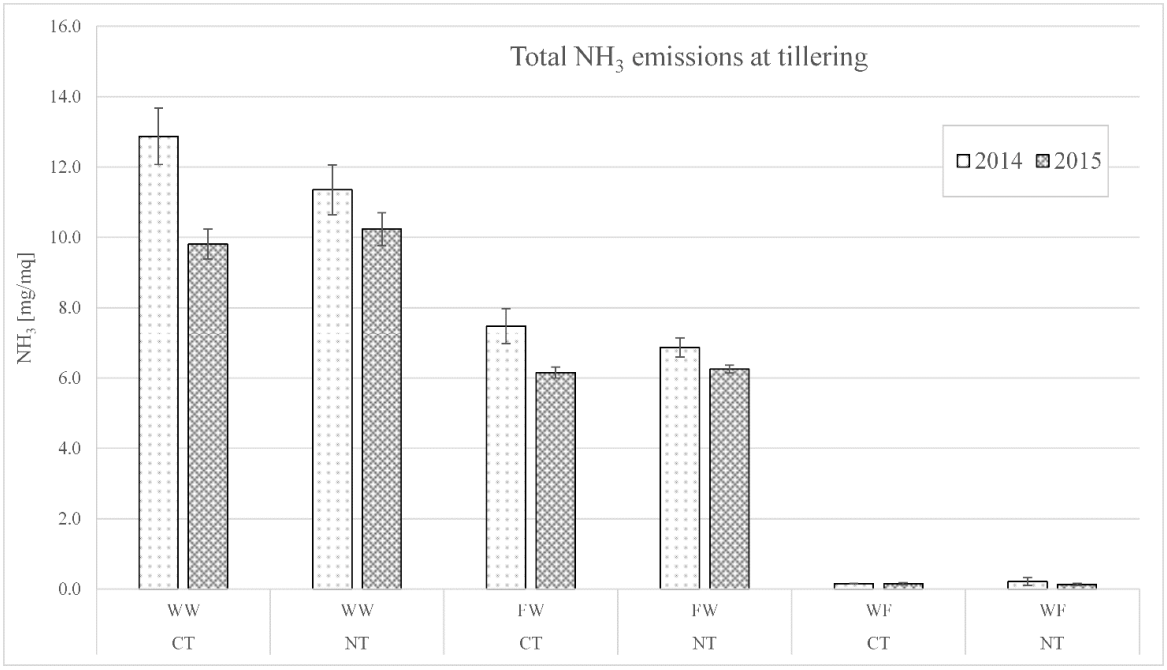

Fig 5.3 Total NH_3 emission at tillering in the 2013-2014 and in 2014-2015 cropping seasons.

Nitrous oxide emissions

Nitrous oxide flux in the 2013-14 ranged from 13.8 to 281.9 μg N_2O m^{-2} h^{-1}. At the start of the measurement period the emission rate, on average, was of 26.8 μg N_2O m^{-2} h^{-1}, with slight differences between the tillage systems. Nitrous oxide emission rate increased during the experimental period reaching the highest flux rate at the third measurement time (february) where NT-WF had an emission of 281.9 μg N_2O m^{-2} h^{-1}; in this sampling, wide differences between treatments were recorded, up to 133.0 μg N_2O m^{-2} h^{-1}. After this peak, nitrous oxide emission rate declined. An additional rise was found in April, despite lower than February, ranging from 72.5 to 151.9 μg N_2O m^{-2} h^{-1}. After April, to the middle of Maythe emission fluxed at the same rate or showed a slight decrease, with the exception of CT-FW. At the end of the cropping season nitrous oxide emission rate rapidly decreased reaching the basal level, on average, of 58.3 μg N_2O m^{-2} h^{-1} (Fig 5.4).

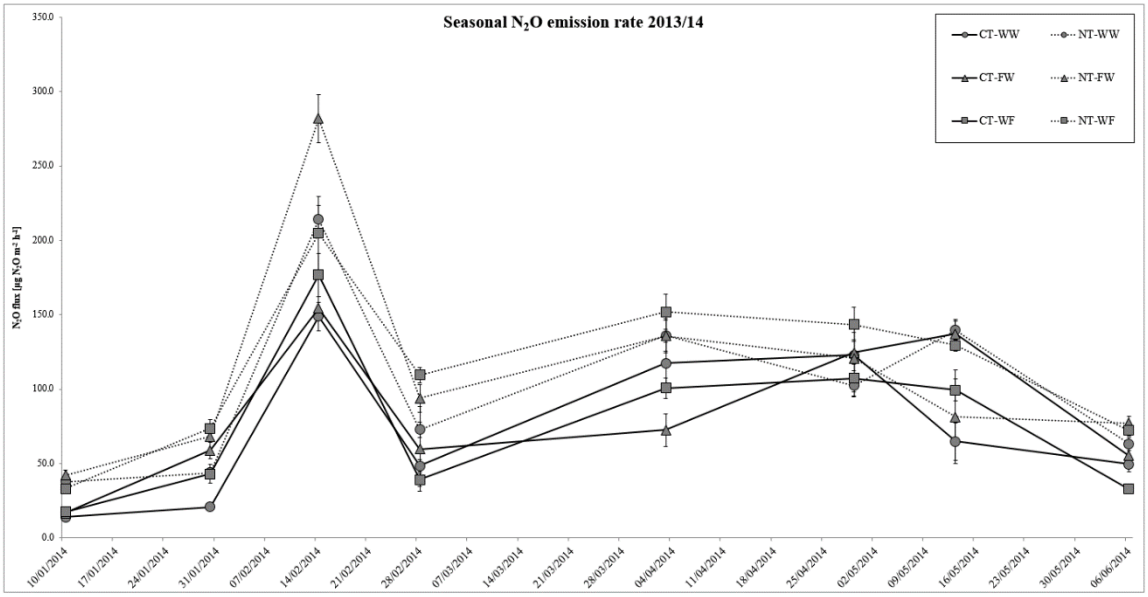

Fig 5.4 N_2O emission course during the 2013-2014 cropping season.

During the second cropping season (2014-15), soil nitrous oxide emission ranged from 10.5 μg N_2O m^{-2} h^{-1} to 278.0 μg N_2O m^{-2} h^{-1}. In the first period of measurement, emission rate

showed the same trend of the first year with an increase of emission at January and a massive peak in February followed by a lower emission rate in early March. In the second, compared to the first year, a low emission rate was recorded also at mid April, whereas in May, emission rate had a newly prominent increase. Such a second peak ranged from 102.2 to 230.4 μg N_2O m^{-2} h^{-1} with significant differences between treatments. In particular, NT-WF showed the highest emission rate (165.7 μg N_2O m^{-2} h^{-1}) whereas CT-WF the lowest (108.1 μg N_2O m^{-2} h^{-1}). At the end of the cropping season, finally, emission rate had a massive decrease reaching in some cases the basal level. On average, at the last measurement time, the emission rate was 59.5 μg N_2O m^{-2} h^{-1} (Fig 5.5).

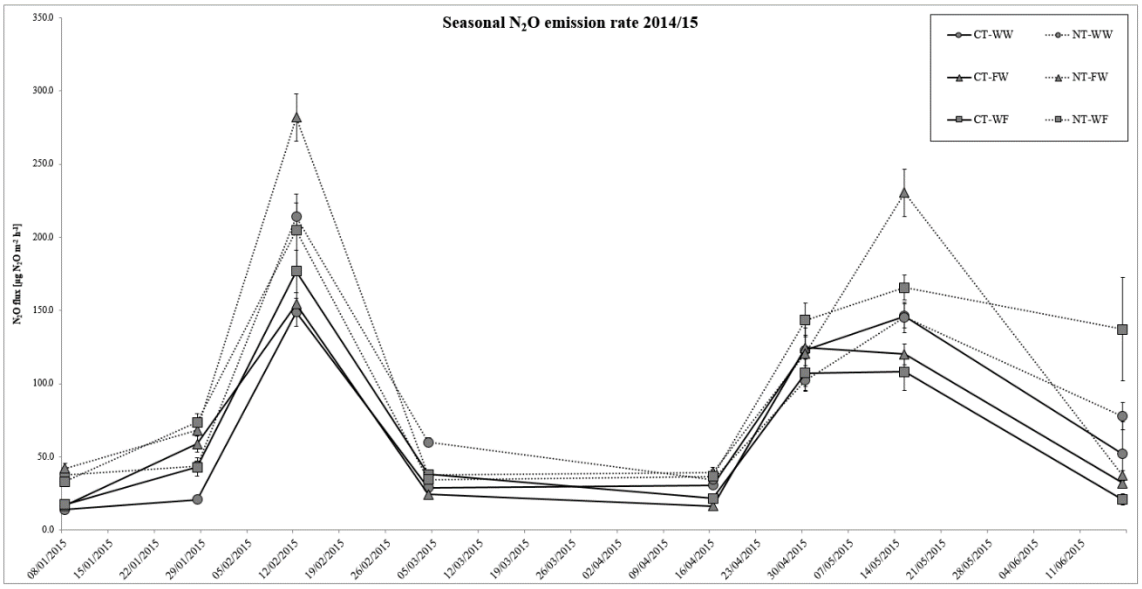

Fig 4.5 N_2O emission course during the 2014-2015 cropping season.

Total nitrous oxide emission ranged from 279.0 mg N_2O m^{-2} to 431.3 mg N_2O m^{-2} and from 242.7 mg N_2O m^{-2} to 394.2 mg N_2O m^{-2}, in the first and in the second year of measurement, respectively. Total nitrous oxide emission was affected by the interaction Tillage x Crop. NT plots, in both year, showed, on average, 30% higher nitrous oxide emissions than CT plots (+114.2 mg N_2O m^{-2}). With regards to the interaction Tillage x Crop, in CT plots no

difference were observed between the crop, whereas in NT, both plots involved in the crop rotation, FW and WF, showed a higher emission than WW plot (+48.9 mg N_2O m^{-2} in FW and +39.5 mg N_2O m^{-2} in WF, respectively) (Fig 5.6).

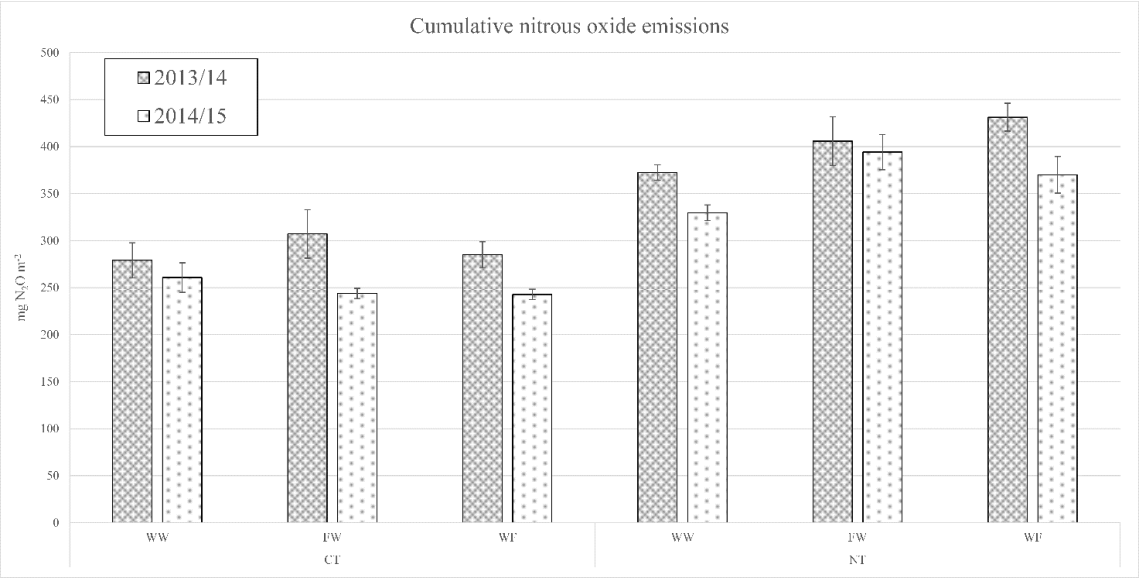

Fig 5.6 Total N_2O emission in the 2013-2014 and in 2014-2015 cropping seasons.

6. Role of plant residue addition on N_2O and CO_2 emission and soil quality: the effect of residue type on two contrasting soils

Aim of the present work was to test the effect of the kind of residues incorporated into the soil on CO_2 and N_2O emission on two contrasting soil. Additional aim was testing the correlation between couples of data drawn with IRGA, PGA and GC.

Materials and Methods

In order to assess the effect of crop residue added on two contrasting soils on CO_2 and N_2O emissions a pot experiment was established during fall 2014 in a greenhouse of the Scotland Rural College (SRUC) of Edinburgh (lat, 55°55' N, long, 3°10' W; 85 m a.s.l.) under controlled condition. The experiment started on 23/10/2014 and taken 7 weeks, finishing on 12/12/2014. The experiment was carried out with two soils and two crop residues, with three replicates. Soil used for the experiment was collected in 9 point per plot from the top 20 cm of a Eutric Cambisol at Bush Estate (lat, 55° 51' N, long, 3° 12' W; 199 m a.s.l.) in Edinburgh (Lothian, Scotland) and of a Chromic Haploxerert (Vertisol) at Pietranera Farm (37°30' N, 13°31 E; 178 m a.s.l.) in Santo Stefano Quisquina (Sicily, Italy) in early October 2014. In particular, soil was collected from the plot "No 3" (Conventional tillage and rotation) at Bush Estate and from the plot "No 8" (Conventional tillage, wheat-faba bean rotation) at Pietranera farm. Selected soil properties of both soils are presented in the Tab 6.1. Further information regarding the soil sampling sites are available in Vinten et al. (1992) and Amato et al. (2013), respectively. The soil was air-dried and passed through a 2 mm mesh and visible roots and organic residues were removed, and then mixed thoroughly before using; for both the water hold capacity on weight basis was measured. Air dried crop biomass of

wheat (cv. Simeto) and faba bean (cv. Gemini), cultivated at Pietranera farm, were ground to pass a 1 mm screen, mixed, and finally used as crop residues.

Soil characteristics	Sicily Pietranera	Scotland Bush Estate
Soil classification	Chromic Haploxerert (Vertisol)	Eutric Cambisol
Soil series	Gessoso-solfifera (sulphurous-chalky)	Macmerry
Texture	Clay-loam	Sandy-loamy
Coordinates	37.3 N, 13.3 W	55.9 N, 3.2 W
Altitude	178	199
Slope [%]	7	6
Clay [%]	52.5	
Silt [%]	21.6	
Sand [%]	25.9	
Carbon exchange capacity [$cmol_+ kg^{-1}$ soil]	35	
pH	8.1	6.6
Water content at field capacity (pF 2.5) [%]	38	36
Water content at permanent wilting point (pF 4.5) [%]	16	20
Organic matter [%]	2.4	4.3
Total N [%]	0.13	0.21

Tab 6.1 Soil properierties of the soil involved on the experiment

For the experiment, a complete randomized factorial design with three replication was adopted; where used 18 pot, with 10 cm of diameter and 25 cm height: 9 pot per soil, three for each crop residues and three control pot without residues. Each pot was filled with 1.5 kg of soil, where was mixed the crop biomass in reason of 5 g per kg of soil, leaving 2-3 cm away from the top edge margin and filling the bottom part of the pot with sand. Then, pots will be brought to 60-70% of the water hold capacity where remain over the entire experiment duration of 7 weeks. After each measurement epoch, water was added to replace the evaporation losses and the pot were randomized inside the greenhouse. During the experiment, the soil temperature was recorded by a temperature data logger (EL-USB-3, Lascar Electronics, United Kingdom).

CO_2 and N_2O soil emission were measured three time a week (Monday, Wednesday and Friday), for overall of 22 epochs, using two different equipment, an Infrared Gas Analyzer

(IRGA, EGM-4 CO_2, PP system, USA) and a Photoacustic Gas Analyser (PGA, INNOVA 1412, LumaSense Technologies A/S, USA). The measurement time was always between the 9:00 and the 15:00 and each time the equipment order was reversed. The EGM-4 was equipped with a SRC-1 Soil Respiration Chamber equipped with a fan, with of 10 cm of diameter and 15 cm height, sealed on top of it by an airtight rubber. The air from the chamber was send to the analyser at flow rate of 0.1 l min^{-1}. After 15 seconds of flushing the chamber was placed above the pot, equilibrated for another 15 seconds, than the CO_2 concentration was measured every 5 seconds and the flux was calculated from the centration increase over time until a good linear fit was obtained.

INNOVA 1412 was equipped with a PVC chamber with a 10 cm of diameter and 10 cm height, connected to the equipment by two small rubber pipe on the chamber top, and sealed above the pot by a rubber seal. The analyser automatically pumped ~0.1 l min^{-1} of air from inside the chambers and performed the analysis with a 5 seconds sampling integration time and a fixed flushing time, 8 seconds for the chamber and 3 s for the tubing. The equipment performed a built-in compensation for water and cross interferences. Before the flux measurements, the instrument analyze ambient air for about 30 min until readings for CO_2 and N_2O were stabilized. The overall time for sampling and measurement of carbon dioxide and nitrous oxide concentration and dew-point temperature was approximately of 70 seconds; once measurement was made every two minutes.

Gas flux measurement, CO_2 from both, IRGA and PGA, and N_2O from PGA were validated by gas chromatography. CO_2 and N_2O emissions were measured using the static closed chamber technique (Hutchinson and Mosier 1981; Clayton et al. 1994; Singh et al. 1999). A chambers made of polyvinyl chloride (PVC), with 10 cm of diameter and 15 height had a lid with a gas sampling port were sealed above the pots for 60 min. Before and after this period gas samples were collected in portable evacuated glass vials (Scott et al. 1999), transported

to the lab and, than, analyzed by a gas chromatography (Agilent 7890a, Agilent Technologies Ltd, Stockport, UK) equipped with a thermal conductivity detector (TCD) and an electron capture detector (ECD). Fluxes of CO_2 and N_2O were calculated from the increase in concentration in the chamber corrected for the chamber air temperature using the following relation (Jantalia et al. 2008):

$$f = \frac{\Delta C}{\Delta t} \times \frac{V}{A} \times \frac{m}{Vm}$$

where, $\Delta C/\Delta t$ is the gas increment during the chamber closure time, V is the volume of the chamber, A is the soil area, *m* is the molecular weight of the gases and *Vm* is the gas molar volume corrected for the ambient temperature.

The total amount of N_2O and CO_2 emissions were cumulated using the emission rates between two consecutive monitoring days by the following equation (Cai et al. 2012):

$$Cumulative\ emission\ of\ N_2O\ or\ CO_2 = \sum_{i=1}^{n} (F_i + F_{i+1})/2 \times (t_{i+1} - t_i) \times 24$$

where *F* are the emission flow of N_2O and CO_2, *i* is the i^{th} measurement, (t_{i+1}-t_i) is the time lenght between two adiacent measurements and n is the total measurement number.

Crop biomass dry matter, ether extract, total nitrogen and crude protein were analysed following AOAC (1995) while, structural carbohydrates, like, cellulose, hemicellulose, neutral detergent fiber (NDF), acid detergent fiber (ADF), acid detergent lignin (ADL) and acid insoluble ash (AIA) where analysed according to Van Soest et al. (1991).

Total Carbon of biomass and soil were performed by an automated analyser (Flash 2000, Thermo-Finnigan, Glasgow, UK).

From each pot two soil samples were collected: one superficial from the surface until 5 cm of depth and one from 5 until 15 cm of depth. Soil pH was measured in a 1:5 (v/v) suspension of soil in water. Dissolved organic carbon content in the soil were determined by a total organic carbon analyser (DC-80, Rosemount Analytical, Inc. Dohrmann Division, USA) after the removal of inorganic carbon by acidifying the sample. Concentration of NH_4-N and NO_3-N were determined from 10 g of soil extracted with 100 ml of 2M KCl (1:5 ratio); than the extract was measured determined by continuous flow analysis autoanalyser (SAN SYSTEM, Skalar Analytical B.V., Netherland)

The analysis of variance, ANOVA, of soil and emissions was performed by SAS software (SAS Institute 2008). Treatment means were compared using Fisher's protected LSD test at the 5% probability level.

The comparison between GC, IRGA and PGA, respectively, for CO_2, CO_2 and N_2O, were performed. Further, the soil CO_2 emission rate measurement from IRGA and PGA were compared over the 22 sampling dates. Techniques evaluation were made by a linear regressions analysis and evaluated by the coefficient of determination (R^2) of the adjusted linear equations (SAS Institute 2008).

Results

Temperature

The temperature inside the greenhouse during the experiment are reported in the Fig 6.1. The temperature ranged from a minimum of 17°C to the maximum of 28.5°C. The mean temperature of the period was of 20.5°C.

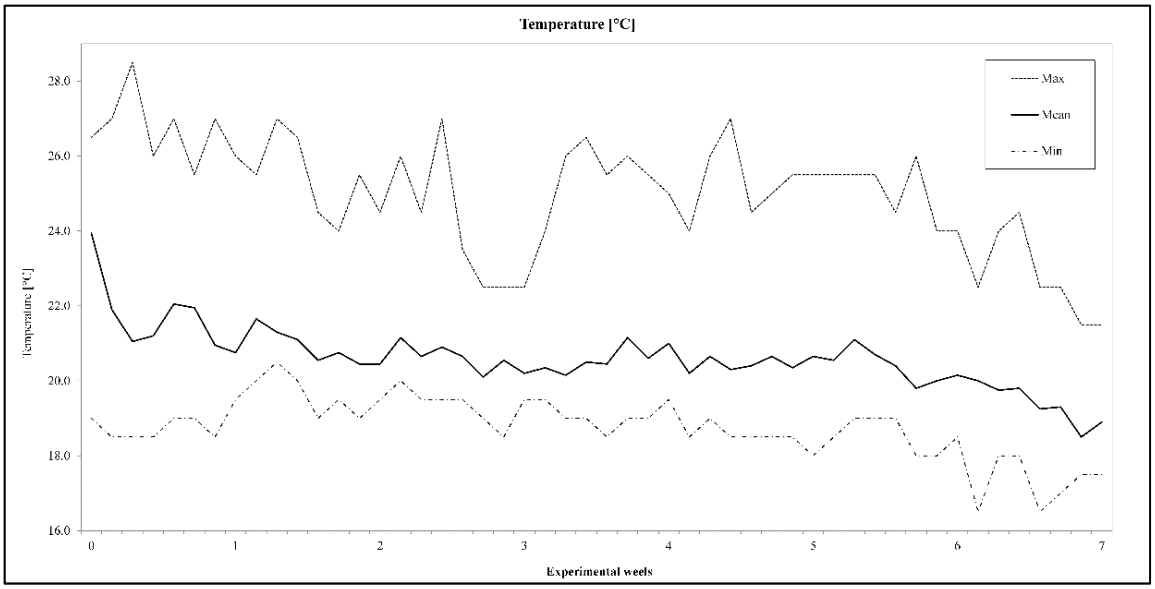

Fig 6.1. Daily minimum, maximum and medium temperature during the experiment.

Crop biomass

The average chemical composition and the incidence in botanical fractions of crop biomasses, expressed as percentage, are reported in Tab 6.2.

Surprisingly, nitrogen and relative crude protein content of faba bean and durum wheat were comparable (1.4% vs 1.3% and 8.6% vs 8.2%). This can was due to the different collection period of biomass, which took place at maturation for faba bean and at heading for the wheat. With regards to the botanical fractions, marked differences were among the biomasses. In particular, faba bean had higher ADF (+66.5 %), ADL (2.9 times more), cellulose (+60.2 %)

and NDF (+18.9) than wheat, while shown a lower content of hemicellulose (-50.9 %). Observing other biomass parameters most of all are very similar between the two crops, with only the ADL ash content higher in wheat than in faba bean (Tab 6.2).

Residue characteristics	Faba Bean	Durum Wheat
Dry Matter	91.8	91.8
Organic Matter	91.8	92.1
N content	1.4	1.3
Crude protein	8.6	8.2
Ether extract	1.1	1.7
Acid detergent fibre (ADF)	48.0	28.8
Acid detergent lignin (ADL)	10.0	3.5
Cellulose	34.1	21.3
Neutral detergent fibre (NDF)	54.0	45.4
Hemicellulose	7.5	15.3
Ash	8.2	7.9
ADL Ash	0.4	3.2

Tab 6.2 Chemical composition and proportion of botanical fractions of the crop residues used in the experiment

Soil Traits

Interaction between soil and residue type for all the soil traits at the end of the experiment was strong and significant (Tab 6.3).

At the beginning of the experiment, the Sicilian soil used in the present study had a high pH (8.1) and high clay and low total C content (1.39%), whereas the Scoland soil had a neutral to subacid pH (6.6), low clay and high C content (2.48%). As expected, the addition of organic residue mostly increased DOC in both the top- and sub-layer of the Sicilian soil (on average by 52.5% compared to unamended control), whereas its effects on the Scotland soilwere null (Tab 6.3).

Soil incubation, either with or without biomass incorporation, decreased soil pH by 0.86 in the Scotland soil and 0.33 in the Sicilian soil. The effect of the addition of organic residues to the soil pH varied with both the soil and kind of biomass incorporated: in the Scotland soil, addition of wheat residues significantly decreased pH in the top- and sub-layers if comparing to the unamended control whereas addition of faba bean residues did not

influence soil pH. In the Sicilian soil, no effect of the addition of organic residues on soil pH were found in both soil layers (Tab 6.3).

The concentration of ammonium-N was higher in the Sicilian than in Scotland soil, especially in the sub-layer. The role of the addition of organic residues to the soil ammonium-N depended on the soil and kind of biomass added: addition of durum wheat residues increased soil ammonium-N in top-layer of both soils (+40% in the Eutric Cambisol and +102% in the Chromic Haploxerert), whereas ammonium-N in the soils amended with fababean residues was similar to those of the controls. In the sub-layer of the Scotland soil, the effect of the addition of the organic residues was similar to that observed in the top-layer, whereas both residues strongly increased the ammonium-N of Sicilian soil comparing to the unamended control (+133% in faba bean and +454% in wheat residues) (Tab 6.3).

The concentration of nitrate-N in both layers was dramatically higher in the Scotland soil compared to the Sicilian soil and this occurred irrespective of the addition of organic residues. In the Scotland soil, addition of fababean residues reduced nitrate-N more than wheat residues, especially in the sub-layer, if compared to the unamended control. In the Sicilian soil, nitrate-N in both layers did not vary by the addition of plant residues (Tab 6.3).

		Sicily			Scotland			*P*		
		Faba bean	Durum wheat	No addition	Faba bean	Durum wheat	No addition	*Soil*	*Residue Type*	*S × T*
0-5 cm soil layer										
DOC	mg C kg^{-1} soil	42.5	43.2	33.6	73.5	67.6	67.2	*<.001*	*0.000*	*0.007*
pH	-	7.7	7.8	7.8	5.8	5.4	5.9	*<.001*	*0.009*	*0.019*
Ammonium N	mg N kg^{-1} soil	1.6	3.3	1.7	0.9	1.3	0.9	*<.001*	*<.001*	*<.001*
Nitrate N	mg N kg^{-1} soil	0.4	2.4	0.3	104.6	149.6	164.5	*<.001*	*0.001*	*0.001*
Ammonium:Nitrate N ratio	-	4.3	1.4	6.5	0.009	0.008	0.006	*<.001*	*0.001*	*0.001*
5-15 cm soil layer										
DOC	mg C kg^{-1} soil	75.9	83.0	48.1	86.4	93.1	91.8	*<.001*	*0.000*	*<.001*
pH	-	7.7	7.7	7.8	5.9	5.6	5.8	*<.001*	*0.037*	*0.043*
Ammonium N	mg N kg^{-1} soil	13.5	32.0	5.8	1.1	1.5	0.9	*<.001*	*<.001*	*<.001*
Nitrate N	mg N kg^{-1} soil	0.5	0.5	0.8	36.9	43.3	66.4	*<.001*	*<.001*	*<.001*
Ammonium:Nitrate N ratio	-	25.7	62.9	7.3	0.030	0.034	0.014	*<.001*	*<.001*	*<.001*

Tab 6.3. Effect of crop residue type on Dissolved Organic Carbon (DOC), pH, Ammonium and Nitrate Nitrogen content and Ammonium:Nitrate ratio in 0-5 cm and 5-15 cm soil layers of a Sicilian and Scottish soils.

Ammonium:Nitrate N concentration ratio (NH_4^+:NO_3—N) extremely differed by the soil under study: in the unamended controls, it was 6.467 in the Sicilian soil and 0.006 in the Scotland soil. In the latter, addition of organic residues to the soil did not influence the NH_4^+:NO_3—N of either the top- or sub-layer. In the top-layer of Sicilian soil, the addition of organic residues reduced the NH_4^+:NO_3—N, especially when faba bean residues were added. In the sub-layer, an opposite result was found and thus addition of organic residues increased the NH_4^+:NO_3—N, especially when wheat residues were added (Tab 6.3).

CO_2 and N_2O emissions

Carbon dioxide emission trend measured with IRGA and PGA are presented in Fig 2 and Fig 3, respectively. CO_2 fluxes, measured with IRGA, ranged from a minimum value of 0.11 g m^{-2} h^{-1} to a maximum value of 3.64 g m^{-2} h^{-1}. For almost the entire experimental period Scotland soil had higher CO_2 emission flux than Sicilian soil. At the beginning of the experiment the two soil reached the maximum emission flux at the first and second day of measurement with fluxes of 3.58 g m^{-2} h^{-1} for the Scotland soil and 1.42 g m^{-2} h^{-1} for the Sicilian soil (Fig 6.2).

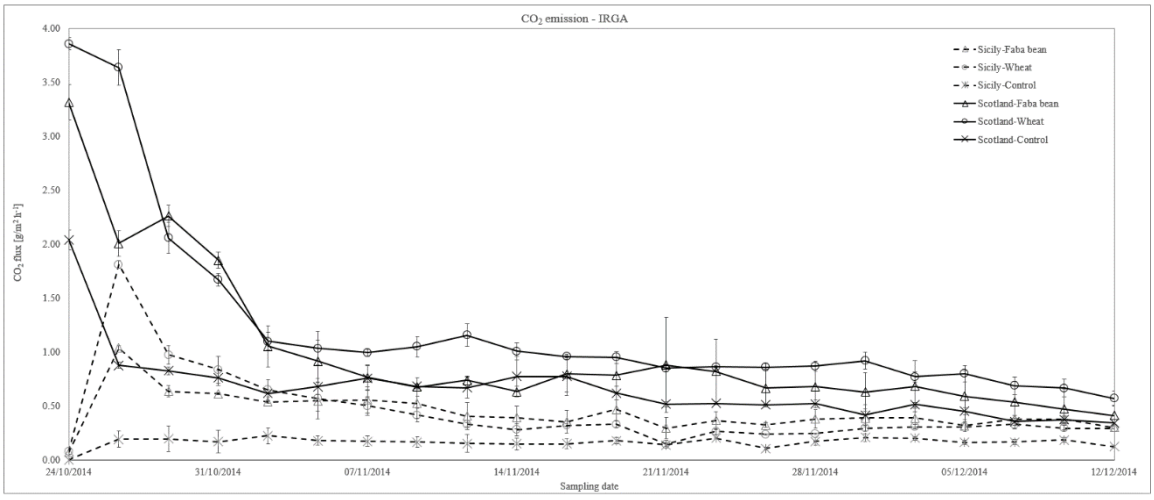

Fig 6.2 CO_2 emission course during the experimental period in Sicilian and Scottish soils amended with faba bean and wheat biomass, measured with IRGA.

The highest carbon dioxide fluxes were recorded in both soils amended with wheat straw while the lowest emission were observed in the unamended controls. The differences in emission magnitude, between the two soils, were massive in the first two weeks of measurement, where were emitted the 53.8% and 46.2% of total carbon dioxide, in Scotland and Sicilian soil, respectively. Afterwards, the differences between the two soils have diminished and the emissions slightly descending until the end of the experimental period. With regard to the CO_2 emissions measured with PGA they almost shown the same trend of the measurement done with IRGA. However, in the first part of the experimental period, was possible to observe higher emissions flux in PGA than with IRGA, especially in the Scotland soil. In the following period, until the end of experiment, there were no substantial differences between the techniques (Fig 6.3).

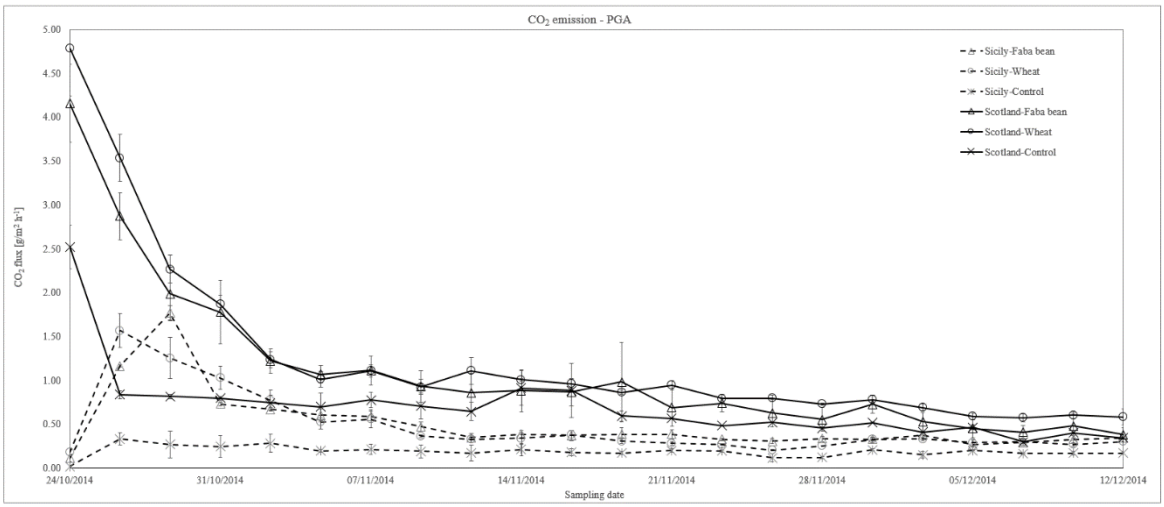

Fig 6.3 CO_2 emission course during the experimental period in Sicilian and Scottish soils amended with faba bean and wheat biomass, measured with PGA.

Total CO_2 emission was 74% lower in the unamended Sicilian soil (198 g CO_2 m^{-2}) compared to the Scotland soil (765 g CO_2 m^{-2}). Addition of plant residues to the soil increased total emission to a different extent depending on the soil under study (interaction Soil x Residue Type significant): in the Scotland soil, addition of faba bean and wheat consisted in an increase of 24% and 88%, respectively, of the total CO_2 emission. In the Sicilian soil, no differences were found between the kind of biomass incorporated, which, on average, increased total CO_2 emission by 171% comparing to the unamended control (Fig 6.4).

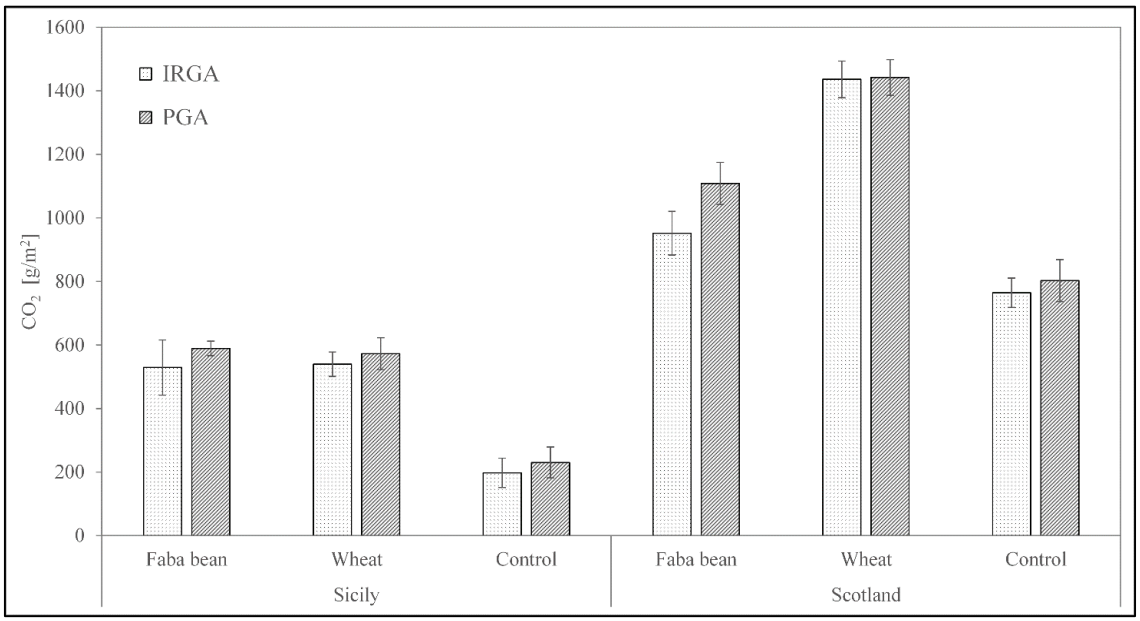

Fig 6.4 Total CO_2 emission in Sicilian and Scottish soils amended with faba bean and wheat biomass measured with IRGA and PGA.

Nitrous oxide emissions fluxes course across the experimental period, measured with PGA, is presented in the Fig 5. N_2O fluxed during the experiment ranged from 0.022 to 0.348 mg m^{-2} h^{-1}. Looking to each soil, nitrous oxide emissions ranged from 0.024 mg m^{-2} h^{-1} to 0.117 mg m^{-2} h^{-1} and from 0.022 mg m^{-2} h^{-1} to 0.348 mg m^{-2} h^{-1}, respectively, in the Scotland and Sicilian soils (Fig 6.5).

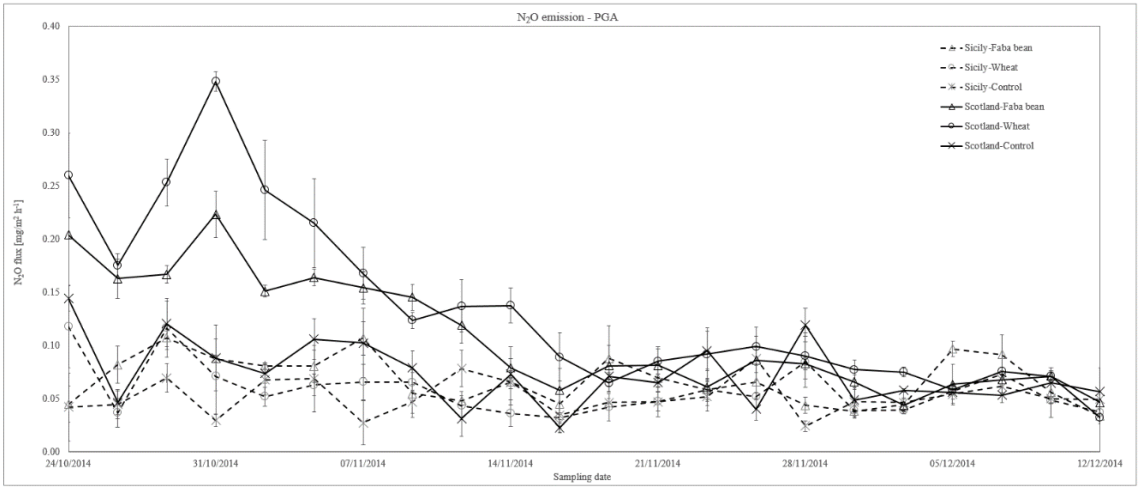

Fig 6.5 N_2O emission course during the experimental period in Sicilian and Scottish soils amended with faba bean and wheat biomass, measured with PGA.

The highest flux were measured in both soil amended with wheat straw. Scotland soil reached the emission peak between the third and the tenth measurement epoch while the Sicilian soil shown a more constant development of the emission during the trial. In particular, during the first period of experimentation marked differences between amended and unamended soil were observed in Scotland soil (Fig 6.5).

umulative nitrous oxide emission in the unamended control was 30% higher in the Scotland soil (85.1 mg N_2O m^{-2}) than in Sicilian soil 59.9 (mg N_2O m^{-2}). Crop residue addiction had a different effect in each soil (interaction Soil x Residue Type significant). In the Scotland soil the highest nitrous oxide emission was observed in the pots amended with wheat (159.8 mg N_2O m^{-2}, +88% w.r.t control) followed by the pots amended with faba bean (127.0 mg N_2O m^{-2}, +49% control), while in the Sicilian soil, faba bean (80.8 mg N_2O m^{-2}, +35% w.r.t control > wheat (67.2 mg N_2O m^{-2}, +12 w.r.t control) (Fig 6.6).

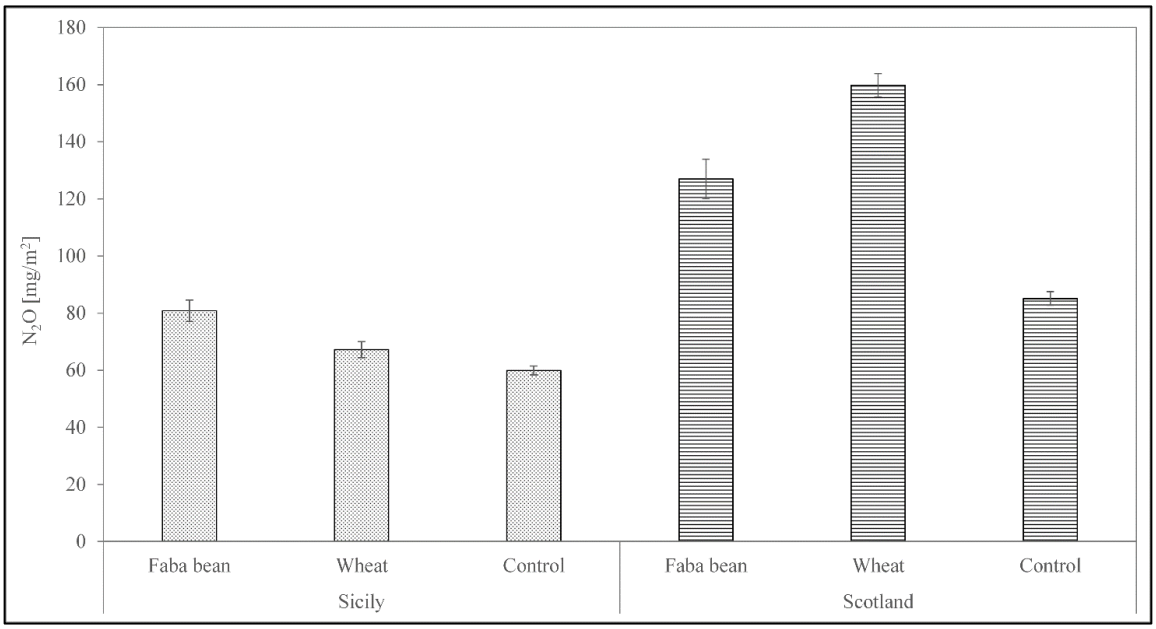

Fig 6.6 Total N_2O emission in Sicilian and Scottish soils amended with faba bean and wheat biomass measured with PGA

Measurement technique evaluation

The results of validation with gas chromatography of the gas fluxes measurement performed with IRGA and PGA, for CO_2 and N_2O, are reported in the Fig 6.7.

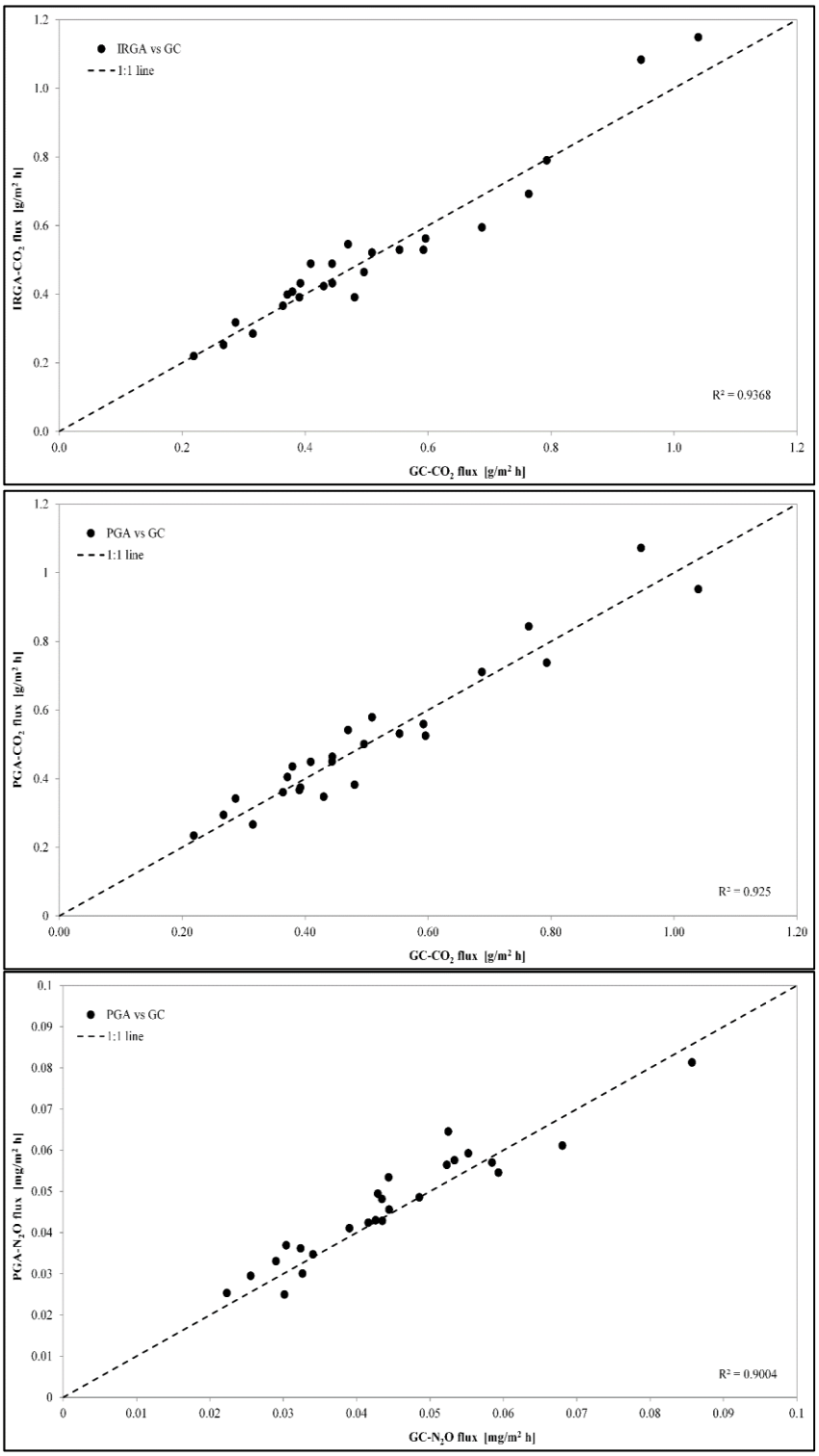

Fig 6.7 Relationship between CO_2 and N_2O absolute concentration determined by GC, IRGA and PGA.

Overall, no significant differences were for IRGA and PGA in CO_2 measurement in comparison with GC, showing a determination factor of 0.95 and 0.925, respectively. With regards to the nitrous oxide measurement, the linear regression between GC and PGA shown a good relation between the results (R^2=0.90), although PGA-N_2O are 10% higher of GC-N_2O measurement, overestimating the emissions (Fig 6.7).

The comparison between IRGA and PGA carbon dioxide measurement, across the entire experimental period (more than 600 measurements), shown an higher correlation between the two instruments (R^2=0.95) Fig 8. However, cumulated CO_2 emission measured by PGA were 9% higher, on average among the treatment, than those measured by IRGA (Fig 6.8).

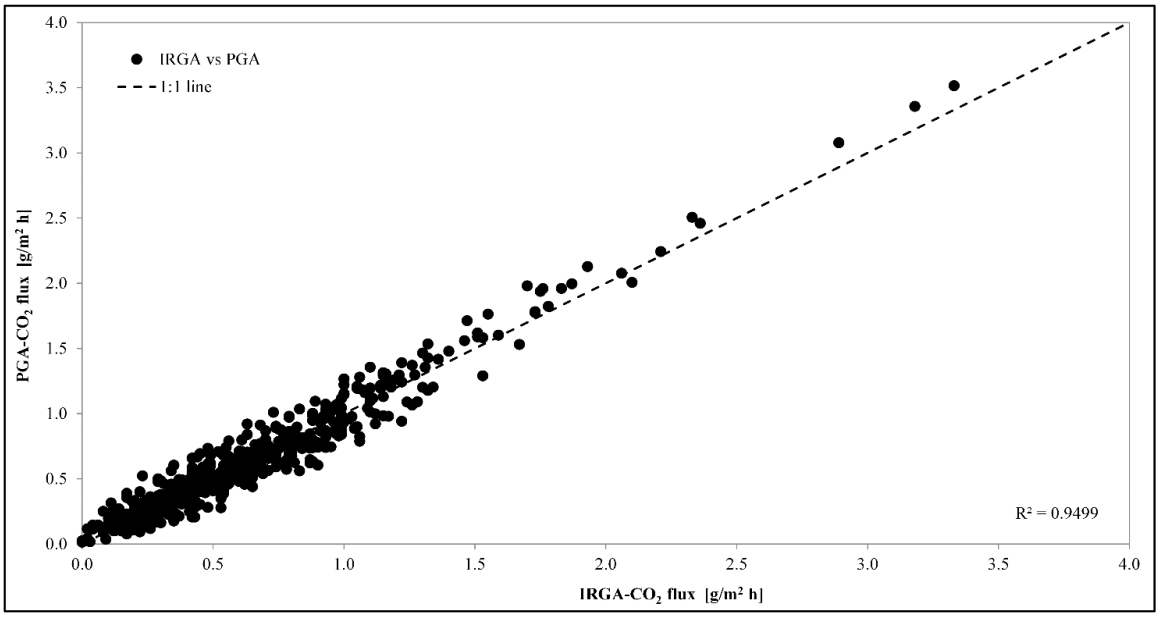

Fig 6.8 Relationship between CO2 absolute concentration determined by IRGA and PGA.

7. Discussion

The effect of tillage and crop sequence on soil quality, microbial community and its activity and nitrogen losses

In the present work, I studied the combined effect of the long term application of two contrasting soil tillage systems (mouldboard ploughing, CT, comparing to a direct sowing in no tillage system, NT) and crop species (continuous wheat, WW, wheat after faba bean, FW, and faba bena after bean WF) on a wide range of soil microbial and biochemical proprieties and ammonia and nitrous oxide emissions (experiment 1, 2, 3). In addition, the role of plant residue on NO_2 and CO_2 emission and soil quality was deepened in a greenhouse experiment with two contrasting soil (experiment 4).

On the whole, the effect of tillage on TOC and TON was promiment and soils from CT plots showed higher amounts of total organic carbon and nitrogen. Other experiments showed that yield of wheat under no tillage is similar to that under conventional tillage after 5-10 years of continuous application of the technique (Pittelkow et al., 2015). And in the present experiment similar grain yield between wheat in the tilled and NT systems were found (Amato et al., 2013). Thus it is likely that similar amounts of biomass were returned to the soil in both tillage systems. Reducing or avoiding soil tillage were indicated as a strategy to preserve soil organic matter, especially in Mediterranean soils (Laudicina et al., 2012) and that bigger aggregates were found in the NT soil comparing to the CT soil in the same experiment (Barbera et al., 2012). Similar results were found in other experiments conducted in similar soils (Menéndez et al., 2008) where in no tillage system was observed an increase of soil organic matter; however, in this study the organic matter content of the soil and the total nitrogen showed contrasting trends. These results strongly agree with both the higher microbial biomass, especially fungal, in NT than CT. Other authors reported that the fungal

component of the soil microbiota is the most important driver of the stabilization of the organic matter (Fontaine et al., 2011; Tisdall et al., 1997; van Groenigen et al., 2010) and that under N limiting conditions, stabilization of OM by bacteria is favoured (Fontaine et al., 2003; Shimel et al., 2004). And indeed, the present experiment was performed under N limiting conditions since the crop always strongly respond to the N fertilization (Giambalvo et al., 2009). In addition, MBC/TOC, BR, and qCO_2 were higher in the continuous wheat than the rotation (+17.1%, +48.9%, +29.9%, respectively), which suggest that the absence of a legume in the rotation can consist in an excess of carbon and relative lack of N for the microbial consortium (Insam and Haselwandter, 1989; Yan et al., 2003). And similar results were found in other soils when nitrogen was added to a continuous wheat system (Campbell et al., 1991). The results from the present experiments likely depended on the high C:N ratio of the wheat residues, since both MBC and MBC/MBN were 33.5% and 24.7% higher, respectively in wheat cultivated plots (irrespective of the crop sequence) than fababean.

The effect of tillage and crop sequences on N transformation and losses mostly depended on the total N content of the topsoil. In particular, no tillage consisted in a general increase of DEA, total and nitrification bacteria (by means of 16S and amoA, respectively), with lesser differences among crops. The high dependence of such traits on the soil conditions, rather than on soil management, were also found by Smith et al. (Smith et al., 2010), which suggested that the effect of the agronomical practices on 16S and AmoA mostly depend on the time course of the crop. Other experiments (Kong et al., 2010) also showed that amoA abundance in soil mostly depend on the amount of organic fertiliser or plant residues of the previous crop and that soil texture, especially the clay fraction, and structure are main drivers of amoA abundance. In the present study, no organic fertiliser was applied and the amount of crop residues strongly varied among crops. However, few differences in amoA among crops were found. This may be due to the high clay content of soil which could have

contributed in reducing the effect of both crop and tillage on organic matter mineralization and ammonia oxidation, as also observed elsewhere (Alluvione et al., 2013). The present result strongly agree with the differences in ammonia emission among crops, which strongly correlated to the total ammonium-N fertilised applied in the crop, as also found by others (Sommer et al., 2004) and did not vary according to the tillage system. Other experiments found that ammonia emission of NT soil can be higher than ploughed soils (Rochette et al., 2009a) and this is mostly due to the lack of incorporation of residues and fertiliser in NT soils (Rochette et al., 2009b). However, in the present study, the N fertiliser was not incorporated in the soil in either CT or NT and this explain the absence of difference in ammonia emission. With regard to the effect of the different crop management on ammonia emission, according with Black et al. (1985), NH_3 losses increased with the increased rate of nitrogen fertilizer applied.

Total N_2O emission during the cropping season in the field experiment ranged from 250 to 450 mg N_2O m^{-2}. In contrast to the ammonia emission, avoiding tillage (i.e. the application of the NT comparing to CT) increased N_2O emission. Moreover, minor differences between of the crop species and sequence were observed in NT and no differences between them in CT. This result agree with Bayer et al. (2015) where the application of no tillage system has highlighted the differences between crop sequences. Similarly, nosZ abundance was higher in NT than CT. The abundance of some soil bacterial gene region, including nosZ, was associated with total N_2O emission (Morales et al., 2010). Such bacteria were also indicated as main drivers of the N_2O emission and the composition of the bacterial population, especially nitrifying, strongly linked to land use rather than the above ground vegetation (Jangid et al., 2011). In the present experiment, I also found that both the crop species and sequence have minor impacts on total N_2O emission. However, the highest N_2O emission was lower than 3 kg N ha^{-1}. Such value, despite similar to that observed by other authors

(Oorts et al., 2007; Passianoto et al., 2003), could be due to the relatively good distribution of the rainfall, which may have contributed to the reduction of the total N emissions.

Role of plant residue addition on NO_2 and CO_2 emission and soil quality: the effect of residue type on two contrasting soils

In the present experiment, we evaluated the role of kind of plant residue incorporated into the soil on N_2O and CO_2 emission and soil quality of two contrasting soils, an Eutric Cambisol with low pH and high SOC and a Chromic Haploxerert with high pH and low SOC. Emissions and soil quality parameters varied according to both the kind of biomass added and the soil.

The total CO_2 (measured by PGA) and N_2O emission of the unamended Cambisol were 249% and 40% higher than the unamended Haploxerert, respectively. The differences of N_2O emission mirrored those of the TON, which was 62% higher in the Cambisol if compared to the Haploxerert. The differences in CO_2 emission between the two soils under study were dramatically higher than the differences in term of soil C content (the Cambisol showed only 78% more TOC and 100% more DOC than the Haploxerert). Such discrepancy suggests that the Cambisol has a relatively high respiration rate, which likely depends upon the higher SOC protecting ability of high-clay Haploxerert than the low-clay Cambisol (Baldock and Skjemstad, 2000; Krull et al., 2003; Lutzow et al., 2006; Six and Paustian, 2014), as also showed elsewhere (Alluvione et al., 2013). However, the difference of CO_2 emission between soils reduced when an organic residue (either faba bean or wheat) was added to the soil. In particular, the Cambisol emitted +88% and +152% more CO_2 than the Haploxerert when faba bean and wheat residues were added, respectively. And similar differences were found for N_2O emission between soils amended with organic residues.

Other studies showed that an increasing clay content (by means of artificial soils) consisted in an acceleration of the decomposition rate of the raw organic matter added and argued that the SOC protecting ability of clay is mainly addressed to the stabilised OM in the microaggragates (Six and Paustian, 2014; Velthof et al., 2002; Wei et al., 2014).

In our experiment, such effect mostly depended on the CO_2 emission rate during the first week of incubation. In particular, the addition of organic residues consisted in an increase of the emission rate in both soils during the whole experiment, as expected. However, the rate of such emission within the first week of incubation rapidly declined in the Cambisol whereas it sharply increased in the Haploxerert. After the first week of incubation, the CO_2 emission rate in all treatments declined and differences between soils or organic residues addition were constant until the end of the experiment. A similar trend of N_2O emission rate was found in the Cambisol, which however showed a longer transient effect (up to 25 days after the establishment of the experiment) with a milder decline in the emission rate than CO_2. Whereas no differences of N_2O emission rate among treatments were found in the Haploxerert and this is likely due to a high ammonia emission in the Haploxerert but not in the Cambisol. In the present work, we did not measure ammonia emission, but the lower ammonium concentration in the topsoil than the subsoil of the Haploxerert and its high pH suggest that an active loss of N as ammonia could have reduced the amount of N available for the mineralization process and its loss as nitrate (Mosier et al., 1998).

The transient effects of the CO_2 and N_2O emission rates likely depended on both the increased gas diffusivity due to the soil disturbance to establish the experiment and the rapid decomposition of the highly-labile organic fraction (either added or not) (Baggs et al., 2006; Magid et al., 1999). And indeed, we found that the reduction of the DOC in topsoil compared to the subsoil mirrored the amount of CO_2 emitted, especially in the low-C Haploxerert whose TOC was mostly influenced by the addition of the organic residues.

The soils under study showed very different total mineral N content (100 and 5 mg N kg^{-1} soil in the unamended Cambisol and Haploxerert, respectively) and ammonium:nitrate N concentration ratio (7.00 and 0.01, respectively). The incorporation of plant residues, either wheat or faba bean, consisted of contrasting effects on the ammonium and nitrate concentrations of each soil under study. In particular, addition of plant residues increased the ammonium content of the Haploxerert but not that of the Cambisol and such increases were more evident when wheat residues were added. At the same time, addition of plant residues reduced the total nitrate content of the Cambisol but not that of the Haploxerert, and such an effect was more evident when fababean residues were added. Such result points out to a net immobilization process in the subsoil of both the Haploxerert and the Cambisol.

The effect of the addition of the organic residues on soil mineral N concentration, especially nitrate, and CO_2 and N_2O emissions were more evident when wheat residues were added compared to faba bean residues. The mineralization rate of an organic residue added to the soil mostly depends on its C:N ratio and to a lesser extent to its lignin:N ratio and fibre content (Trinsoutrot et al., 2000; Vigil and Kissel, 1991). However, in the present study, the difference in the C:N ratio of the residues used (38.6 in faba bean and 40.7 in wheat) does not explain the difference in soil mineral N concentration and CO_2 and N_2O emissions between crop residues. Thus, it is more likely that mineralization rate of faba bean residues was lower than wheat residues due to a different lignin, acid detergent, and neutral detergent fibre content (+188.3%, +66.5%, +18.9%, respectively in faba bean comparing to wheat).

8. Conclusions

The present research aimed to study the effect of tillage system and crop system on soil microbial dynamics and biochemical process related to the the nitrogen emission in the Mediterranean environment.

The combination of field surveys and laboratory tests allowed to depict a clear picture of the treatments effects on soil microbiota dynamics and nitrogen emissions during the cropping cycle. The specifics analysis on denitrification enzyme activity, bacterial genes abundance and crop residue decomposition also permitted to obtain important information on key aspects of the biochemical processes of the soil and their importance to structure proper management techniques to preserve soil quality.

The information obtained from this research showed that the adoption of the no tillage can greatly impact the soil microbiota, improving its activity and determining a change on the community structure and functioning. However, despite several economic and agronomic benefits may arise from the application of no tillage system, both surveys in the field as well as laboratory analysis showed that the application of this technique can lead to an augmented risk of emissions of nitrous oxide.

With regards to the crop, this study showed how the crop type can directly affect some soil microbial group whereas the fertilization strategy can affect the ammonia emissions. Moreover, the interaction between tillage system and crop sequences indicated that a two-year crop sequence can lead to higher nitrous oxide emissions than wheat monoculture in no tilled soil characterized by higher soil microbial activity.

Finally, this study demonstrated the importance of long-term experiment in order to obtain important information on the biochemical processes of the soil due to the stabilization of the treatments effects.

9. References

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Weed seedbank size and composition in a long-term tillage and crop sequence experiment

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Summary

Knowledge of the effects of agricultural practices on weed seedbank dynamics is essential for predicting future problems in weed management. This article reports data relative to weed seedbank structure after 18 years of continuous application of conventional tillage (CT, based on mouldboard ploughing) or no tillage (NT) within three crop sequences (continuous wheat, W_W; wheat–faba bean, W_F; and wheat–berseem clover, W_B). Tillage system did not affect the size of the total weed seedbank, but altered both its composition and the distribution of seeds within the soil profile. In particular, the adoption of CT favoured some species (mainly *Polygonum aviculare*), whereas the continuous use of NT favoured other species (*Papaver rhoeas, Phalaris* spp. and *Lactuca serriola*). The effects of tillage system on weed seedbank size and composition were less pronounced in the W_B cropping system than in either the W_W or W_F. Compared with W_F and W_B, W_W resulted in an increase in total weed seedbank density (about 16 000 seedlings m^{-2} in W_W, compared with 10 000 and 6000 seedlings m^{-2} in W_F and W_B, respectively) and a reduction in weed diversity, with a strong increase in some species (e.g. *Polygonum aviculare*). Our results for the effect of NT application on weed seedbank size and composition suggest that farmers should only apply such a conservative technique within an appropriate crop sequence.

Keywords: no tillage, mouldboard ploughing, crop rotation, wheat, faba bean, berseem clover, mediterranean environment.

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Introduction

Although important advances in weed control technologies have been made in the past decades, weeds are still a major concern in agricultural systems, as they are able to cause significant losses in crop yield and quality. Weeds remain one of the most detrimental factors for crop performance, because weed flora continually change in response to new control measures (Sosnoskie *et al.*, 2006). Several studies have documented how weed flora respond to changes in agricultural practices (Staniforth & Wiese, 1985; Froud-Williams, 1988). Crop sequence and tillage system are two primary practices that affect weed population dynamics (Ball, 1992). Crop rotation is an effective method of controlling weeds; many studies have documented changes in both the weed seedbank and aboveground weed communities in response to varying crop

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sequences (Blackshaw *et al*., 2001; Légère *et al*., 2005). Crop rotation can increase weed diversity and can reduce weed density compared with monocultures (Liebman & Dyck, 1993), mainly because the selective pressure on weed flora exerted in monoculture systems can over time favour the build-up of species with a phenotype, phenology and physiology similar to that of the crop (Koocheki *et al*., 2009). The choice of crops and the sequence in which crops are grown can markedly influence weed community dynamics, because of their different biological cycles, end use, competitive ability against weeds, cultural management practices (fertilisation, seedbed preparation, etc.) and particularly weed control measures (the use of herbicides, mechanical operations, etc.).

Tillage can affect the weed community, causing a vertical redistribution of seeds through the soil profile and changes in soil characteristics (which in turn determine changes in soil habitability and, as a consequence, advantage or disadvantage different weed species) and dictating weed control management strategies [e.g. presowing use of non-selective, broad-spectrum, systemic herbicides under no tillage (NT)]. Hence, it is not surprising that several studies have shown strong variations in the size and composition of the weed seedbank in response to changing tillage systems (Dorado *et al*., 1999; Sosnoskie *et al*., 2006). In general, both the abundance and diversity of soil weed seeds increase with decreasing tillage. In particular, Tørresen *et al*. (2003) have observed an increase in annual grasses, perennial weeds and wind-dispersed species under NT. Such floral changes under reduced tillage (RT) or NT have been interpreted by some authors as steps in an ecological succession (Swanton *et al*., 1993; Zanin *et al*., 1997). However, other research has shown no alteration of the weed community in response to the application of conservative tillage techniques (Plaza *et al*., 2011).

The composition and abundance of weed species in crop fields can be affected by interactions between tillage and rotation (Murphy *et al*., 2006), although many observations have been contradictory. Doucet *et al*. (1999) reported that weed density was affected more by the tillage system than the cropping system. Along these same lines, Bàrberi and Lo Cascio (2001) concluded that the tillage system influenced weed seedbank structure to a much greater extent than did crop rotation. While Brainard *et al*. (2008) stated that the impact of a particular crop sequence is often less important than the management practices used in that sequence. However, Ball (1992) reported that cropping sequence was the most dominant factor influencing species composition in the weed seedbank.

Most studies reporting changes in the size and composition of weed seedbank as a consequence of variation in the agronomic practices have been based on short- or mid-term experiments (<10 years). However, the effects on weed communities of changing the tillage system and the crop rotation materialise only with time, and they can require several years to stabilise (Dick & Daniel, 1987). The aim of this study was to evaluate the long-term effect of tillage system, crop sequence and their interaction on the weed seedbank. Data were collected in an experiment set-up in 1991 to determine the influence of the continuous use of conservation tillage techniques (RT and NT) on the performance of crops in cereal–legume rotation systems typical of the semi-arid Mediterranean environment. In particular, we compared the size, composition and diversity of the weed seedbank between conventional tillage (CT, based on mouldboard ploughing) and NT within three crop sequences: continuous wheat (W_W), wheat–faba bean (W_F) and wheat–berseem clover (W_B) seed crop. Understanding how agricultural practices can affect the weed community over the long term is essential for planning control strategies able to reduce the use of chemical herbicides by favouring, at the same time, the development of a weed community with little impact on the agroecosystem.

Materials and methods

Site characteristics, experimental design and management

A long-term field experiment was started in the 1991–1992 growing season at Pietranera farm, which is located about 30 km north of Agrigento, Sicily, Italy (37°30′N, 13°31′E; 178 m a.s.l.), on a deep, well-structured soil classified as a Chromic Haploxerert (Vertisol). Soil characteristics (measured at the beginning of the experiment and referring to the 0–0.40 m layer) were as follows: 52.5% clay, 21.6% silt, 25.9% sand, pH 8.1 (1:2.5 H_2O), 1.40% total C (Walkley Black), 1.29 g kg^{-1} total N (Kjeldahl), 36 mg kg^{-1} available P (Olsen), 340 mg kg^{-1} K_2O (exchangeable potassium), cation exchange capacity 35 $cmol_+$ kg^{-1}, 0.38 cm^3 cm^{-3} water content at field capacity (pF 2.5) and 0.16 cm^3 cm^{-3} permanent wilting point (pF 4.5). The climate of the experimental site is semi-arid Mediterranean, with a mean annual rainfall of 552 mm, mostly in autumn/winter (74%) and in spring (18%). The dry period is from May to September. The mean air temperature is 15.9°C in autumn, 9.8°C in winter, 16.5°C in spring and 24.7°C in summer.

The experiment was set up as a strip-plot design with two replications. Three soil tillage systems (CT, RT and NT) acted as vertical treatments and three crop sequences (W_W, W_F and W_B) as horizontal

treatments. The seedbank study was performed only on samples collected in NT and CT plots. CT consisted of one mouldboard ploughing to a depth of 0.30 m in the summer, followed by one or two shallow harrowing operations before planting. NT consisted of sowing by direct drilling. The plot size was 370 m^2 (18.5 × 20.0 m). Each year, both rotations (W_F and W_B) were duplicated in reverse order so as to obtain data annually from all crops. In NT plots, weeds were controlled before planting with glyphosate [*N*-(phosphonomethyl) glycine] at a dose of 533–1066 g a.e. per ha, depending on the development of weeds.

The planting date of all crops varied by year, but was always in December. Wheat and faba bean were always harvested in June, whereas berseem clover was always harvested in the first half of July. During the wheat growing season, a mixture of post-emergence herbicides was applied to all plots to control both monocotyledons and dicotyledons (varying the active ingredients during the experimental period); on the whole, the herbicides applied controlled weeds efficaciously. During the faba bean growing season, weeds were controlled mechanically by shallow hoeing (with minimum soil disturbance) when the faba bean plants were at the third-leaf stage; if necessary, the operation was repeated at the seventh-leaf stage. During the berseem clover growing season, weeds were controlled by cutting all plants (berseem clover and weeds) to a stubble height of *c*. 8 cm when the berseem clover had basal shoots *c*. 5 cm long. More details on how the trial was performed are reported in Amato *et al.* (2013a) and in Table S1.

Seedbank sampling and analysis

Sampling of the weed seedbank was carried out in August, at the end of two growing seasons, 2007–2008 and 2008–2009 (the 17 and 18th years, respectively, after the beginning the experiment). Each year, sampling was carried out only in the plots where wheat was grown; this means that for W_W, sampling was done each year in the same plot, whereas for W_F and W_B, sampling was done in both plots used for the rotations.

In both years, eight soil cores of 30 cm depth were randomly taken in the central part of each plot using a 5 cm diameter manual steel probe. Considering the high abundance of actual weed flora in the plots (observed visually during the previous growing seasons) and based on Dessaint *et al.* (1996), who reported that the intensity of sampling can be reduced when weed density is high, this number of samples was considered sufficient to adequately represent the weed seedbank variability within each plot. Soon after the sampling, each core was subdivided into three subcores corresponding to 0–5, 5–15 and 15–30 cm soil layers. Separately for each layer, the eight subcores were pooled four by four to obtain two composite samples from each plot. Soil samples were kept in a dark room at 4°C until processing. A total of 144 (3 crop sequences × 2 tillage systems × 3 depths × 2 years × 2 subcores × 2 replications) soil samples were used for the weed seedbank study. The study was made using the seedling emergence method (Bàrberi & Lo Cascio, 2001) by placing each soil sample in a tray (30 × 20 × 5 cm) over a 2 cm thick layer of coarse sand that had previously been sterilised in an oven at 105°C for 72 h. A dense mesh was placed in between soil and sand to avoid mixing and to facilitate periodic soil stirring. Trays were placed in a cold glasshouse (i.e. at ambient temperature) for 12 months starting at the end of November of each year and were watered regularly by sprinkler irrigation. To favour dormancy breakdown, irrigation was suspended after 6 months for a period of 15 days, after which soil samples were stirred. Weed seedlings that emerged were identified and counted by species at regular time intervals and then removed.

Calculations and data analysis

Species richness (i.e. the total number of species observed), Shannon's diversity index (H_{SH}) and Shannon's evenness index (E_{SH}) were compared across tillage and rotation treatments. H_{SH} was calculated as follows:

$$H_{SH} = -\sum_{i=1}^{S} P_i(\ln P_i), \quad (1)$$

where

$$P_i = \frac{N_i}{N_{total}}, \quad (2)$$

where N_i is the number of individuals of species *i*, N_{total} is the total number of individuals per soil sample and S is the total number of species found. Subsequently E_{SH} was calculated using the following equation:

$$E_{SH} = \frac{H_{SH}}{\ln S} \quad (3)$$

Data from each year were analysed separately for each soil layer, and the homogeneity of variances was assessed using Bartlett's test before combined analyses were performed. Data can be considered as coming from a split strip-plot design, with time (random) as a whole plot and tillage system (vertical) and crop sequence (horizontal) as a strip plot with two replicates. According to Schabenberger and Pierce (2002), the linear model to analyse such data contains four

different experimental error sources of variability associated with the plot, the columns, the rows and their intersection. In Table S2, sources of variability and degrees of freedom for a single soil layer are reported. Analysis was carried out in the R environment (R Development Core Team, 2011). Moreover, a canonical discriminant analysis (CDA) was performed (SAS Institute, 2008) using data on the 15 primary weeds detected, to establish the importance of each weed species in discriminating among the six cropping systems (combinations of the two tillage systems and the three crop sequences). Canonical variable means (centroid values) were calculated for each tillage system/crop sequence combination, and the significance between means was determined using Mahalanobis distance.

Results

Ecophysiological and biological groups and relative density in the total seedbank of all weeds are shown in Table S3. A total of 46 species were detected during the study, 72% of which were therophytes (annuals), 19% hemicryptophytes (biennials and perennials) and 9% geophytes (perennials). Tillage system significantly affected the number of weed species in different ways, depending on crop sequence (Table 1). For instance, in the upper soil layer (0–5 cm), the number of weed species detected was higher in NT than in CT, with differences between these two tillage systems higher under W_W and W_B than W_F. The opposite was true in the lower soil layer sampled (15–30 cm). In each layer (0–5, 5–15, 15–30 and 0–30 cm), total weed seedling density (expressed as number of seedlings per square metre) differed significantly among the three crop sequences in the order $W_W > W_F > W_B$ (Table 2). No variation was observed due to tillage system in the whole layer sampled (0–30 cm); however, in both the upper and the intermediate layers, weed seedling density was significantly higher in NT than CT, whereas in the lower layer, the opposite was true. The differences between CT and NT in both the upper and the lower layers were higher in W_W than in W_F or W_B (the crop sequence × tillage system interactions were significant at $P < 0.01$).

Total weed seedling density by biological group is given in Table 3. In all treatments, the most numerous group were the therophytes (91.6%, on average). Crop sequence significantly affected total weed seedling density in the therophytes group, with values higher in W_W than W_F or W_B (in the order $W_W > W_F > W_B$). The densities of hemicryptophytes varied by tillage system (higher in CT than in NT, on average), whereas significant interactions were found between tillage system and crop sequence for the densities of both biennials and geophytes (higher in NT than in CT under W_W and W_B, but not under W_F for biennials, and higher in NT than in CT only under W_F for geophytes). No effect of tillage system was found for therophytes.

The seedling density of the major weed species (Table 4) together accounted for almost 90% of the total weed seedlings, regardless of the treatment

Table 1 Number of weed species detected in each soil layer

	W_W		W_F		W_B		*P*-value		
Soil layer (cm)	CT	NT	CT	NT	CT	NT	CS	TS	CS × TS
0–5	15.5	18.0	17.5	18.0	15.0	17.5	0.866	0.101	0.045
5–15	13.5	13.5	13.0	12.0	12.5	13.0	0.528	0.187	0.009
15–30	16.5	12.0	16.5	9.0	14.0	11.5	0.251	0.074	0.035
0–30	25.0	22.5	27.5	22.5	22.0	23.0	0.479	0.660	0.004

CT, conventional tillage; NT, no tillage; W_W, continuous wheat; W_F, wheat–faba bean; and W_B, wheat–berseem clover; CS, crop sequence; TS, tillage system.

Table 2 Weed seedling density (number of seedlings per square metre) detected in each soil layer in the six cropping systems

	W_W		W_F		W_B		*P*-value		
Soil layer (cm)	CT	NT	CT	NT	CT	NT	CS	TS	CS × TS
0–5	1616	7562	1591	4687	861	2221	<0.001	<0.001	0.002
5–15	3147	4943	2565	4466	1178	2633	0.002	0.001	0.473
15–30	11 078	3680	4537	2429	2827	2189	<0.001	0.028	0.002
0–30	15 840	16 185	8692	11 582	4866	7043	<0.001	0.179	0.360

CT, conventional tillage; NT, no tillage; W_W, continuous wheat; W_F, wheat–faba bean; and W_B, wheat–berseem clover; CS, crop sequence; TS, tillage system.

Table 3 Total weed seedling density (number of seedlings per square metre) by biological group detected in the six cropping systems. Data refer to the 0–30 cm layer

	W_W		W_F		W_B		*P*-value		
	CT	NT	CT	NT	CT	NT	CS	TS	CS × TS
Therophytes	15 230	15 292	7596	10 188	4232	6253	<0.001	0.224	0.361
Biennial species	130	555	274	390	83	489	0.845	0.020	0.022
Hemicryptophytes	286	120	565	49	335	169	0.354	0.046	0.094
Geophytes	195	218	257	955	217	132	0.039	0.028	0.004

CT, conventional tillage; NT, no tillage; W_W, continuous wheat; W_F, wheat–faba bean; and W_B, wheat–berseem clover; CS, crop sequence; TS, tillage system.

applied. For most of these species, the effect of tillage system on the total seedling density significantly varied with varying crop sequence. For instance, the seedling density of *Polygonum aviculare* L. was higher in CT than in NT, with differences between these two tillage systems higher under W_W than W_F or W_B. The opposite was true for *Papaver rhoeas* L. The total seedling densities of *Lactuca serriola* L. and *Lolium* spp. were higher in NT than in CT under W_B and W_W, but not under W_F. The total density of seedlings for *Anagallis arvensis* L. was significantly affected by crop sequence (in the order $W_W > W_F > W_B$) and tillage system (NT > CT). Both H_{SH} and E_{SH} were significantly affected by crop sequence, being lower in W_W than in W_F or W_B (Fig. 1), whereas no effect of tillage system was found on these indices.

The CDA based on data on seedling density of the major weed species clearly discriminated the six cropping systems (Fig. 2). The first two canonical variables accounted for about 72% of the total variance, which can be considered adequate for a bidimensional representation. CAN1 accounted for 37.0% of the total variance and was positively influenced by *P. rhoeas* and *A. arvensis* and, to a small extent, *Ecballium elaterium* (L.) A. Rich., *Phalaris* spp., and *L. serriola*. CAN2 explained 35.2% of the variance; the weed species with the greatest influence were *P. aviculare*, *A. arvensis* (both positive) and *E. elaterium* and *Sinapis arvensis* L. (both negative). CAN1 separated NT-W_W and NT-W_F cropping systems from CT-W_B and NT-W_B, while CAN2 separated CT-W_W from CT-W_F.

Discussion

Tillage system and crop sequence interacted to determine weed species richness, but the differences among treatments were, on the whole, rather small. It is noteworthy that after 18 years, the continuous use of NT in different crop rotations did not result in substantial changes in weed seed diversity. Regarding this,

Table 4 Total seedling density (number of seedlings per square metre) for the 15 primary weed species detected in the six cropping systems. Data refer to the 0–30 cm layer

	W_W		W_F		W_B		*P*-value		
Species	CT	NT	CT	NT	CT	NT	CS	TS	CS × TS
Anagallis arvensis	3929	5958	662	2889	357	2402	<0.001	0.001	0.945
Chenopodium vulvaria	266	49	799	217	14	78	0.054	0.018	0.058
Diplotaxis tenuifolia	204	120	62	49	315	148	0.053	0.386	0.544
Ecballium elaterium	152	121	139	917	120	84	0.013	0.004	0.003
Lactuca serriola	109	519	262	292	77	465	0.870	0.018	0.004
Lolium spp.	60	695	25	24	133	317	0.011	0.011	0.003
Papaver rhoeas	1686	5910	3295	5666	249	791	0.003	0.004	0.018
Phalaris spp.	124	621	140	237	244	75	0.018	0.110	<0.001
Polygonum aviculare	7687	542	832	133	2357	99	<0.001	0.004	<0.001
Portulaca oleracea	0	20	14	36	0	673	0.015	0.055	0.013
Ridolfia segetum	124	111	207	27	12	46	0.150	0.184	0.034
Sinapis arvensis	12	99	432	15	104	15	0.129	0.143	0.016
Sonchus asper	201	211	303	159	135	170	0.691	0.217	0.106
Stellaria media	353	221	14	72	63	92	0.227	0.505	0.584
Veronica hederifolia	171	324	27	162	0	940	0.018	0.019	0.034

CT, conventional tillage; NT, no tillage; W_W, continuous wheat; W_F, wheat–faba bean; and W_B, wheat–berseem clover; CS, crop sequence; TS, tillage system.

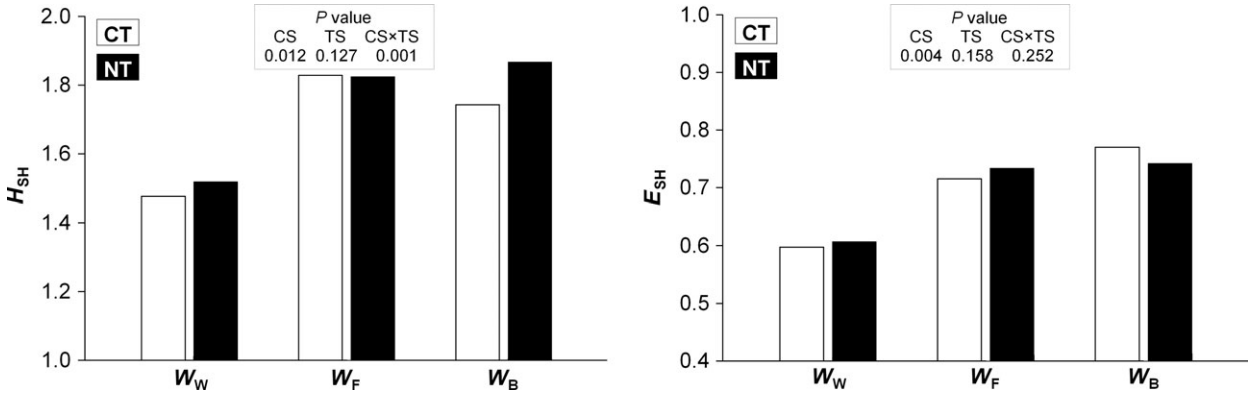

Fig. 1 Shannon's diversity index (H_{SH}) and Shannon's evenness index (E_{SH}) in the six cropping systems (conventional tillage, CT; no tillage, NT; continuous wheat, W_W; wheat–faba bean, W_F; and wheat–berseem clover, W_B). Data refer to the 0–30 cm layer.

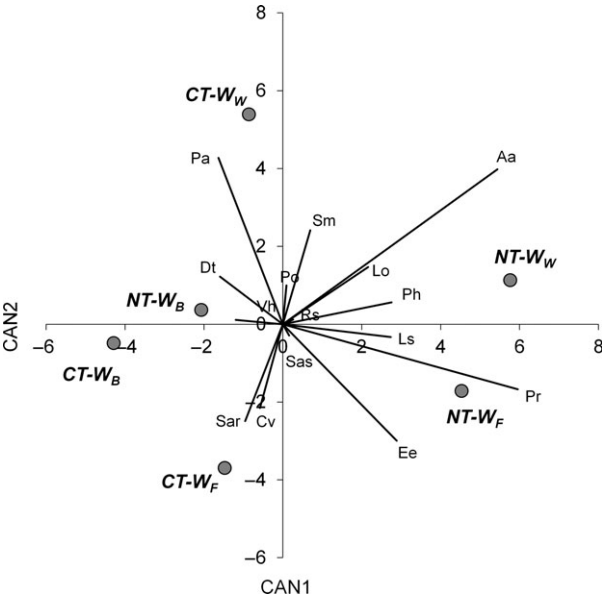

Fig. 2 Canonical discriminant analysis ordination biplot of the six cropping systems centroids. CAN 1, first canonical variable; CAN 2, second canonical variable. CT, conventional tillage; NT, no tillage; W_W, continuous wheat; W_F, wheat–faba bean; and W_B, wheat–berseem clover. The direction and length of each line indicate the degree of association between each weed species and cropping system. Only the 15 primary weeds are displayed. Pr, *Papaver rhoeas;* Aa, *Anagallis arvensis;* Pa, *Polygonum aviculare;* Vh, *Veronica hederifolia;* Ls, *Lactuca serriola;* Ph, *Phalaris* spp.; Cv, *Chenopodium vulvaria;* Ee, *Ecballium elaterium;* Lo, *Lolium* spp.; Sas, *Sonchus asper;* Dt, *Diplotaxis tenuifolia;* Sm, *Stellaria media;* Po, *Portulaca oleracea;* Sar, *Sinapis arvensis;* Rs, *Ridolfia segetum*. Comparisons of Mahalanobis squared distances showed highly significant differences ($P < 0.01$) in the compositions of weed communities among all cropping systems.

contradictory results can be found in the literature. For instance, Dorado *et al*. (1999) and Sosnoskie *et al*. (2006) observed greater weed species richness in crop rotations compared with monocultures and when the tillage intensity decreased, whereas Fried *et al*. (2008) found a higher number of weed species in deeply tilled fields compared with those in which NT or minimum tillage were applied. After 12 years of applying four tillage systems in two crop rotations, Bàrberi and Lo Cascio (2001) found that the number of weed species in the total seedbank did not substantially vary among treatments. They argued that although management practices can exert a considerable effect on the emergence and growth of weed species, they can have little or no effect on the reserve of biodiversity in the soil; this is mainly because the seed longevity of many weed species can serve as a buffer against environmental variability to reduce the risk of extinction. The discrepancy between the reported results shows that the impact of different soil tillage techniques and crop sequences on weed species richness is likely to be highly site specific; this is not surprising given the intrinsic variability in climatic conditions, soil characteristics, management practices, agronomic history and duration of the experiments.

Both diversity indices (H_{SH} and E_{SH}) were significantly affected by crop sequence, being higher in the two crop rotations than in the wheat monoculture. This result is in agreement with the findings of Légère *et al*. (2005) and Sosnoskie *et al*. (2006). The crop sequences together with their associated cultural practices (sowing time, weed management strategies, fertilisation, etc.) modified the physical, chemical and biological conditions of the soil, which may have differentially influenced the emergence, growth and capacity of species to produce seeds, thus modifying their relative abundance. Tillage system did not influence either H_{SH} or E_{SH}. This result is in line with the findings of Légère *et al*. (2005), who observed that tillage had little effect on weed diversity indices, but played an important role in determining the composition of the weed community.

Total weed seedling density was significantly influenced by crop sequence (in the order $W_W > W_F > W_B$).

The introduction of berseem clover into the crop sequence resulted in a dramatic reduction in potential weed infestation; this result can be explained by the fact that the seeds of berseem clover develop after a spring cut, before dissemination of weeds occurs. The spring cut, together with the excellent regrowth ability of berseem clover (Giambalvo *et al.*, 2011; Amato *et al.*, 2013b), greatly limits weed seed production. The reduction in total weed seed density detected in W_F compared with W_W is difficult to explain. In fact, compared with wheat, faba bean has a sparser canopy (as a result of a greater row spacing) and a slower growth rate in the early stages of the crop cycle, both characteristics that favour the emergence and growth of weeds (Frenda *et al.*, 2013); moreover, during the faba bean growing season, weeds were controlled mechanically by shallow hoeing, which did not always guarantee an optimal result (Giambalvo *et al.*, 2012). Therefore, it seems that the negative aspects were widely counteracted by the positive effects of disturbance.

According to many authors (Dorado *et al.*, 1999; Blackshaw *et al.*, 2001; Sosnoskie *et al.*, 2006), crop sequence markedly influences the weed seedbank by creating environmental conditions that differentially affect species emergence, development and dissemination. Some species, such as *P. aviculare* and *A. arvensis*, were markedly more abundant in W_W than in W_F or W_B, whereas the seedling density of *P. rhoeas* was lower in W_B than in W_W or W_F. According to Menalled *et al.* (2001), crop sequence effects cannot be distinguished from associated cultural practices or, in particular, from the effects of weed control strategies that, in our study, differed widely in relation to the crop species.

Tillage system did not affect total weed seedling density, but markedly influenced the distribution of weed seeds through the soil profile. According to Ball (1992), NT left a greater proportion of seeds near the soil surface (particularly evident in W_W), whereas in CT, weed seeds were more or less uniformly distributed through the tillage layer. It should be noted that, in spite of 18 years of continuous use of NT, the seedling density in the lower layer (15–30 cm) of NT plots was particularly high (on average, 2766 m^{-2}); it is likely that cracks and fissures in the soil when it dries out (during summer) can cause the movement of weed seeds down the soil profile.

The continuous use of NT led to an increase in eight species (*P. rhoeas, A. arvensis, V. hederifolia, L. serriola, Phalaris* spp., *Lolium* spp., *E. elaterium* and *P. oleracea*), even if, for most of these species, the magnitude of the differences between the two tillage systems was markedly affected by crop sequence. Other authors have found a progressive increase in most of these species due to a reduction in tillage intensity (Dorado *et al.*, 1999; Bàrberi & Lo Cascio, 2001). From an agronomic point of view, the increased abundance of these species (e.g. *L. serriola*) represents a serious weed management concern for NT cropping systems. For many species, particularly for *P. rhoeas*, the superiority of NT over CT (in terms of seedling density) was less under W_B compared with both W_W and W_F. This result is probably attributable to the weed control strategy adopted during the berseem clover growing season (i.e. the spring cut of the vegetation), which drastically reduced the probability of seed dissemination for many weed species, thus masking the effect of tillage system in the W_B crop sequence. The severe reduction in seedling density of many weed species in W_B probably led to a release of ecological niches that were then occupied by other species. This may have been true for both *P. oleracea* and *V. hederifolia,* whose density increased only in the NT–W_B system. This result can be explained considering the prostrate or semi-prostrate growth habit of these two species and the late spring emergence of *P. oleracea*; both characteristics probably allowed plants to avoid the cut made during the berseem clover growing season, thus increasing the probability of seed dissemination.

In our experiment, *P. aviculare* showed a preference for mouldboard ploughed soil, in agreement with data from Dorado and López-Fando (2006). For this species, germination is markedly affected by both fluctuations in soil moisture conditions experienced by seed during dormancy and sensitivity to light (Batlla *et al.*, 2007), factors that are modified by tillage system. Ghersa and Martínez-Ghersa (2000) reported that the CT system tends to generate a more persistent weed seedbank compared with NT, as a consequence of the different vertical distribution through the soil profile and the different level of exposure to predation. In our study, only some weeds with persistent seeds were more abundant in CT than NT (*P. aviculare*, *C. vulvaria*), whereas others were more abundant in NT system (*P. rhoeas*, *V. hederifolia*). Probably, in these cases, the effects of tillage system could have been masked by other agronomic factors (i.e. different crop sequences and the associated weed control strategies).

The CDA allowed us to discriminate among the different cropping systems, highlighting how the interaction of the treatments applied (tillage system and crop sequence) affected the weed seedbank in both quantitative and qualitative terms. The presence of berseem clover in the crop rotation markedly influenced the composition of weed community, masking, at the same time, the effects of tillage system (so that the two CT-W_B and NT-W_B systems were plotted very close to each other). In contrast, NT exerted great pressure on

weed communities in both the W_W and W_F cropping systems, which were plotted near each other and distant from all other treatments, whereas crop sequence (W_W vs. W_F) differentiated weed community composition only under CT. Some authors (Swanton *et al*., 1993; Zanin *et al*., 1997) have offered an ecological interpretation of weed flora dynamics under different tillage systems. In particular, Zanin *et al*. (1997) reported that a reduction in the mechanical disturbance of soil can result in marked changes to flora, with a tendency for weeds to undergo succession towards a more mature community, with an increased importance of biennial, hemicryptophytes and geophytes species. Other studies have highlighted the fact that a reduction in soil disturbance generally results in an increase in the occurrence of perennial weeds in many arable cropping systems (Buhler *et al*., 1994; Stevenson *et al*., 1998). Although biennial weed species were generally favoured in NT systems in our study, the data did not allow us to clearly demonstrate the existence of a gradient reflecting ecological community succession.

In conclusion, this weed seedbank analysis performed within a long-term field experiment provided useful information about the effects of some agronomic practices on weed population dynamics in wheat-based Mediterranean cropping systems. Although studies on weed seedbanks do not provide information on above-ground weed, they are fundamental to understanding and predicting the evolution of weed communities, as the seedbank reflects the history of the field. Our results suggest that crop rotation and tillage technique both act as filters that often interact with each other in determining the composition and abundance of weed species in the soil seedbank. In particular, compared with crop rotations (wheat–faba bean and particularly wheat–berseem clover), the continuous monoculture of wheat resulted in an increase in total weed seedbank density and, at the same time, a reduction in weed diversity, with a marked increase in some species, some of which are potentially hard to control. In contrast, tillage system had no effect on the size of the weed seedbank, but significantly modified its composition, as well as the distribution of weed seeds down the soil profile. Indeed, the adoption of a CT technique (based on mouldboard ploughing) favoured some weed species (mainly *P. aviculare*); in contrast, the continuous use of NT led to an increase in weed seeds in the upper soil layer and a significant increase in the seed density of some problematic species, such as *P. rhoeas, Phalaris* spp., and *L. serriola*. In any case, the effects of tillage system on weed seedbank size and composition were enhanced in both the wheat–faba bean cropping system and the continuous monoculture of wheat, but weakened in the wheat–berseem clover cropping system. From a practical point of view, these results suggest that, although NT is environmentally friendly because it mitigates soil erosion, reduces energy use and enhances wildlife habitat, farmers should only apply such a soil conservation technique within an appropriate crop sequence.

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Supporting Information

Additional Supporting Information may be found in the online version of this article:

Table S1 Details on the management of the crops in the experiment.

Table S2 Sources of variation and degrees of freedom for a single soil layer.

Table S3 Weed populations in the six cropping systems (CT, conventional tillage; NT, no tillage; WW, continuous wheat; WF, wheat–faba bean; and WB, wheat–berseem clover) classified into biological group and ecophysiological group. Data refer to the 2 years of the experiment.

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Valuing long-term sites and experiments for agriculture and ecology

Microbial biomass carbon dynamics in a long-term tillage and crop rotation experiment under semiarid Mediterranean conditions

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Summary

Microbial biomass carbon (MBC) of soil is an important ecological indicator of nutrient cycling and soil fertility. In addition, it responds to the changes of soil fertility more rapidly than soil organic matter. The aim of the present work was to evaluate the effect of a long-term implementation of a conservative soil management strategy (No Tillage [NT]) compared to the inversion tillage (conventional tillage [CT]) on the soil MBC in a range of crops - continuous durum wheat (WW), wheat after fababean (FW) and faba bean after wheat (WF).

MBC of NT plots was higher than CT. In addition, the content of MBC varied with sampling time during the growing season; this variation did not show a consistent pattern with tillage techniques or the crop species. Further research is needed to elucidate the effects of tillage and crop on the MBC dynamics within the soil profile in the semi-arid Mediterranean environment.

Key words: Microbial biomass carbon, no tillage, crop rotation, Mediterranean environment

Introduction

Agricultural soils found in the semi-arid Mediterranean environment are characterised by low levels of soil organic matter due to limited C inputs resulting from agricultural systems with intensive tillage systems combined with long bare fallows and removal of crop residues for animal feed. Sustainable crop management strategies, including conservation tillage, crop residue return, and appropriate crop rotation could thus improve soil quality.

Soil microorganisms are the living fraction of the soil organic matter and source-sink of major plant nutrients (C, N, P, and S) (Laudicina *et al*., 2012*a*). Microbial biomass C (MBC), being more responsive over short periods than total organic C, is commonly used as indicator of soil quality and of changes in soil management (Laudicina *et al*., 2012*b*). The impact of management practices on the flow of carbon through soil is also largely mediated by soil microorganisms (van Groenigen *et al*., 2010). Crop rotations can change soil habitat by affecting nutrient status, depth of rooting C input from roots (rhizosphere products as root exudates, mucilage, sloughed cells, etc.), amount and quality of residue, aggregation/microbial habitat, and can stimulate soil microbial diversity and activity (Laudicina *et al*., 2012*a*). Tillage systems influence physical, chemical, biological properties and stratification and distribution of soil organic matter and nutrients (Mathew *et al*., 2012). No

tillage (NT) is a common conservation practice which can lead to a wide range of benefits such as reducing soil erosion, C emissions, cultivation costs and the conservation of soil moisture (Jordan *et al*., 2000). The surface accumulation of MBC under NT has been demonstrated in many studies (Minoshima *et al*., 2007; Helgason *et al*., 2009), although not observed in some reports (Drijber *et al*., 2000; Carpenter-Boggs *et al*., 2003). The underlying mechanisms driving MBC accumulation in NT are therefore linked to a wider range of factors than tillage treatment alone.

Studying seasonal fluctuations in MBC may provide a more complete perspective to understand the effect of the tillage system and crop rotation on soil microbial biomass. Temporal variations in MBC may be driven by environmental factors (e.g. temperature and moisture), crop type and growth (Debosz *et al*., 1999), and are directly linked to the residue decomposition, organic matter turnover and nutrient cycling (McGill *et al*., 1986).

The aim of the present work was to evaluate, in a semi-arid Mediterranean environment, the long-term effect (23 years) of tillage system and crop rotation on the dynamics of the whole MBC pool during a cropping season. At the same experimental site, Laudicina *et al*. (2014, 2015) have shown that tillage system and crop rotation affect SOM quality more than quantity and that crop rotation improves soil biochemical properties more than tillage management. However, such studies were carried out only on the 0–15 cm soil layer and on the active microbial biomass fraction (i.e. MBC was investigated by glucose-induced respiration).

Materials and Methods

Experimental site

The trial was conducted under rain-fed conditions at the Pietranera farm, which is located about 30 km north of Agrigento, Sicily, Italy (37°30′ N, 13°31′ E; 178 m a.s.l.), on a deep, well-structured soil classified as Chromic Haploxerert, with a slope of about 7%. Soil characteristics (measured at the beginning of the experiment (0–40 cm layer) were 525 g kg^{-1} clay, 216 g kg^{-1} silt, 259 g kg^{-1} sand, pH 8.1 (1:2.5 H_2O), 14 g kg^{-1} total organic C, 1.29 g kg^{-1} total N, 36 mg kg^{-1} available P, 35 cmol kg^{-1} cation exchange capacity, 0.38 cm^3 cm^{-3} water content at field capacity (matric potential = -0.01 MPa), and 0.16 cm^3 cm^{-3} at the permanent wilting point (matric potential = -1.5 MPa). Based on the climatic data gathered from a weather station located within 500 m of the experimental site (34 years of observations), the climate is semi-arid Mediterranean, with a mean annual rainfall of 572 mm, concentrated mostly during the autumn–winter period (September–February; 76%), and spring (March–May; 19%). A dry period occurs from May to September. Mean air temperatures are 15.9°C in fall, 9.7°C in winter, and 16.5°C in spring.

Experimental design and crop management

The long-term field experiment was established in autumn 1991 as a strip-plot design with two replications. A detailed description of the experiment is given by Giambalvo *et al*. (2012) and Amato *et al*. (2013). In this study, the experimental factors tested were tillage (conventional tillage and no tillage) and crop (WW, continuous wheat; WF, faba bean after wheat; FW, wheat after faba bean). Conventional tillage (CT) consisted of one mouldboard ploughing to a depth of 30 cm in the summer, followed by one or two shallow harrowing (0–15 cm) operations before planting. No tillage (NT) consisted of sowing by direct drilling. Plot size was 370 m^2 (18.5 m × 20.0 m). In NT plots, weeds were controlled before planting with glyphosate at a dose of 533 to 1066 g a.e. ha^{-1}, depending on the development of weeds. Every year, WW and FW plots were broadcast fertilized with 69 kg ha^{-1} of P_2O_5 before planting. Nitrogen fertilizer was broadcast on the soil surface at 120 kg N ha^{-1} in WW plots and 80 kg N ha^{-1} in FW plots. These fertilizer rates credited 40 kg N ha^{-1} from the previous grain legumes, a value similar to that observed by Giambalvo *et al*. (2004). The total amount of N fertilizer was split with 50% applied immediately before planting (as diammonium phosphate and urea) and 50% applied at mid-tillering (end of March; during this experiment, it was

before the 2nd soil sampling) as ammonium nitrate. WF plots were broadcast fertilized with 46 kg ha^{-1} P_2O_5 before planting and received no N fertilizer. Crop planting was always in December using a no-till seed drill with hoe openers under both CT and NT, making the appropriate sowing depth adjustments to ensure a homogeneous planting depth (3–5 cm). Faba bean *cv*. Gemini was sown at 40 viable seeds m^{-2} with an inter-row spacing of 75 cm. No rhizobial inocula were applied before planting because soil has a native rhizobial population. Durum wheat was planted in rows spaced 16 cm apart at 350 viable seeds m^{-2}. In WW and FW plots, weeds were controlled by applying post-emergence herbicides (by varying the active ingredient during the experimental period) at the early growth stage of the crop. In WF plots, weeds were controlled mechanically by shallow hoeing (with minimum soil disturbance) when plants were at the third-leaf stage; if necessary, the operation was repeated at the seventh-leaf stage. No treatment against disease or pests has being applied since 1992. Faba bean were harvested in late June or beginning of July each year, leaving standing straw and uniformly spreading crop residues. Wheat was harvested also in late June or beginning of July each year and stubble (about 20–25 cm from the soil surface) was left standing. Wheat straw was baled and removed from the field. The soil surface covered by mulch in the NT treatments was always >30%.

Soil sampling and analysis

During the cropping season 2013/2014, two soil samples (each composed of three mixed subsamples) per plot were collected separately from the 0–15 cm and 15–30 cm soil layers in December 2013 (before sowing), April 2014 (wheat heading), and July 2014 (wheat harvest). Soil samples were air-dried and then gently sieved to pass through a 4 mm mesh sieve. Visible pieces of crop residues and roots were removed by hand before sieving and then samples were stored in plastic bags at room temperature until analysis.

MBC was determined by the fumigation-extraction method (Vance *et al*., 1987). Moist (50% WHC) soil aliquots (equivalent to 25 g oven-dry soil) were fumigated with alcohol-free chloroform in vacuum desiccators for 24 h in the dark. After removing the chloroform by vacuum extraction, soil samples were extracted with 0.5 M K_2SO_4 (4 K_2SO_4:1 g soil, *v/w*) for 45 min on a horizontal shaker (70 rpm). Unfumigated soil samples were similarly extracted and used as a control. All soil extracts were filtered through Whatman 42 paper and analysed for organic C by acid dichromate oxidation. MBC was estimated as the difference between the organic C from fumigated and unfumigated samples multiplied by a k_{EC} of 2.64. Data reported are the arithmetic means of four samples and are expressed on an oven-dry basis (105°C) of soil. Before performing parametric statistical analyses, normal distribution and variance homogeneity of the data were checked by Kolmogorov–Smirnoff goodness-of-fit and Levene's tests, respectively. Two-way ANOVA with repeated measures was performed with tillage (CT, conventional tillage and NT, no tillage) and crop (WW, continuous wheat; FW wheat after faba-bean and WF, faba bean after wheat) as factors. Fisher values (F) were used to individuate the different degree of variance in MBC explained by single or interacting experimental factors. Statistical analyses were carried out with SAS statistical package.

Results

In the 0–15 cm layer, MBC ranged from 346 to 756 mg kg^{-1} in NT plots and from 186 to 472 mg kg^{-1} in CT ones. In the 15–30 cm layer, MBC was not affected by tillage. In both the 0–15 and 15–30 cm layers, MBC showed lower values in WF plots compared to WW and FW ones. The content of MBC in the 0–15 cm layer was strongly affected by both tillage (P<0.001) and crop (P<0.001), whereas, in the 15–30 cm layer, the effect of tillage on MBC varied significantly by crop (tillage × crop significant at P<0.001) (Table 1).

In 0–15 cm layer of CT plots, MBC increased slightly during the cropping season, whereas in NT plots, fluctuations in the content of MBC were observed during the cropping season (Fig. 1).

Moreover, MBC increased slightly with time in WW whereas fluctuations were observed during the cropping season in both FW and WF. MBC did not show significant differences among sampling occasions in the 15–30 cm layer (Table 1 and Fig. 2).

Table 1. *Fisher (F) and P values of microbial biomass C (MBC) determined on soil samples taken at 0–15 cm and 15–30 cm depth in December 2013, April 2014 and July 2014 calculated by two-way analysis of variance (ANOVA) with repeated measures*

Experimental factors	0–15 cm		15–30 cm	
	Test of Between-Subjects Effects			
	F	*P value*	*F*	*P value*
Tillage	94.0	**0.002***	3.3	0.166
Crop	19.3	**0.002**	37.6	**<0.001**
Tillage * Crop	0.8	0.502	25.8	**0.001**
	Tests of Within-Subjects Contrasts			
time	3.9	**0.050**	2.2	0.159
time * Tillage	12.0	**0.008**	0.7	0.527
time * Crop	5.1	**0.013**	0.4	0.800
time * Tillage * Crop	1.3	0.331	0.6	0.658
Greenhouse-Geisser	0.945		0.866	
Huynh-Feldt-Lecoutre	1.365		1.186	

Experimental factors were tillage system (conventional tillage, CT; no tillage, NT) and crop rotation (continuous wheat, WW and wheat-faba bean rotation, WF and FW) across time. Greenhouse-Geisser and Huynh-Feldt-Lecoutre correction factors for the effect of time and interaction between time and other treatments are provided. Values in bold are significant at $P \leq 0.05$

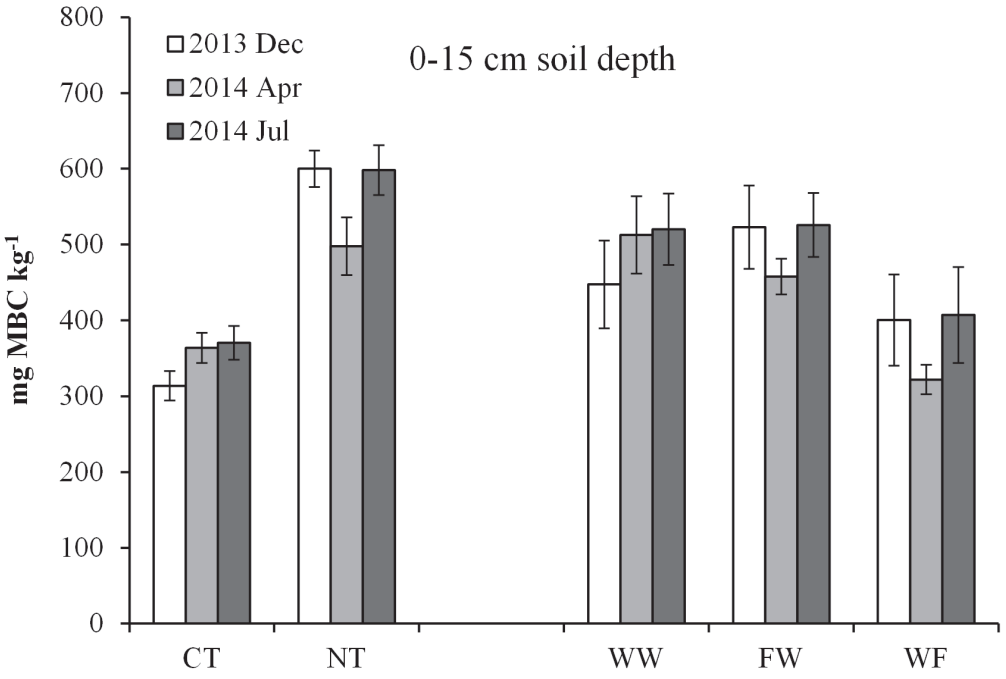

Fig. 1. Microbial biomass carbon (MBC) in the 0–15 cm soil layers collected in December 2013 (before sowing), April 2014 (wheat heading) and July 2014 (wheat harvest).

Data reported are means ± standard errors. Experimental factors were: tillage system (conventional tillage, CT, and no tillage, NT) and crop (WW, continuous wheat; FW wheat after faba-bean; WF, faba bean after wheat) across time. No interaction between treatments was observed.

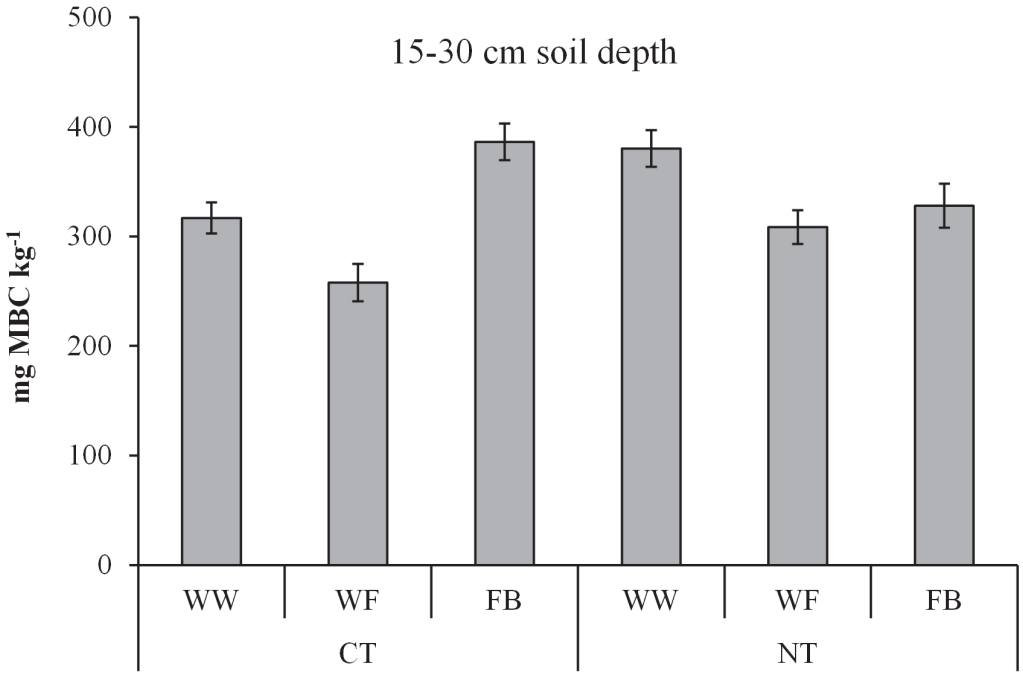

Fig. 2. Microbial biomass carbon (MBC) in the 15–30 cm soil layers. Data reported are means ± standard errors of three samplings collected in December 2013 (before sowing), April 2014 (wheat heading) and July 2014 (wheat harvest).

There was no effect of sampling occasion. Experimental factors were: tillage system (conventional tillage, CT, and no tillage, NT) and crop (WW, continuous wheat; FW wheat after faba-bean; WF, faba bean after wheat).

Discussion

MBC measured in the 0–15 cm layer was slightly higher than that previously determined by substrate induced respiration (Laudicina *et al*., 2014, 2015). Such a difference is not unexpected given the difference in the method used to determine MBC (Dilly, 2006). MBC of NT plots was higher than CT; a similar result to Mathew *et al*. (2012) and Laudicina *et al*. (2014). Since microbes depend on available soil organic C for energy, and given that in NT plots, plant residues from the previous cropping season were not removed, this is likely to increase C availability for microbes. Adoption of NT systems can promote the formation of C-enriched microaggregates within macroaggregates (Six *et al*., 2000) due to a range of factors including reduced activity of mineralizer bacteria and oxygen permeability in the microaggregates (Laudicina *et al*., 2012*b*). Luo *et al*. (2010) also showed that NT adoption can change the distribution of the organic carbon along the soil profile and that its effect on total soil organic carbon depends on the crop rotation and span from no effect under continuous cropping to a positive effect under rotation. We found higher MBC in the WW and FW plots compared to WF in the 0–15 cm layer which may results from differences in the applied agronomic practices (including fertilization, weed management, etc.), root distribution and amount and composition of root exudates between wheat and faba bean.

In the 0–15 cm layer, the content of MBC varied with time (interactions time × tillage and time × crop both significant at $P<0.05$) and no univocal trend could be inferred from the data. The topsoil is more exposed both to the effects of tillage management and crop residues input, as well as the climatic factors (above all temperature and rainfall) and hence we would expect higher in-season variability.

In conclusion, in the present experiment, carried out under semiarid Mediterranean conditions, both tillage system and crop strongly influenced the content of MBC, particularly in the topsoil.

Finally, seasonal variations in the MBC content, although sometimes significant, did not mask the effects of the applied treatments. Further research is needed to elucidate the effects of tillage and crop on the MBC dynamics within the soil profile in the semi-arid Mediterranean environment.

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