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DENITRIFIER AND NITRIFIER ACTIVITIES AND N_2O EMISSIONS OF FINE AND COARSE TEXTURED SOILS OF A MEDITERRANEAN IRRIGATED CROPLAND IN SOUTHERN ITALY.

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EXTENDED ABSTRACT

Nitrous oxide (N_2O) is a climate relevant trace gas, moreover involved in the depletion of stratospheric ozone. Although in the last decades the increased N-input and the large use of irrigation, have greatly increased N_2O emissions from croplands (actually contributing about 50 % of the global anthropogenic N_2O emissions), only scanty data about N_2O fluxes are available up to the present from irrigated croplands of Mediterranean countries, despite the extention of these cropped surface areas, and this is limiting to provide the necessary information in order to validate models predicting N_2O fluxes at a global scale.

It's well known that denitrification and nitrification are the main natural sources of this trace gas and, if N-fertilizers are not used efficiently, great loss of nitrogen can occur via both these processes. Moreover recent studies pointed out the importance to take into account biological parameters such as denitrifier and nitrifier activities in order to develop more reliable N_2O fluxes models.

In this study nitrous oxide emissions, denitrifying and nitrifying activities and their different contribution to N_2O production, were measured in an irrigated cropland in Campania Region (South Italy), with the aim to determine how the changing environmental climate conditions and the agriculture management practices can affect soil bacterial processes and the amount of N_2O evolved by, under Mediterannean climate conditions.

The experimental site, contributing to the FLUXNET network, is the agricultural field of a buffalo zootechnic farm, characterized by an alluvial soil with both clay (relating to most of the cropped surface) and sandy profile inside the same field. Dairy farms are a typical component of the overall regional agricultural section and show a relevant potential for N losses via soil denitrifying and nitrifying activities, since they produce a great amount of organic waste, generally applied as fertilizer N to the cropped soil, and largely relay on irrigation practice to grow fodder plants for

animal consumption.

Both monitoring activities and a manipulation experiment were carried out in the agricultural field.

As far as concern the monitoring activities, measurements of denitrification rate (AIT on intact soil cores) and N_2O fluxes from soil (Static manual chambers) were carried out for the clay soil through the course of the *Lolium italicum* crop (Sep ’04 - Apr ’05) and the *Zea mays* growths both in 2005 and 2006 (May ’05 - Aug ’05). Similar analyses were performed at the sandy site during the winter grass cultivation and the maize crop in 2005, to investigate possible differences between fine and coarse textured soils. At that time also measurements of net nitrification rate (Buried-bag method by intact soil cores) were carried out for both profiles, moreover at the sandy site the relative nitrifier and denitrifier contributions to N_2O fluxes from soil were investigated as well (Short exposure to acetylene method adapted for intact soil cores).

By the manipulation experiment, the effects of different amounts of urea N fertilizer (higher urea N supply **N+** and lower urea N supply **N-** than the rest of the field **C**) on denitrifying activity and N_2O emission from the fine textured soil were tested at the late fertilization time during the maize crop in 2005, in restricted plots inside the agricultural field where determinations of nitrogen metabolism of maize plants were carried out as well (Arena, pers.comm.; Parisi et al., 2006).

The monitoring study showed considerable denitrifying activities (up to about 1500 μg N_2O- N m^{-2} h^{-1}) and N_2O fluxes from soil (up to 570 μg N_2O- N m^{-2} h^{-1}) in the course of the maize cropping cycles, soon after irrigation events following fertilizer N applications, clearly as a result of the combined enhancing effects of high soil temperatures and not limiting soil nitrates and WFPS’s.

Anyway different patterns between clay and sandy soils were noticed according to their different physico-chemical characteristics.

At the clay sites, characterized by higher soil NO_3^- concentrations, organic matter content and WFPS’s, denitrification activities showed the highest values and appeared a foundamental process determining N_2O emissions from soil, as suggested by the significant correlation found between actual denitrification rate and the amount of N_2O evolved from this kind of fine textured soil.

In the coarse textured soil, with lower NO_3^- concentrations, organic matter content and WFPS’s, nitrification activities and related N_2O emissions appeared to be promoted, as suggested by the significant correlation found between N_2O fluxes and the relative nitrifier contribution to the overall amount of N_2O evolved from soil ($N_2O_{nit\%}$).

Beyond confirming for the clay soil the close relations of denitrification rate and N_2O fluxes between each other and with both soil NO_3^- concentration and WFPS, the manipulation experiment pointed out that even in the less fertilized treatment **N-**, at least up to 1 month after the fertilizer N application, soil NO_3^- concentrations were probably enough high to cause no competition between microbial community and plant system for N-mineral source demand, evidently leading to marked N-losses by denitrification (up to about 1500 µg N_2O- N m^{-2} h^{-1}) every time soil moisture promoted the process through that period.

The idea of N-surpluses at the experimental site was supported by the results coming from the investigation of nitrogen metabolism of plants, since all parameters analized didn't exhibit significance differences among **C**, **N-** and **N+** treatments on all sampling dates, suggesting that the different nitrogen fertilizations did not influence at relevant extent maize performance in the field (Arena, pers.comm; Parisi et al., 2006). Moreover it appears in agreement with the findings of a recent emergetic analyses of the zootechnic farm, showing that the system greatly relay on non-stop external inputs of not renewable resources, among which fertilizers N are the main contributing factors (Alfieri, 2005).

Finally, according to the higher NO_3^- concentrations detected, pronounced N_2O fluxes were measured from the soil of the **N+** treatment (about 100 µg N_2O- N m^{-2} h^{-1}), right to the very end of the maize growing season, pointing out that relevant surplus N may cause high N-losses from the system, also enhancing the risk of nitrate leaching through September rains.

Correlation and regression analyses on the whole set of data relating to the fine textured soil in the course of the maize cropping cycles in 2005 and 2006 (from both monitoring activities and the manipulation experiment) pointed out that actual denitrification rate may be a good predictor parameter to develope reliable empirical models and/or a useful tool to parameterise and calibrate exhisting process models in other to achieve more appropriate estimations of N_2O at a local scale.

In fact actual denitrification rate could be effectively predicted by considering its dependence on soil characteristics such as nitrate concentration and WFPS (according to simple Michaelis-Menten kinetic and exponential functions respectively) and appeared in its turn a good predictor parameter for estimating N_2O emissions indirectly, without flux measurement.

N_2O fluxes showed indeed a marked exponential relationship with denitrification rate and simple preticting functions for emission estimates derived also considering their dependence on actual denitrification rate appeared to be more fitting than predicting equations based only on direct measurement of soil nitrates and WFPS's.

Of course the idea of using actual denitrification rate as a predictor parameter for indirect emission estimation need to be supported by further investigations. For istance as far as concern this study there is evidence that the predictive power of actual denitrification rate in the clay soil analysed may drop under accentuated dry conditions (WFPS<40%), when nitrifying activity and related N_2O emissions may be promoted, thus leading to possible underestimation of total emissions from soil.

AKNOWLEDGEMENTS

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Finally, but first of all, I wish to thank my husband Claudio for being a continuous source of inspiration and for his "non-stop input" of psychological and scientific support.

1 INTRODUCTION

1.1 THE ENVIRONMENTAL ISSUE OF N_2O EMISSIONS FROM AGROECOSYSTEMS

It has been estimated that the current greenhouse effect may lead to a 2 °C global temperature rise, with a corresponding warming of 1-3 °C in the Mediterranean region (IPCC, 2001 a; WWF Report, 2005).

This warming, characterized by extremely hot days especially during summer period in inland and southern Mediterranean locations, might on overall reduce precipitations (with longer drought periods in the Southern regions and more intense and strong rains at certain locations in the northern Mediterranean), increase the risk of forest fire (above all in the southern Mediterranean) and reduce crop yield (IPCC, 2001 a; WWF Report, 2005).

Moreover as a result of climate change and reduction in precipitation, it is expected a decrease of surface runoff and water yields, with consequent relevant detrimental effects on the distributions and abundances of plant and animal species (IPCC, 2001 a; WWF Report, 2005).

On this very subject, the current increase of the atmospheric concentration of N_2O, 0.25% per year, i.e. 0.8 ppb yr^{-1} (IPCC, 2001 b), appears an environmental issue of great concern.

In fact N_2O is a powerful greenhouse gas, characterized by a warming potential 200 times as big as CO_2 and responsible for 5% of the total greenhouse effect; moreover it has been shown that it reacts with oxygen radicals in the stratosphere to form nitrogen monoxide, involved in the destruction of stratospheric ozone protecting the earth from biologically harmfull ultraviolet radiation from the sun (Johnston, 1972; Crutzen, 1981).

The raise of atmospheric N_2O concentration is the result of the huge increase of anthropogenic inputs to ecosystems of reactive nitrogen (Nr), that according to Galloway's definition (Galloway, 2004) includes "inorganic reduced forms of N (e.g., NH_3, NH_4^+), inorganic oxidized forms (e.g., NO_x, HNO_3, N_2O, NO_3^-), and organic compounds (e.g., urea, amines, proteins, nucleic acids).

Nitrogen cycle (Fig.1-1) has been in fact dramatically altered in the course of the last century by human population, both at local and global scale, in consequence of the increased demand of nitrogen to grow food. As a matter of fact, the increase in atmospheric N_2O concentration can be primarily attributed to agriculture (contributing up to 80% to global anthropogenic N_2O emissions) as a result of the increased N input into agricultural soils, associated with changes in food production systems (Kroeze et al. 1999; Mosier et al., 2001; IFA and FAO, 2001).

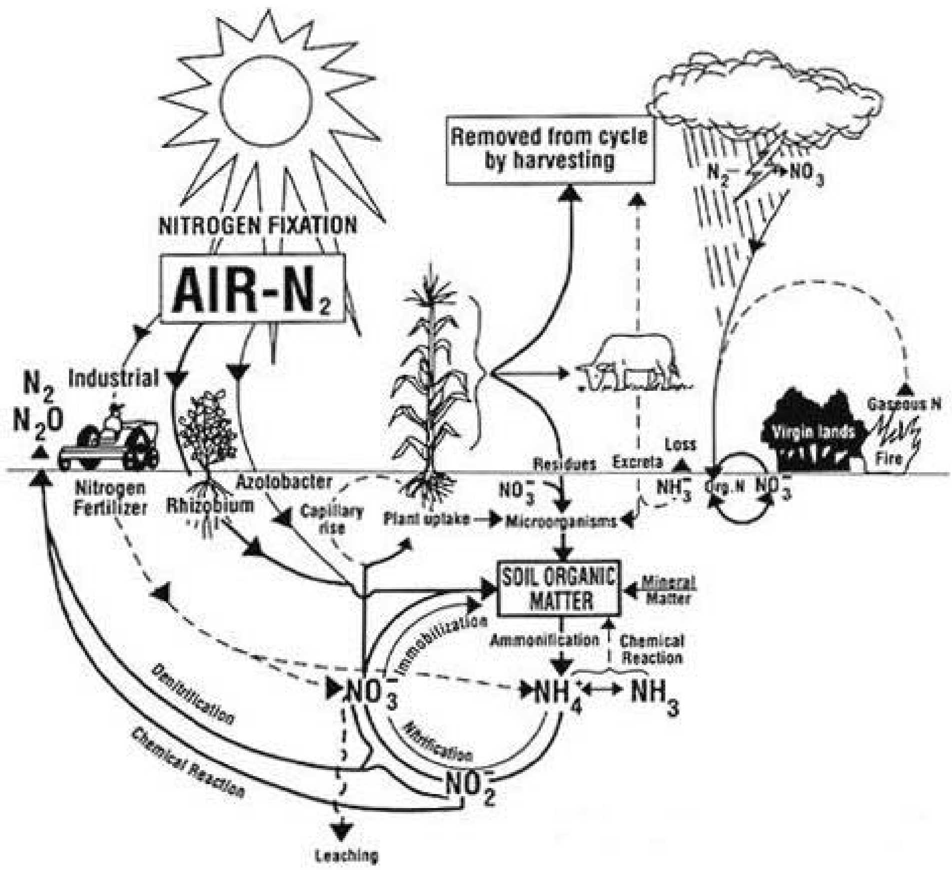


Figure 1-1: The nitrogen cycle in soil (Brown and Johnson, 1996).

As shown in table 1-1, from the latest review of this topic (Galloway et al., 2004), in pre-industrial time (1860) most of the Nr input to terrestrial ecosystems derived from biological nitrogen fixation (BNF) through N_2-fixing organisms and atmospheric deposition by lightning processes, while anthropogenic Nr creation gave only a slight contribution to the overall input and mostly by cultivation of N_2-fixing crops (BNF-cultivation).

However in the early 1990s, Nr creation by anthropogenic activities has increased over a factor of 10 compared to the late-19th century, from 15 Tg N yr^{-1} to 156 Tg N yr^{-1} (and it is expected that by 2050 anthropogenic Nr creation will be 270 N yr^{-1}), becoming the dominant force in the transformation of N_2 to Nr on continents and substantially changing Nr distribution via atmospheric and hydrologic pathways.

Table 1-1: Global creation and distribution of reactive nitrogen Nr (Tg N yr^{-1}) in 1860 and in the early 1990s; predictions for 2050 are shown as well. Modified from Galloway et al., 2004.

	1860	Early-1990s	2050
Nr creation			
Natural			
Lightning	5,4	5,4	5,4
BNF-terrestrial	120	107	98
BNF-marine	121	121	121
Subtotal	246	233	224
Anthropogenic			
Haber-Bosch	0	100	165
BNF-cultivation	15	31,5	50
Fossil fuel combustion	0,3	24,5	52,2
Subtotal	15	156	267
Total	262	389	492

It's noteworthy the main anthropogenic activity increasing Nr was the production of NH_3 from N_2 and H_2 by the Haber-Bosch process, mainly addressed to agroecosystems as mineral-N fertilizers.

Anyway marked anthropogenic Nr creations also occurred in consequence of the increased fossil fuel combustion (since nitrogen as NO is emitted to the atmosphere as a waste product from either the oxidation of atmospheric N_2 or organic N in the fuel) and both intensive and extensive cultivation of N_2-fixing monocultures. Moreover, even if not shown in Table 1-1, a significant contribution has derived from biomass burning as well (40 Tg N yr^{-1}), particularly concerning Mediterranean countries subject to recurring seasonal fires.

Much of the current anthropogenic Nr creation is dispersed to the environment, and besides other crucial consequences such as alteration of forest productivity, acidification of surface waters and coastal eutrophication, it is responsible for the marked increase of greenhouse potential of the atmosphere via N_2O production (Galloway et al. 2004).

Global N_2O emissions have in fact increased from 12 Tg Nyr^{-1} in 1860 to 15 Tg Nyr^{-1} in the early 1990s (Table 1-2), and the soil appears to be the main contributing factor, accounting for about 70% of the overall N_2O emitted annually from the biosphere into the atmosphere.

Table 1-2: Global atmospheric emissions of N_2O (Tg N yr^{-1}) in 1860 and in the early 1990s; predictions for 2050 are shown as well, but no forecast can be made for soil anthropogenic sources. Modified from Galloway et al., 2004

	1860	Early-1990s	2050	Notes
Soils				
Natural	6,6	6,6	6,6	1
Anthropogenic	1,4	3,2	3,2±?	2
Rivers				
Natural	0,05	0,05	0,05	3
Anthropogenic		1,05	3,22	4
Esturaries				
Natural	0,02	0,02	0,02	3
Anthropogenic		0,2	0,9	4
Shelves				
Natural	0,4	0,4	0,4	5
Anthropogenic		0,2	0,32	6
Ocean (natural)	3,5	3,5	3,5	7
Total	12	15,2	18,2±?	8

Moreover, as shown in table 1-2, agricultural soil contribution has greatly increased over the last decades and currently croplands are considered the most relevant terrestrial source on a global scale, contributing in their turn to about 70% of the overall N_2O emitted annually from terrestrial system, that is about 50% of the global anthropogenic N_2O and equivalent to a global warming potential of 1.0 Pg C yr^{-1} (Robertson, 2000).

Among N_2O soil forming processes, biological denitrification and nitrification are considered the principal responsible for the evolution of this gas to the atmosphere, anyway their relative importance can greatly vary depending on local circumstances (Smith and Arah, 1990; Bremner, 1997; Hopkins et al., 1997; Bateman and Baggs, 2005).

Furthermore, besides the environmental issue of N_2O evolution, a major concern is the implication of denitrifying activity in potential losses of Nr from agroecosystems via N_2 production.

In fact, it has been estimated that N_2 losses from agroecosystems via denitrification might be in the order of 10%–40% of anthropogenic Nr input received, and even if estimates are still affected by consistent uncertainties, this finding suggests denitrifying process may represent a permanent sink for a relevant part of Nr created by human action, influencing the amount of Nr storage in terrestrial reservoirs and therefore requiring further investigations to achieve a more detailed understanding of the N budget, both in agroecosystems and at a global scale (Galloway, 2004).

1.2 BIOLOGICAL SOURCES OF N_2O FROM SOIL

Up to the present works to identify sources of nitrous oxide in soils have pointed out that the most of N_2O evolved from soils is produced by biological denitrification and nitrification processes, while on the whole non biological sources such as chemical decomposition of nitrite (Bremner and Nelson, 1968; Nelson and Bremner, 1969, 1970; Nelson 1982; Blakmer and Cerrato, 1986), chemical decomposition of hydroxylamine (Arnold, 1954; Nomik, 1956; Bremner and Shaw, 1958; Alexander, 1977; Nelson, 1978) and reaction of nitrite with hydroxylamine (Arnold 1954; Bremner et al, 1980; Minami and Fukushi, 1986) appear to play a minor part (if any) in both natural and agricultural systems (Bremner, 1997).

As a matter of fact besides bacterial nitrification and denitrification, other biological sources including certain heterotrophic nitrifier fungi (Burth and Ottow, 1983; Killham; 1986; Kuenen and Robertson, 1988; Shoun et al., 1992; Castaldi, 1997), yeasts (Bleakley and Tiedje, 1982) and some non denitrifying nitrate-reducing bacteria (Anderson and Levine, 1986; Smith and Zimmerman, 1981) may be significant in contributing to N_2O production in soil of natural systems (Robertson and Tiedje, 1987). Anyway there’s no clear evidence of them as relevant factors in cultivated soils and they will not be discussed further in this study.

1.2.1 Denitrification

Denitrification can be defined as a respiratory bacterial reduction of nitrate and/or nitrite to gaseous NO, N_2O and N_2 (returned to the atmospheric pool), coupled to electron transport phoshorilation.

Many aerobic microorganisms, both Proteobacteria and Archea, can in fact use NO_3^- as electron acceptor to derive energy from organic compounds when oxygen tension is low (heterotrophic denitrification):

$$\mathbf{5(CH_2O) + 4NO_3^- + 4H^+ \rightarrow 5CO_2 + 7H_2O + 2N_2 + energy}$$

through the stepwise reduction of the intermediates nitrite, nitric oxide, and nitrous oxide, acting as terminal acceptors for electron transport phosphorylation through denitrification enzymes (Fig.1-2):

$$\mathbf{NO_3^- (+5) \rightarrow NO_2^- (+3) \rightarrow NO (+2) \rightarrow N_2O (+1) \rightarrow N_2 (0)}$$

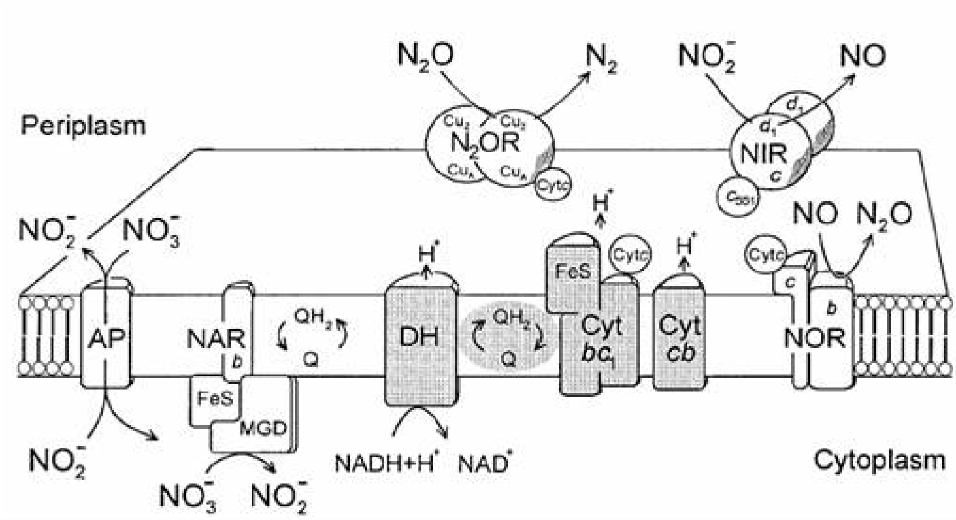


Figure 1-2: Schematic model for the bioenergetic apparatus of bacterial cell in deniftrifying bacteria. NADH dehydrogenase complex (DH), nitrate reductase (NAR), nitrite reductase (NIR), NO reductase (NOR), and N_2O reductase (N_2OR). Modified from Zumft, 1997.

Therefore nitrous oxide is an obligatory intermediate of heterotrophic bacterial denitrification, even if besides the regulating effect of soil physico-chemical parameters (see Section 1.3), the amount of N_2O reduced to N_2 by the labile enzyme nitrous oxide reductase can greatly vary in different microbial species, with some bacteria producing mostly N_2 and others giving various mixture of N_2O and N_2 or only N_2O, through incomplete reduction pathways (Kaplan and Wofsey, 1985; Stouthamer, 1988; Martin et al., 1988; Powlson et al., 1888; Schmidt et al., 1988; Munch, 1989,1991; Robertson and Kuenen, 1991; Zumft, 1997).

For instance Suharti and De Vries (Suharti and De Vries, 2005) found in B. azotoformans (Gram+ bacterium) a quite different profile of enzymes and electron-transfer pathways compared with Gram- bacteria and concluded that the study of both structure and biochemical properties of membrane proteins could improve the knowledge of the wide biological variation in electron-transfer routes and their regulation in denitrifier bacteria.

Besides heterotrophic denitrification, also some chemoautotrophic bacteria can produce N_2O by using NO_3^- as electron acceptor for oxidation of inorganic compounds such as S^{2-} and Fe^{2+} (Golterman, 1991). Anyway this kind of autotrophic denitrification usually occurs in specific location such as shallow water sediments and it's not as important as heterotrophic denitrification in determining N_2O emission from soil (Granli and Bockman, 1994).

1.2.1.1 Factors regulating the process

Key factors affecting anaerobic heterotrophic denitrification in soil are pH, temperature, nitrate and labile organic material availability and, of course, soil aeration (Firestone, 1980; Sahrawat and Keeney, 1986; Robertson, 1995; Bremner, 1997). Depending on the characteristic features of the soil analysed one or more than one among these soil parameters can act as limiting factors for denitrifier activity.

The optimum pH for denitrification is in the range of 7.0 to 8.0 (Bremner and Shaw 1958; Bryan, 1981) even if a recent study (Simek et al., 2004) showed that the optimum pH can greatly vary depending on the kind of laboratory incubation performed (short-term assay or long-term incubation for potential denitrification assessment, DEA and DP respectively) and suggested that more than a single optimum pH, bacterial populations in soil can exhibit different optimum pH ranges, depending on the specific soil characteristics they are adapted to.

When other soil parameters are not limiting, an inverse relationship exists between the rate of denitrification and soil oxygen concentration, with and exponential decay of denitrifier activity at increasing O_2 concentration (Focht, 1974; Smith, 1980; Betlach and Tiedje, 1981; Parkin and Tiedje, 1984; Burton and Beauchamp, 1985; Arah et al, 1991).

Moreover since, besides consumption by soil microorganisms and plant roots, the O_2 content in soil is deeply affected by soil water content, with water displaying air and regulating O_2 diffusion into soil micro and macropores, many studies showed a strong positive correlation between denitrification rate and soil WFPS (Benckiser et al, 1986; Mosier et al, 1986; Bakken et al., 1987; Mancino et al., 1988; Myrold, 1988; Malhi et al., 1990; Smith and Arah, 1990; Parson et al, 1991).

Soil volumetric water content is the predictor parameter employed in denitrification models to estimate the reduction of denitrifying activities at increasing O_2 supply rates (Heinen, 2006) and usually the dependence of denitrification on WFPS is described by a non linear steep curve such as a power reduction function (Grundmann and Rolston, 1987).

Peaks of denitrifying activity had been recorded after irrigation and/or rain events at not limiting values of soil temperature, nitrates and degradable organic material (Ryden et al., 1979; Rolston et al., 1982; Ryden et al., 1983; Aulak et al., 1983; Mosier at al. 1986; Jarvis et al., 1991; Arcara et al., 1999; Vallejo et al., 2001, 2004) and often the increase appeared more marked at WFPS above 60% (Terry et al, 1981; Linn and Doran, 1984; Aulak et al, 1984; Rolston et al., 1982; Grundman and Rolston, 1987; Arcara et al., 1999; Henault and Germon, 2000; Vallejo et al., 2001, 2004).

Anyway the relation between denitrification rate and soil water content appears to be at a certain extent a little be complex. For instance soil wetting-drying cycles can result in more marked denitrifying activities than those one detectable in soils kept at high constant water content (Mulvaley and Kurtz, 1984), while Groffman and Tiedje (Groffman and Tiedje, 1988), showed that, after wetting very dry soils, denitrification rate increased much more markedly than it decreased after drying the soils to the initial low water content.

Afterwards, when soil aeration state favours anaerobic denitrification and other factors are not limiting, a strong dependence of actual denitrification rate on NO_3^- concentration is evident. The relationship is described by Michaelis-Menten Kinetics:

$$\mathbf{V= (V_m \times S)/(S+K_m)}$$

where:

V= denitrification rate

V_m= maximum denitrification rate

S= soil nitrate concentration

K_m= half-saturation constant= soil NO_3^- concentration giving a denitrification rate of 50% of the maximum value

According to this kind of kinetics, usually employed by many N-cycling models to derive potential denitrification rate estimates (Heinen, 2006), when nitrate concentration is in the low-medium range, denitrification rate increases via a first order equation, while when nitrate concentration rises up to not limiting values the process approached a zero-order equation.

Both maximum rate and half-saturation constant can greatly vary depending on soil texture, physico-chemical characteristics and environmental conditions. For instance K_m values reported ranged from 4 mg Kg^{-1} (Klemedtsson et al., 1977) and 25 mg Kg^{-1} (Limner and Steele, 1982), up to 117-138 mg Kg^{-1} (Mahli et al., 1990).

Since nitrogen can be limiting in most terrestrial systems, a usual trend is that denitrification increases at raising nitrates concentrations (Ryden, 1983; Vinther, 1984; Robertson et al., 1987; Samson et al., 1990; Ambus and Lowrance, 1991), however in croplands, after N inputs such as chemical mineral and/or organic fertilization, manuring and incorporation of crops residues, denitrification can reach the plateau (Granli and Bochman, 1994).

Anyway, often (for instance in most mineral soil) the key factor limiting denitrification is the availability of organic material and many investigations indicated under soil anaerobic conditions denitrifying activity is strongly regulated by the amount of easily decomposable organic substances for reduction of nitrate (Burford and Bremner, 1975; Limner and Steele, 1982; Paul and Beuchamp, 1989; Malhi et al., 1990; McCarty and Bremner, 1992; Yeomans et al., 1992).

Finally very slight values of denitrification rate have been reported at temperature of -2°C (Dorland and Beuchamp, 1991) and -4°C (Malhi et al., 1990) and it is assumed values above 5°C are necessary for appreciable denitrifying activity (Aulak et al., 1983; Vinther, 1990).

Since usually biological processes increase exponentially with increasing temperature (up to a level after which a decrease is noticed), in model predicting denitrification rate from soil physico-chemical parameter, the dependence on soil temperature is often calculated according to the Van't Hoff or the Arrhenius laws.

1.2.2 Nitrification

According to the Soil Science Society of America nitrification can be defined as a "*biological oxidation of ammonium to nitrite and nitrate, or a biologically induced increase in the oxidation state of nitrogen*" and it is usually assumed that autotrophic bacteria are responsible for it in most soils, even if some studies suggested heterotrophic nitrifier microorganisms may contribute to nitrification and N_2O emissions related with, more than is commonly believed (Schimel et al., 1984; Tortoso and Huchinson, 1990; Williams et al., 1992; Anderson, 1993).

Lithotrophic nitrifiers are Gram- bacteria, conventionally placed in the family Nitrobacteriaceae (Buchanan, 1917; Watson, 1971; Watson et al., 1989), using the oxidation of inorganic nitrogen compounds as their major energy source:

$$\mathbf{NH_4^+ + 2O_2 \rightarrow NO_3^- + H_2O + energy}$$

Nitrification takes place in two separate steps (Haynes, 1986).

In the first step, ammonia oxidizing bacteria (characterized by the prefix Nitroso-: i.e. Nitrosomonas, Nitrosospira, Nitrosolobus and Nitrosovibrio) generate energy via ammonia oxidation to nitrite:

$$\mathbf{NH_4^+ + 1^1/_2O_2 \rightarrow NO_2^- + 2H^+ + H_2O + energy}$$

It is a two-stage passage where ammonia oxidation is initiated by the enzyme ammonia monooxygenase producing hydroxylamine (Rees and Nason, 1966; Dua et al., 1979; Hollocher et al., 1981; Wood, 1988; Hooper and Terry, 1973):

$$\mathbf{NH_3 + O_2 + 2H^+ + 2e^- \rightarrow NH_2OH + H_2O}$$

The intermediate hydroxylamine (NH_2OH) is the real energy source. In fact during its further oxidation to nitrite by hydroxylamine oxidoreductase (Olson and Hooper, 1983; Hooper et al., 1984; Hooper and DiSpirito, 1985), two of the four electrons derived are transferred back for AMO activity, while the other two are used for energy generation (Fig.1-3):

$$\mathbf{NH_2OH + H_2O \rightarrow HNO_2 + 4H^+ + 4e^-}$$

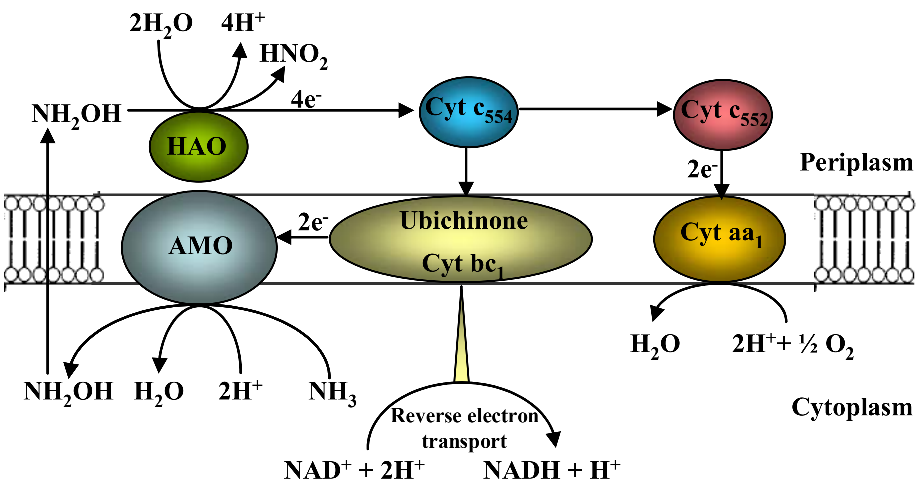


Figure 1-3: Schematic model for the bioenergetic apparatus of bacterial cell in lithoautothrophic ammonia-oxidizing bacteria. Ammonia monooxygenase (AMO), hydroxylamine oxidoreductase (HAO).

In the second step nitrite is oxidized by the enzyme nitrite-oxidoreductase (NO_2-OR; a complex of membrane-associated molybdenum and iron-sulfur enzymes) of nitrite-oxidizing bacteria (characterized by the prefix Nitro-: Nitrobacter, Nitrococcus and Nitrospina):

$$\mathbf{NO_2^- + H_2O \rightarrow NO_3^- + 2H^+ + 2e^-}$$

During oxidation of nitrite to nitrate, the additional oxygen atom of nitrate is derived from water (Aleem et al., 1965) and the two electrons released originate the electron flow through cytoplasmatic membrane for the ATP generating electron transport (Aleem, 1968; Aleem and Sewell, 1981; Tanaka et al., 1983) (Fig.1-4).

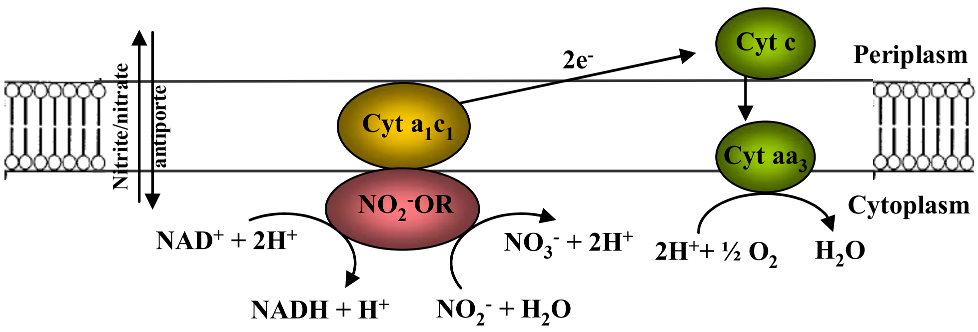


Figure 1-4: Schematic model of the bioenergetic apparatus of bacterial cell in lithoautothrophic nitrite-oxidizing bacteria: electrons from nitrite are transferred via a- type and c-type cytochromes to a cytochrome oxidase of the aa3-type. Nitrite-oxidoreductase (NO_2-OR).

N_2O is not an obligatory intermediate of nitrification process, anyway autothrophic bacteria can produce nitrous oxide by enzymatic reduction of nitrite, when O_2 supply is limiting in soil (nitrifier denitrification) (Fig.1-5).

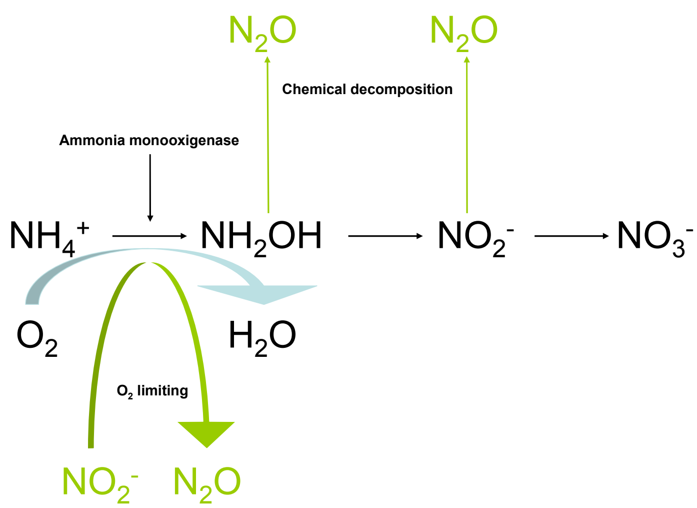


Figure 1-5: N_2O production via enzymatic reduction of nitrite used as electron acceptor under anaerobic conditions. Pathways for N_2O production via chemical decomposition are shown as well.

Autothrophic ammonium-oxidizing bacteria such as *Nitrosomonas europea* can use NO_2^- as an alternative electron acceptor under anaerobic conditions, reducing NO_2^- to N_2O by the enzyme nitrite reductase (Nomik 1956; Yoshida and Alexander, 1970; Ritchie and Nicholas, 1972; Poth and Focht, 1985; Fireston and Davidson, 1989; Groffman, 1991), preventing the accumulation of toxic level of nitrate as well (Shank et al., 1962; Mancinelli and McKay, 1983).

1.2.2.1 Factors regulating the process

Since in soil NH_4^+ oxidation proceeds more rapidly than recovering of NH_4^+ pool via mineralization (agricultural fertilized field may greatly diverge from this), the availability of NH_4^+ is assumed to be a main factor limiting nitrification in most soils (Shmidt, 1982; Haynes, 1986; Hutchinson and Davidson, 1993; Skiba and Smith, 2000).

Other key factors regulating nitrification in soil are pH, O_2 supply, water content, phosphate availability, allelopathic compounds and temperature (Haynes, 1986; Bremner et al., 1997).

Similarly to denitrification, autothrophic nitrification has its optimum pH close to neutral or slightly sub-alkalyne values (Fotch and Verstraete, 1977; Bock et al, 1986) and increase at raising soil temperature, with optimum temperature ranges depending on the specific climatic regions (Granli and Bockman, 1994).

Otherwise, since it is an aerobic process, a positive relationship with O_2 concentration was found in many investigations (Khdyer and Cho, 1983; Keeney et al., 1985) and both conceptual models (Skopp et al., 1990) and laboratory and field experiments (Goodroad and Keeney; 1984; Tietema, 1992; Franzluebbers, 1999) showed maximal denitrification rates at WFPS between 50% and 60%, when there was a compromise between favouring effects on NH_4^+ diffusion and limiting effects on O_2 supply.

Anyway the optimum WFPS for nitrifier activities appears not to be universal across different soil types, for instance many authors found the optimum for nitrification rates at both much higher WFPS's, up to 80%- 90% (Doran et al., 1990; Schjonning et al., 2003) and much lower WFPS's, down to 42% (Franzluebbers, 1999).

This wide range in predicted water content for maximum nitrification rate is probably the result of more than one factors.

A first critic topic is that some investigations have been conducted on undisturbed soil samples, while others (almost the majority) have been performed on homogenized and sieved soil samples, but soil exposed to such treatments can be quite different in soil structure from equivalent undisturbed field soils (Schjonning et al., 1999; 2002).

On this very subject Schjonning (Schjonning et al., 2003) carried out a study to examine the effect of the soil water regime on microbial activity in undisturbed soil cores and verify the validity of the conceptual model of Skopp (Skopp et al., 1990), where the maximum nitrification rate is calculated considering the WFPS value balancing limiting effects of substrate and O_2 diffusion inside soil. It's noteworthy he found that the parameter WFPS was not able to normalize soil type differences in water regime relevant to the rate of aerobic microbial activity in undisturbed soil cores and that the relative gas diffusivity was a better predictor of net nitrification than the soil air content. Therefore he suggested a more complex approach including soil type dependent expressions for solute and gas diffusivity (Olesen et al., 2001; Moldrup et al., 1999; 2001), when extrapolating aerobic microbial activity at a field scale, where diffusional constraints for gas and solute, deeply affected by the soil structure, might be not completely predictable by the use of soil WFPS.

1.3 FACTORS CONTROLLING N_2O PRODUCTION VIA HETEROTROPHIC DENITRIFICATION AND AUTOTROPHIC NITRIFICATION

Soil is a quite heterogeneous system, with aerobic and anaerobic microsites not homogeneously distributed along its profile, consequently nitrification and denitrification can occur simultaneously and N_2O can be evolved from soil via both these processes (Nielsen et al., 1996; Abbassi and Adams, 1998; 2000).

As illustrated by the "hole in the pipe" model of Davidson (Davidson, 1991), shown in Figure 1-6, besides being influenced by the rates of nitrification and denitrification (flow through the pipes: level I), N_2O emissions depend on how soil physico-chemical parameters affect the ratio of end-products via both the processes (the size of holes and orifices of the pipes: level II) and on how much fast, after being produced, N_2O gas can diffuse through gaseous phase of soil to the atmosphere (level III).

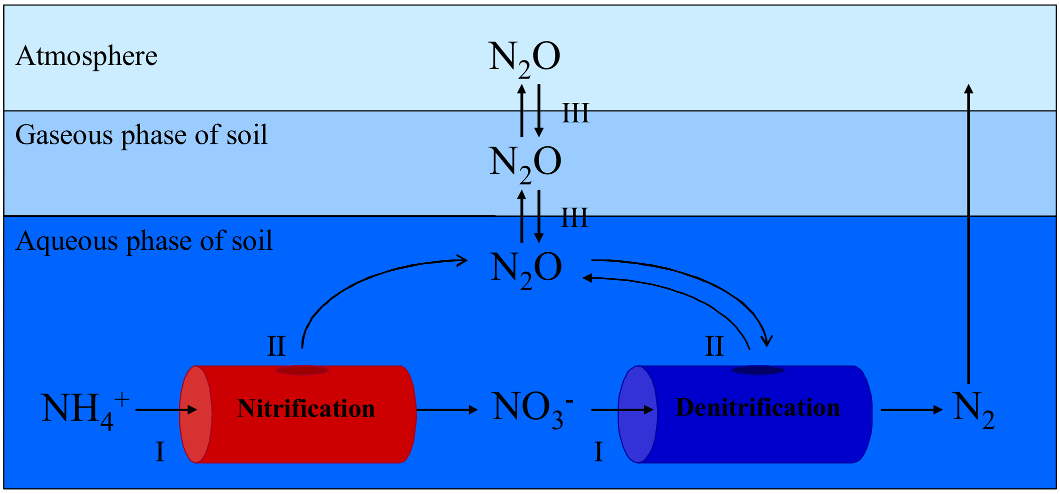


Figure 1-6: The three levels of regulation of N_2O fluxes from soil, according to the "hole in the pipe" model (Redrawn from Davidson, 1991). Further explanations inside the text.

Therefore the amount of N_2O produced and the balance between nitrification and denitrification as the prevailing process determining nitrous oxide emission is something difficult to predict, since regulated by many soil characteristics which can act simultaneously and on different levels, as explained in detail in the following sections.

1.3.1 Soil aeration state

N_2O emission dependence on soil aeration is quite complex since many factors can regulate at the same time gas diffusivity inside soil profile (see sections 1.3.2 and 1.3.7). Anyway many studies showed that the highest N_2O fluxes from soil can be detected when soil conditions are suitable for both nitrification and denitrification (Fotch, 1974; Kralova et al., 1992; Smith and Patrick, 1983), since for both processes N_2O is produced at intermediate O_2 availability (Khdyer and Cho, 1983).

As far as concern denitrification, no N_2O production occur at very high redox potentials in soil since denitrification is hindered, while at O_2 concentration not limiting the process, as O_2 inhibites nitrous oxide reductase at a greater extent than nitrate and nitrite reductases, a decrease of the

N_2O/N_2 ratio is noticed at decreasing oxygen supply rates (Fotch, 1974; Smirnov et al., 1979; Fireston et al., 1980; Smith et al., 1983; Tiedje, 1988; Masscheleyn et al., 1993). Anyway, since denitrification rate increases under developing anaerobic conditions (see section 1.3.1), on the whole N_2O emissions increase at decreasing soil aeration (Dowell and Smith, 1974), up to a level when no gas exchange is allowed between soil and air any more, and N_2 is the only final product of denitrification (Granli and Bockman, 1994).

Similarly, in laboratory incubations of soils supplied with NH_4^+ substrate, Keeney (Keeney et al., 1985) found increasing N_2O production via nitrification at decreasing O_2 concentrations, while no production at all was observed when soils were completely anaerobic.

1.3.2 Soil water content

Soil water content (expressed as water filled pore WFPS or air filled pore space AFPS) has numerous effects on N_2O emission from soil, since water is necessary for microbial activity, regulates soil aeration (with air porosity decreasing at increasing value of WFPS) and can act as a carrier for N_2O, also preventing its diffusion to the atmosphere.

Many authors recorded marked amount of N_2O dissolved in soil water, from 1-300 μg N_2O-N l^{-1} (Dowdell et al., 1979; Terry et al.,1981; Minami, 1987; Minami and Ohsawa, 1990), up to 500-10000 μg N_2O-N l^{-1} (Amudson and Davidson, 1990). After being dissolved in soil solution N_2O may be leached through percolating water in wet seasons or be gradually released to the atmosphere during drier periods.

Since oxygen gradient along the soil profile is strongly affected by the soil water content, a decrease of the N_2O/N_2 ratio via denitrification is noticed at increasing values of soil moisture (Murakami and Kumazawa, 1987; Rolston et al, 1978; Rolston et al., 1982; Aulak et al., 1984; Christensen, 1985; Weier et al., 1993). Anyway, as already pointed out in section 1.3.1., N_2O emission by denitrification are mostly determined by the rate of the process, so that the amount of N_2O evolved from soil usually increases with raising WFPS's, up to a level when water severely hinder gas exchange between soil and air and most of N_2O is reduced to N_2 (Granli and Bockman, 1994).

Differently the product ratio N_2O/NO_3^- of nitrification tend to increase with raising soil water content up a level soil aeration becomes restrictive for aerobic microbial activity (Goodroad and Keeney, 1984).

The relationship between soil WFPS and the amount of N_2O produced via both nitrification and denitrifiaction can be described by the widely accepted model of Davidson (Davidson, 1991) (Fig.1-7).

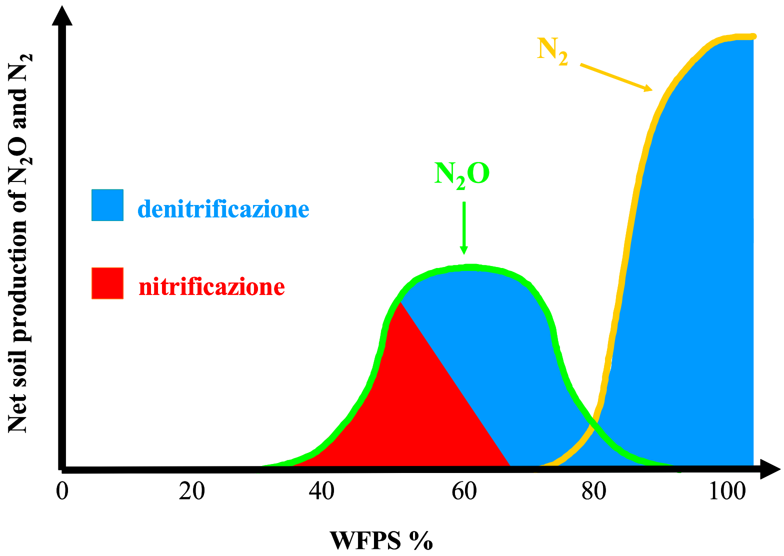


Figure 1-7: Model of net soil production of N_2O and N_2 via nitrification and denitrification in soil (redrawn from Davidson, 1991). Further explanations inside the text.

According to this model at very low values of soil water content no or slight N_2O emissions are detected from soil since microbial activity is low and nitrification converts quantitatively NH_4^+ to NO_3^- at optimum O_2 supply.

With raising water content, N_2O via nitrification increases and when O_2 diffusion decreases also N_2O production through denitrification becomes relevant, up to soil WFPS so high that nitrification is inhibited and denitrification is greatly promoted, producing mostly N_2.

Anyway the values of soil WFPS at which the maximum N_2O fluxes are detected, usually close to field capacity, can greatly vary in different soils, from 45% to 75% (Parton et al., 1988; Klemedtsson et al., 1988 b; Davidson, 1992) up to 85% (Klemedstsson et al., 1988; Hansen et al., 1993), and as already pointed out in section 1.2.2, it might be difficult comparing results coming from different kinds of soil sample handling and extrapolating findings from soil sieved samples to a field scale (Schjonning et al., 2002; 2003).

Moreover N_2O emissions appear to be much higher when dry and wet conditions alternate in soil as compared to prolonged wet periods, and peaks of N_2O fluxes have been often detected following the increase of soil water content after dry conditions in field studies over a wide range of soil types and water contents, (Terry et al., 1981; Mosier et al., 1981; Parton et al., 1988; Hansen et al., 1983; Skiba et al., 1992).

For the effects of irrigation practices on N_2O emission from soil in agricultural systems, see section 1.4.2.4.

1.3.3 Soil nitrogen availability

When soil water content in not limiting, usually a marked increase in N_2O emissions from soil is detected at raising soil mineral-N concentrations.

N_2O fluxes via denitrification increase since NO_3^- stimulates denitrification rate (see section 1.2.1) and enhance the N_2O/N_2 product ratio of the process (Nomik, 1956; Blakmer and Bremner, 1978; Bremner, 1978; Fireston et al., 1980; Vinther , 1984; Christensen, 1985; Ottow et al., 1985, Kroeze et al, 1989).

In fact NO_3^- inhibit or retards the activity of the enzyme nitrous oxide reductase (Blakmer and Bremner, 1978; Cho and Mills, 1979) even if it's not clear yet if this retardation is due to a real enzymatic inhibition or simply NO_3^- is preferred as an electron acceptor instead of N_2O during denitrification. Moreover besides the general assumption that NO_3^- concentration in the range of 10 to 30 mg NO_3^--N Kg^{-1} are sufficient to retard N_2O reduction, the required concentration can vary among soil types depending on the combined effects of other soil parameters, such as pH (Blakmer and Bremner, 1978; Cho and Mills, 1979), water content (Terry and Tate, 1980; Aulakh et al., 1984; Bowman, 1990).

Also N_2O production via nitrification increases when the substrate NH_4^+ increases, as shown by laboratory incubations performed on *Nitrosomonas europea* in liquid cultures and in soil (Yoshida and Alexander, 1970; Blakmer et al., 1980), anyway not so many studies were conducted on this topic compared to N_2O losses via denitrification.

See section 1.4.2.1. for the effects of application of mineral N fertilizers on N_2O emission from soil in agricultural systems.

1.3.4 Soil pH

Soil pH seems to affect N_2O production in soil not in a simple manner, depending on which one between nitrification and denitrification is the prevailing process occurring in soil (Granli and Bockman, 1994).

Since nitrous oxide reductase in much more sensitive to acidic condition than nitrate reductase, many studies showed a decrease of the N_2O/N_2 when pH increased from acidic to neutral or sub-alkaline values (Nomik, 1956; Burford and Bremner, 1975; Eaton and Patriquin, 1989). Anyway denitrification rate has its optimum pH close to neutrality (see section 1.2.1) and Smith (Smith et al., 1983) found the highest emission rates from soil-water suspension in the range of pH between 6 and 7.

As a result when denitrification is the prevailing process in soil, usually a decrease of N_2O fluxes from soil (mostly acid soil with pH below 5-6) can be observed at increasing values of soil pH.

Even, the dependence of N_2O emissions via nitrification appears to be more complex, since different authors got very contrasting results, with some detecting an increase of the product ratio N_2O/NO_3 at increasing pH (Goodroad and Keeney, 1894; Bremener and Blackmer, 1980; 1981), while others revealing a decrease of both nitrification rate and the amount of N_2O evolved by (Martikainen and De boer, 1993). Therefore there’s no clear evidence of a general trend for N_2O production through nitrification with changing soil pH.

1.3.5 Soil temperature

An inverse relationship between the product ratio N_2O/N_2 of denitrification process and soil temperature has been pointed out by several studies in laboratory incubations of soil (Nomik, 1956; Keeney et al., 1979; Vinther, 1990), while for nitrification N_2O appeares the predominant reaction product at increasing soil temperature (Yoshida and Alexander, 1970; Bremener and Bkackmer, 1981; Goodroad and Keeney, 1894).

Anyway besides the effects of temperature on reaction products of nitrification and denitrification, rates of both these processes increase at soil raising soil temperature (see sections 1.2.1 and 1.2.2) determining on the whole an increase of N_2O fluxes from soil up to temperature of 20-40 °C.

Several study (Nomik, 1956; Yoshida and Alexander, 1970; Freney et al., 1979; Keeney et al., 1979; Goodroad and Keeney, 1894) showed in fact a marked increase of N_2O emission from soil and pointed out the phenomenon could be described with confidence by the Arrhenius or Van't Hoff exponential equations.

1.3.6 Soil organic matter content

Many studies have found a significant positive correlation between N_2O fluxes and soil organic matter content (Bremner and Bkackmer, 1981; Robertson and Tiedje, 1984; Arcara et al., 1985; Iqbal, 1992) and as a matter of fact organic soil appear to produce more N_2O than mineral ones (Duxbury et al., 1982; 1984).

As far as concern denitrification, several investigation have reported a reduction of the ratio N_2O/N_2 at increasing soil content of easily degradable carbon materials, since they appeared to promote a complete reduction of N_2O to N_2 (Nomik, 1956; Smirnov et al, 1979; Elliot et al., 1990).

Anyway, at raising soil labile carbon materials, N_2O emissions from soil through denitrification increase as well, since they promote denitrification rate as reaction substrate (see section 1.2.1) and can lead to O_2 consumption and development of anaerobic microsites in soil, via a generalized enhancement of microbial activity (see section 1.3.1).

Similarly, O_2 supply limitations caused by the enhancement of microbial activity following an increase in soil organic carbon content, can reduce the rate of nitrification rate and increase the amount of N_2O evolved by (see section 1.3.1). However if organic matter has a high C/N ratio, stimulating immobilization of NH_4^+ in soil, nitrification can drop because of plant competition for mineral-N demand and N_2O emissions decrease as well (Granli and Bockman, 1994).

For the effects of chemical and animal organic fertilizers and crop residues incorporations on N_2O emissions from soil in agricultural systems see section 1.4.2.1.

1.3.7 Soil texture

Depending on their own physico-chemical characteristics, soils with different textures usually exhibit very different propensity for N_2O emissions, anyway the relationship between soil texture and the amount of N_2O evolved from soil is complex.

Since clay soils are characterized by higher water holding capacity and colloids content (retaining mineral-N in soil) than sandy soil, they tend to be higher N_2O emitters (McKenney et al, 1980; Webster and Dowdell, 1982; Matson et al., 1990).

However, considering that soil porosity and water content are key parameters influencing gas diffusion, in clay soils with very high WFPS, N_2O diffusion out of the soil can became restricted, and a relevant amount of N_2O can be reduced to N_2 before it can escape from soil (Arah et al., 1991). On the contrary sandy soils, characterized by lower potential for N_2O emissions, allow the N_2O formed to escape easily (Granli and Bockman, 1994).

Since denitrification is an anaerobic process it is usually favoured in fine textured soil, while nitrification, requiring aerobic conditions, preferably occurs in coarse textured soils, anyway the total amount of N_2O produced and the prevailing process responsible for, can easily change depending on changing soil physico-chemical characteristics.

For the effects of tillage on N_2O emissions from soil in agricultural systems see section 1.4.2.4.

1.4 THE ROLE OF AGROECOSYSTEMS AS SOURCE AN SINK OF ATMOSPHERIC N_2O

Agroecosystems can be defined as natural systems managed by humans for the primary purpose of producing food and other socially valuable non-food goods.

Of course the main factor of differentiation of agricultural systems from natural ones, is the removal of plant and animal biomasses from the system, causing a loss of energy and material sources able to undermine the natural balance of the system. Moreover besides the natural physical, chemical and biological components, new selected plants and pedigree breeds have been introduced by humans to get benefits for themselves and their livestock.

Therefore in comparison with natural systems, agroecosystems are characterized by a much simpler composition of plant and animal species and by simpler energy flows and materials interchanges between components.

As a result to maintain their unsteady balance and the high yields of intensive agricultural production, agroecosystems need a series of non-stop inputs, such as: fertilizers, fuel, irrigation, pesticides, machine tools, etc (Fig. 1-8).

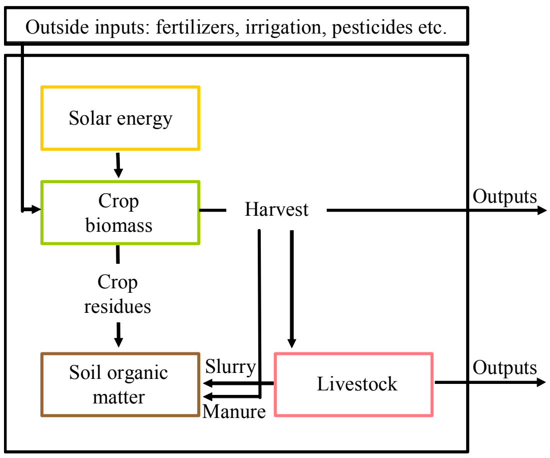


Figura 1-8: Simplified agroecosystem functioning (Modified from Caporali, 1997).

Several agricultural practices necessary for the proper functioning of the economic production system and outputs, can deeply affect N_2O emission from agricultural soils as well, and, as already pointed out in section 1.1, recent estimates have pointed out agricultural soils account for about 50% of the global anthropogenic N_2O flux (IPCC, 2001).

According to the IPCC Guidelines for National Greenhouse Gas Inventories (IPCC, 1997) three different kind of N_2O emissions from agricultural soils can be distinguished in agroecosystems

(Fig.1-9):

- *Direct soil emissions* following nitrogen inputs to soil as synthetic fertilizer, animal waste, biological nitrogen fixation, nitrogen from crop residues, sewage sludge application and organic matter mineralized through cultivation of organic soils (OM%>5%)
- *Direct soil emissions* from animal production induced by grazing animals
- *Indirect emissions* taking place outside the system, after nitrogen is lost from the system as NO_x and NH_3 or through leaching and runoff

Moreover further N_2O emissions occurring during storage and handling of manure, before its spreading over the field, are taken into account also.

Anyway the current protocol has some objective limits in estimating N_2O emission from soil, since it derive the amount of N_2O evolved from agricultural soil as simple percentage of the total N input to soil (default values= 1,25%), without considering N_2O fluxes can depend on N input in a more complex manner, with several factors interfering such as plant competition for mineral-N demand, local climatic and environmental conditions, irrigation etc.

Moreover it not include in the N_2O budget assessment for agricultural system the process of soil uptake of atmospheric N_2O.

It's now widely accepted (may be not unanimously) besides being a source for N_2O, soils also can be a sink for atmospheric N_2O. Several investigations have in fact reported small N_2O uptake in soil (Ryden, 1981; 1983; Duxbury and Mosier, 1993; Nobre, 1994; Neftel et al., 2000), but a total understanding of this phenomenon has not been achieved yet.

Even if the general assumption is that a net consumption via denitrification may occur when O_2 supply to active microsites is hindered or NO_3^- concentration is very low (Arah and Smith, 1989; Davidson, 1991; Granli and Bockman, 1994; Sierra et al., 1994), there are some contradditions.

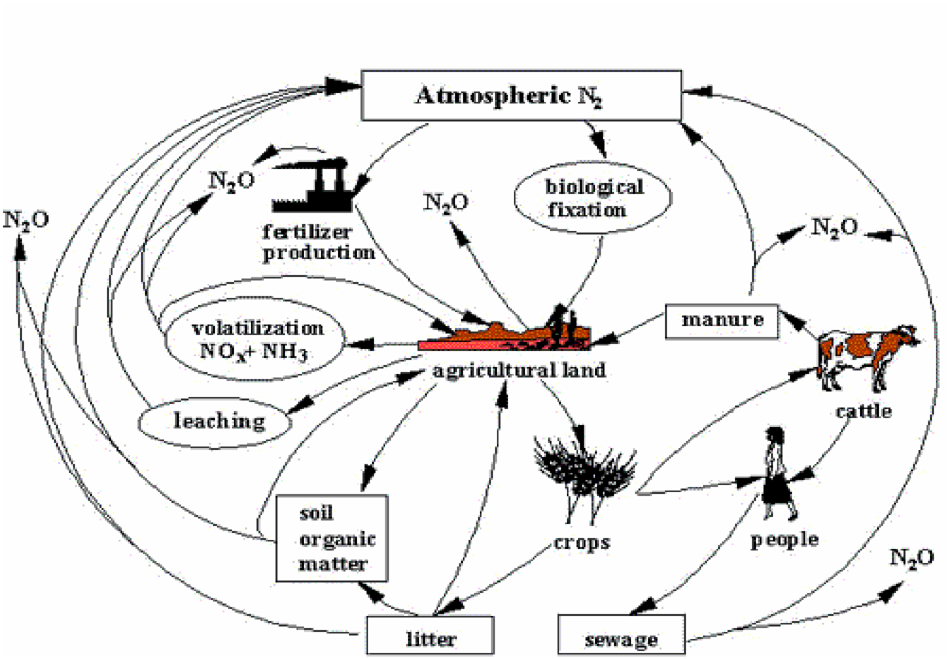


Figure 1-9: Diagram of agricultural soil N cycle and nitrous oxide production (Mosier et al., 1998).

Anaerobic soil should have a great potential for N_2O.uptake, since most N_2O is usually reduced to N_2 (see section 1.3.1), anyway this condition is usually gained at high soil water content, preventing gas diffusion from air to soil. Otherwise N_2O bidirectional exchange should be easier in well aerated soil, where however N_2O reduction to N_2 is not favoured.

1.4.1 Agricultural practises affecting direct soil emission of N_2O in croplands

1.4.1.1 Mineral and organic N input

In most agricultural soil nitrogen is limiting for the growth of the crops, so that the need to increase food production together with the use of high-yielding varieties have greatly increased N fertilizer inputs to agroecosystems. Anyway intensive cultivation itself can be a main cause of soil erosion and loss of fertility and as a result, nowdays, farmers are attempting to boost yields by using more and more fertilizers on soils gradually loosing their productivity.

A crucial environmental issue is that inappropriate use of fertilizer N, besides relevant consequences such as NO_3^- leaching from soil system (Fig.1-10), also can cause huge N_2O emissions from fertilized cultivated lands.

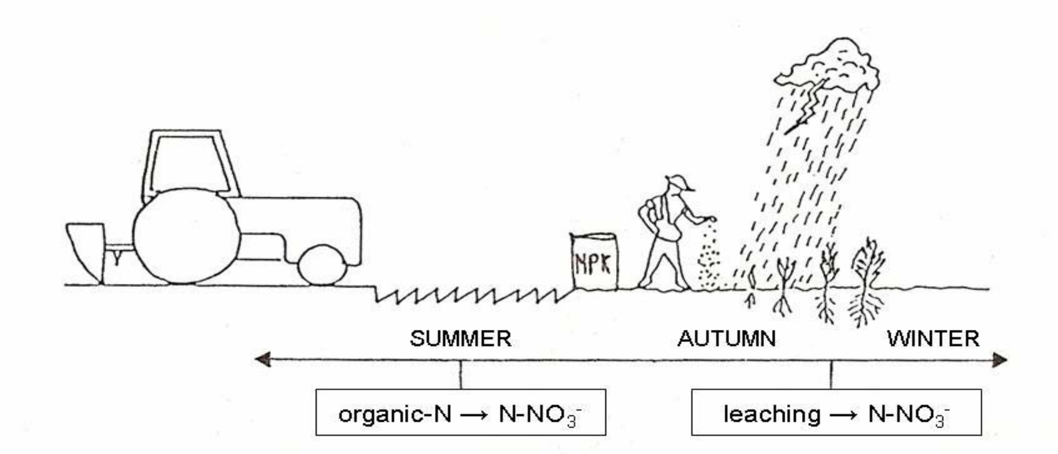


Figure 1-10: Pathway for NO_3^- leaching from agricultural fields under Mediterranean climate conditions. Nitrates accumulate in soil between cropping seasons as a result of mineralization of soil organic matter (enhanced by plaughing) and nitrification of the ammonium so formed. Afterwards when farmers spread fertilizer N for the winter crops there is the higher risk for NO_3^- leaching through the first autumn heavy rains (from Caporali, 1996).

As shown in more detail in sections 1.2.1, .1.2.2 and 1.3.3 availability of mineral N (NO_3^- and NH_4^+) is a key controller for microbial processes involved in N_2O evolution from soil, therefore crop soils potential for N_2O emissions greatly increases through application of mineral-N fertilizers, such as NO_3^-, NH_4^+, NH_4NO_3 and NH_3 compounds.

Also manure, slurry and crop residues can greatly enhance N_2O emission from soil, even though on the whole, their return to soil is considered a beneficial practice, useful to conserve soil organic material and productivity.

Spreading of animal manure as slurry increases soil NH_4^+ concentration, since up to 60%-70% of the N in slurry is present as NH_4^+, urea and uric acid, while solid manure and crop residues contain both organic materials and easily mineralizable N. Moreover application of manure, also as slurry, to the soil surface can favour the development of temporary anaerobic conditions leading to peak of denitrifying activity and N_2O emissions related with.

Several studies found a marked increase in N_2O emissions after application of fertilizer N, either mineral (Bremner and Blackmer, 1980; Bremner et al., 1981; Duxbury et al., 1982; Conrad et al., 1983; Su et al., 1990; Tsuruta et al, 1993; Dambreville et al., 2006) or organic (Arcara et al., 1999; Vallejo et al., 2003; 2004; Mcswiney and Robertson, 2005).

This increase of N_2O fluxes was often recorded soon after N supply or after a lag period of some days and was referable either to denitrification (Arcara et al., 1999; Vallejo et al., 2003; 2004; Dambreville et al., 2006) or to nitrification (Hutchinson and Brams, 1992). Moreover N_2O peaks separated in time were detected after the application of NH_4^+ compounds as a consequence of nitrifying activities followed by bacterial denitrification, as NH_4^+ was nitrified in soil (Freney et al, 1985).

Many investigation have been performed as well to determine the effect of different fertilizer types on N_2O emission from soil.

Among mineral-N fertilizers NH_3 appears to promote the highest emission rates (Huchtinson and Mosier, 1979; Bremner and Blackmer, 1980; Bremner et al., 1981; Breitenbeck and Bremner, 1986), anyway as far as concern Europe this is not a topic of great concern, since usually NH_3 is not used as fertilizer (IFA, 1999).

As regards differences between mineral and organic fertilizers, Christensen (Christensen, 1985) found higher N_2O emissions from grassland soils after mineral N (NO_3^-) application than after slurry application, while Dambreville (Dambreville et al., 2006) didn't find any difference in denitrifying activity and N_2O production of indisturbed soil cores, in relation to the long-term effect of pig slurry applications compared to mineral fertilization.

Anyway in soil where the availability of labile organic material is limiting for microbial activity, manure can produce more N_2O than mineral N fertilizers (Christensen, 1983; Christensen, 1985; Benkiser et al., 1987; Bowmann, 1990; Van Cleemput et al., 1992) and, on the whole, the combined application of manure and mineral fertilizers can lead to amplified N_2O emission rates.

1.4.1.2 Irrigation

Irrigation represents a fundamental tool in increasing productivity of cultivated lands, above all in the Mediterranean regions, where aridity and cyclical water deficit are permanent characteristics during summer period.

Today almost 40% of the global harvest comes from irrigated croplands (about 16%-17% of the world's cropped surface area), anyway the number of water stressed countries around the world has dramatically increased in the course of the last few decades, with drought conditions especially bad in Mediterranean countries such as Spain, Portugal and large part of the United Kingdom, Italy and France (WWF Report, 2006). Therefore currently much effort is placed on achieving irrigation strategies (for instance improving the efficiency of irrigation methods) to combine good crop yields and the need to keep water consumption law, also to avoid problems relating to soil salinization and waterlogging.

Several authors detected peaks of N_2O fluxes from crop soils following irrigation events evidently as a result of enhanced denitrifying activities under restriced aeration state (Freney et al., 1985; Ryden and Lund, 1980; Teira-Esmatges, 1998; Sànchez et al., 2001; Vallejo et al., 2004) and there's evidence high emissions can occur when irrigation is performed simultaneously or soon after N supply (Ryden et al., 1979; Mosier and Hutchinson, 1981; Webster and Dowdell, 1982; Su et al., 1990; Hutchinson and Brams, 1992).

Anyway this topic, together with strategies to reduce irrigation effects on N_2O emission from soil, have not been adequately investigated yet, above all in the Mediterranean region, and this sounds like a non sense, since N_2O gas itself contributes to the greenhouse effect, a main factors responsible for developing drought conditions all around the world.

1.4.1.3 Tillage and compaction

Ploughing is usually performed in traditional farming systems to improve soil aeration and seeds incorporation in soil at sowing time. Anyway the continuous use of conventional tillage systems, by enhancing accessibility of crop residues and soil stable organic matter for soil microbes, can accelerate the deplection of soil organic matter, leading to a loss of soil fertility and increasing the risk of soil erosion (Martel and MacKenzie, 1980; Hussain et al., 1999).

There are only few and contrasting investigation about the effect of soil tillage on N_2O emission from soil.

Some authors (Matthias et al., 1980; Bremner and Blackmer, 1980) found increased N_2O fluxes from soil for a short period after the mechanical disturbance by tillage and ascribed the phenomenon to the release of soil air enriched in N_2O. On the other hand others detected higher denitrification rates and N_2O emission from indisturbed soil than for ploughed ones (Burford et al., 1981; Aulak et al., 1984; Lind and Doran, 1984; Staley et al., 1990) and, finally, Elmi (Elmi et al., 2003) didn't find any difference at all for denitrification and N_2O emission between no-tilled soil and soil cultivated by conventional and reduced tillage systems.

Up to present, few investigations have been conducted about the effect of soil compaction by tractor traffic on N_2O emission as well, but al least they got concordant results.

In fact all studies showed soil compaction appear to increase both denitrification rate (Bakken et al., 1987; Torbet and Wood, 1992) and N_2O emission rates (Hansen et al., 1993; Ruser et al., 2006), evidently in consequence of a reduction of the soil macro-pore volume restricting O_2 availability inside the soil profile.

1.5 OBJECTIVE OF THE RESEARCH

It's well known bacterial nitrifying and denitrifying activities are the most important among soil microbial processes involved in N_2O emission from soil (Davidson, 1991; Groffman, 1991; Hènault and Germon, 1995) and a general assumption is that clay soils tend to be high N_2O emitters via bacterial denitrification, while in well aerated soils nitrification and associated N_2O emissions can be promoted (Granli and Bockman, 1994). Anyway a complete understanding of how soil characteristics regulate these processes and their relative contribution to N_2O fluxes from soil, has not been achieved yet, although essential for realistic and appropriate modelling of N trace gas emissions.

As far as concern agroecosystems, many studies focused on the effects of fertilizer N and irrigation practice on N_2O emissions and soil bacterial processes involved in their production, however these topics have been rarely investigated in southern Mediterranean countries (Teira-

Esmatges et al., 1998; Arcara et al., 1999; Sànchez et al., 2001; Vallejo et al., 2001; 2004) despite the huge extention of irrigated cropped surface areas under Mediterranean climate conditions and their great potential for N losses through denitrifying and nitrifying activities, as a result of the combined favouring effects of high temperatures and cyclic wetting of soil by irrigation practice.

The surface of irrigated land in Mediterranean has doubled in the last 40 years and is still increasing at the present time (WWF Report, 2006), therefore the lack of data concerning emissions of N_2O from Mediterranean agricultural fields appears limiting in order to provide the necessary information to validate current models predicting N_2O fluxes at a global scale.

Moreover most of the studies investigating the role of bacterial denitrification and nitrification in N losses from soil as a function of soil physico-chemical parameters, were performed through techniques disturbing the natural characteristics, structure and oxygen gradient of soils (i.e. measurements on sieved and/or wetted soil samples both not amended and amended with mineral N substrates, analyses of small soil aggregates and experiments with flow-through incubation systems), thus providing results hard to be extrapolated at a field scale and often not related to in-situ measurements of N_2O fluxes from soil.

The aim of this study was to investigate through a process study how the changing environmental climate conditions and the agriculture management practices can affect soil bacterial denitrification and nitrification and the amount of N_2O evolved by, in an irrigated cropland under Mediterranean climate conditions.

The measurements were carried out in the agricultural field of a buffalo zootechnic farm, a typical component of the overall agricultural section in Campania Region (ISTAT, 2000), showing a relevant potential for N losses as N_2O and N_2 via soil denitrifying and nitrifying activities. Dairy activity through intensive farming produce in fact great amount of organic waste generally applied to the cropped soil and largely relay on water supply to grow fodder plants for animal consumption, with a number of irrigated hectares accounting for about 80% of the total cropped surface area (ISTAT, 2000).

Moreover, since at the experimental site soil has an alluvial origin, with alternate clay and sandy profiles inside the same agricultural field, differences of microbial activity and N_2O fluxes from soil between fine and coarse textured soil were investigated as well.

Measurements of bacterial denitrifying and nitrifying activites and of their different contribution to N_2O production in soil were all performed on intact soil cores, without altering the structural characteristics and the status of both C and N availability of soil, in order to achieve results as representative as possible of the real processes occurring in the field and relate them to in-situ measurements of N_2O fluxes from soil.

2 MATERIALS AND METHODS

2.1 SITE DESCRIPTION

The experimental site is part of Iemma zootechnical farm, located in Borgo Cioffi near Eboli, about 25 Km NE from Salerno (4486080 N, 496470 E), in the middle of Piana del Sele flatland, in Campania region (Fig.2-1).

Figure 2-1: Farm location inside Campania Region.

2.1.1 Climate

At the site climate is Mediterranean, characterized by dry summers and mild winters (Fig.2-2). The mean annual air temperature is approximately 19 °C and the annual precipitation is 490 mm (Di Tommasi, 2003).

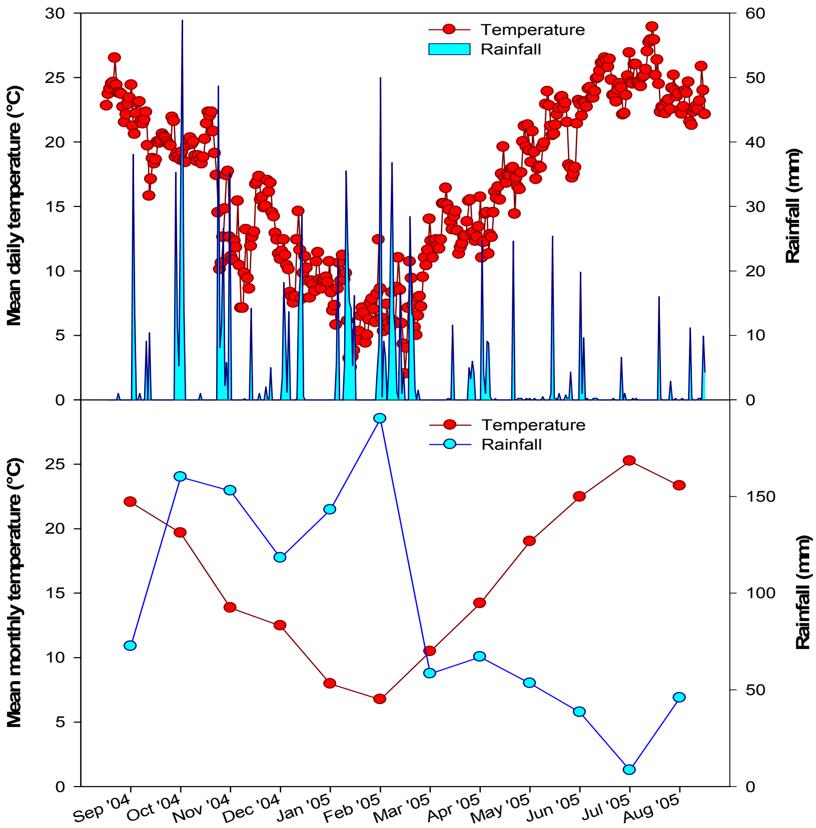


Figure 2-2: Climatic data from the agricultural field of Borgo Cioffi during the growing season of *Lolium italicum* (September '04–April '05) and *Zea mays* (May '05– August '05).

2.1.2 Soil

The parent material has alluvial origin, with alternate sandy clay layers and stones present only on the surface of patchy areas.

Two different soil profiles have been detected in the same field along an E-W transect (Table 2-1), showing different soil physico-chemical characteristics (Table 2-2).

Table 2-1: Texture distribution of East and West soil profiles at the experimental site of Borgo Cioffi (Di Tommasi, 2003).

Profile	Sand	Silt	Clay	Classification
East	29.8	22.1	48.1	Clay
West	75.1	12.5	15.0	Sandy

Table 2-2: pH, organic matter content, bulk density, field capacity (FC) and water filled pore space (WFPS) at field capacity, in the soil top-layer (0-15 cm) of sandy and clay profiles (Mean values from this study).

Profile	pH	OM %	Bulk densidy (g cm^{-3})	FC (g_{water} $g^{-1}{}_{dry\ soil}$)	WFPS at FC
Sandy	7.65	3.7	1.15	0.206	45.4%
Clay	7.63	7.8	1.01	0.391	78.3%

The East profile, relating to the most of the field (about 2/3), is characterized by clay texture in the top 0.2 m, high carbonate content and hydromorphic traits related to winter waterlogging. In fact the water table level in the field may rise from 5-6 m depth during summer period up to 1 m depth beneath soil surface during winter time.

The West profile, more heterogeneous and complex, is characterized by sandy texture, lower carbonate content and less evident waterlogging features.

2.1.3 Farm management

The farm extends on about 50 hectares and produces mainly dairy products (mozzarella, ricotta, etc.) from on-farm produced milk by about 400 adult buffaloes.

As far as concern nutrient cycle, it features as a semi-closed system (Fig.2-3). Most agricultural fields are in tilled to grow fodder plants (corn, alfa alfa or winter grass crops such as *Lolium italicum*) for fresh animal consumption or silage. Occasionally, during winter period, part of the fields is given for rent to raise vegetables such as Florence fennel and cauliflowers. Buffalo dejections from paddocks are stored in ponds and manure as slurry sewage is used as main source of nutrients for crops needs. Anyway besides manuring, additional mineral and/or organic chemical fertilizers are spread also.

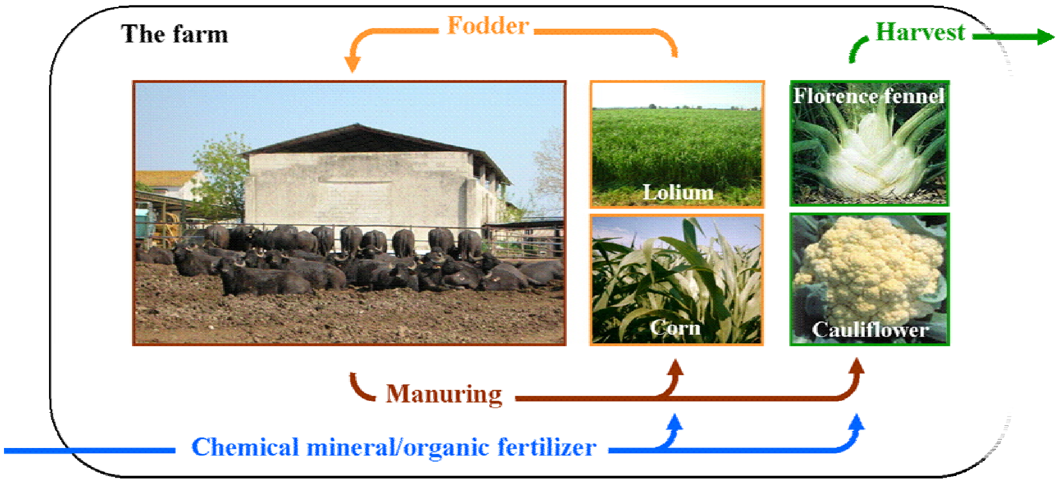


Figure 2-3: Nutrients cycle inside the zootechnical farm.

Soil tillage schemes are conventional with ploughing being performed before establishing the main crop, moreover they also may include superficial harrowing by tiller or disks, down to a depth of about 0.15 m.

A centre pivot irrigation system is used to supply crops with water during summer period, but it cannot cover the whole agricultural land so that the side of the field is irrigated by means of pumps.

Finally for row crops, herbicides are spread soon after sowing to control weeds.

Tillage schemes, sowings and fertilizations performed in the field during this study are listed below (Fig.2-4).

Zea mays crop in 2006 was preceded a mixed crop of *Lolium italicum* and *Trifolium Alessandrinum*, supplied with 2.5 q ha^{-1} NPK 11-22-16 Hydro at the sowing time.

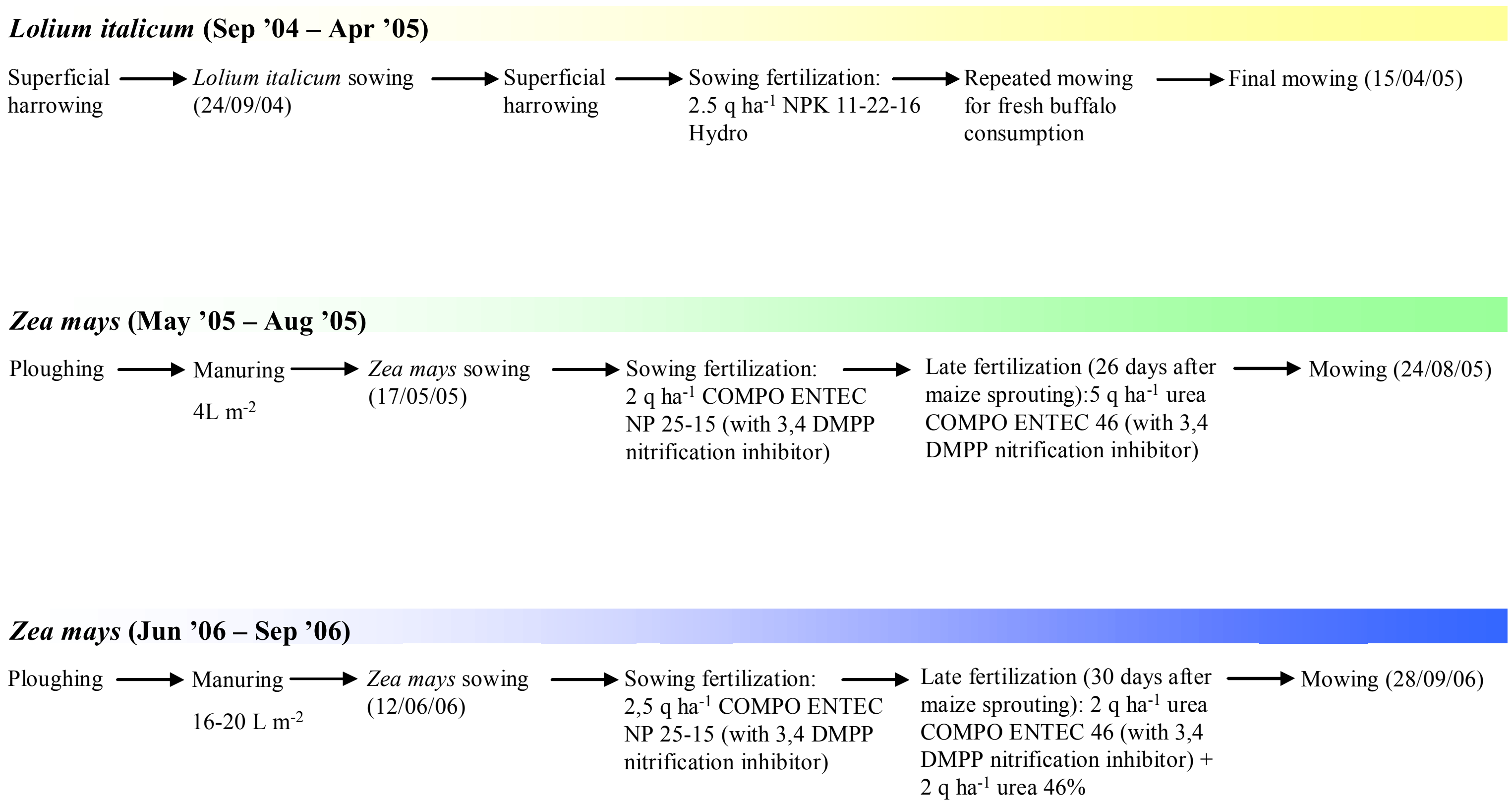


Figure 2-4: Illustrative scheme of the agricultural practices performed in the field in the course of the *Lolium italicum* and the *Zea mays* growths.

2.2 LABORATORY AND FIELD METHODS

Both monitoring activities and one manipulation experiment were performed at the experimental field.

The monitoring study in the course of the first year of measurements concerned bacterial denitrification, nitrification and N_2O fluxes from soil surface both in clay and sandy soils throught the *Lolium italicum* (Sep ’04 – Apr ’05) and the *Zea mays* (May ’05 – Aug ’05) growths. Moreover at the sandy site measurements to assess the relative contribution of nitrifying ($\mathbf{N_2O_{nit\%}}$) and denitrifying bacteria ($\mathbf{N_2O_{den\%}}$) to N_2O fluxes from soil were carried out during the *Lolium italicum* crop up to the first stages of the *Zea mays* growing season.

Further determinations of denitrifying activity and N_2O fluxes from soil were performed at the clay site in the course of the *Zea mays* crop in 2006 (Jun ’06 – Sep ’06).

By the manipulation experiment, the effects of different amounts of mineral N fertilizer on denitrifying activity and N_2O emission from clay soil were tested in restricted plots inside the agricultural field during the corn crop in 2005.

On each sampling date ancillary analyses for soil physical-chemical characterization were performed as well.

To avoid disturbance of soil aeration status, all measurements related to denitrifying and nitrifying activities and to the amount of N_2O evolved by, were carried out on intact soil cores sampled by an Eijkelkamp split tube soil sampler (∅= 5 cm, h= 40 cm) (Fig.2-6).

2.2.1 Soil physico-chemical parameters

2.2.1.1 Soil pH

10 g of fresh sieved soil (2mm mesh) were shaken twice for 20 minutes with deionized water on a shaking machine. After soil being sedimented, the pH was measured inserting a glass electrode in the solution (Methron 665 Dosimat, Hanna Instruments).

Sometimes to get round the slow sedimentation of soil with clay texture, samples were centrifuged for 10 minutes at 5000 rpm after being shaken.

Figure 2-5: The split tube soil sampler for intact soil cores.

2.2.1.2 Soil mineral-N

During *Lolium italicum* and *Zea mays* 2005 grows (manipulation experiment included), only soil NO_3^- -N was determined, while during *Zea mays* crop in 2006, extractions of mineral-N (NO_3^-+ NH_4^+) by K_2SO_4 solution were performed.

<u>Extractable NO_3^- -N</u>

10 g of fresh sieved soil (2mm mesh) were shaken twice for 20 minutes with deionized water on a shaking machine. After soil being sedimented, the solution was filtered through a Whatman 42 filter paper (15-20 µm mesh) and analysed by colorimetric reaction and spectrophotometry (LASA

50 DrLange portable spectrophotometer)

To get round the slow sedimentation of soil with clay texture, samples were often centrifuged for 10 minutes at 5000 rpm before being filtered.

After being collected, soil sample were stored at 4°C and always extracted before 24 hours. Soil extracts were analysed as soon as possible anyway, when not possible, they were stored frozen.

Extractable NO_3^- -N and NH_4^+ -N

15 g of fresh sieved soil (2mm mesh) were shaken with a 0.5 M K_2SO_4 solution for 2 hours on a shaking machine. After soil being sedimented, the solution was filtered through a Whatman 42 filter paper (15-20 µm mesh) and analysed by ion-selective electrodes (ISE, STANDARD METHODS nr. 4200).

2.2.1.3 Soil water content

20 g of fresh sieved soil (2mm mesh) were weighed in small glass cups and placed in an oven at 75°C for 48 hours. After getting completely dry, soil samples were cooled in a desiccator and reweighed. Soil gravimetric water content was then expressed as percentage:

$$\textbf{Water content} = ((\mathbf{mass}_{\mathbf{wet\ soil}} - \mathbf{mass}_{\mathbf{dry\ soil}}) / \mathbf{mass}_{\mathbf{dry\ soil}}) \times \mathbf{100}$$

2.2.1.4 Soil bulk densit, WFPS and WHC

Soil bulk density can be defined as the mass of dry soil per unit volume of bulk soil. Soil bulk density measurements were performed on the undisturbed soil cores collected from the experimental field for denitrifying activity assessment (∅= 5 cm, h= 15 cm).

Soon after sampling, fresh soil intact cores were weighed and, after finishing denitrification rate analyses, they were placed in an oven at 75°C, till getting completely dry. After that soil samples were cooled in a desiccator and reweighed.

The bulk density is then equal to:

$$\textbf{Soil bulk density } (\mathbf{g\ cm^{-3}}) = \mathbf{mass}_{\mathbf{dry\ soil}} / \mathbf{volume}_{\mathbf{intact\ soil\ core}}$$

The soil water filled pore space WFPS, often expressed as percentage, is given by the ratio between volumetric soil water content θ_v (i.e. the volume of water per unit volume of soil) to total porosity of soil ε (i.e. the volume of pore space per unit volume of soil):

$$\textbf{WFPS} = (\theta_v/\varepsilon) \times 100$$

Knowing gravimetric soil water content, soil bulk density and soil particle density, volumetric soil water content θ_v and total porosity of soil can be easily calculated from the following relationships:

$$\theta_v = (\theta_v \times \textbf{bulk density})/\ \textbf{density of water}$$

and

$$\varepsilon = 1\text{- }(\textbf{bulk density/particle density})$$

In this study no direct measurements of soil particle density were carried out, so it was assumed to be 2.65 g/cm^3 (Rowell, 1993).

The soil water holding capacity WHC, or field capacity FC, is the amount of water that the soil can hold, resisting what drains away through gravitational pull and is greatly dependent upon the soil particle size and organic matter content. At field capacity air occupies the large pore spaces while water coats the soil particles and organic matter, filling the small pore spaces.

Soil WHC measurements were performed only once throught the present study. Undisturbed soil cores (∅= 5 cm, h= 15 cm) collected in the field, were oven dried and placed in Hilgard soil cups equipped with a Whatman #2 filter paper on the screen inside.

Afterwards the cups were placed into a shallow pan of water allowing only the bottom few centimeters of the cups to become wet.

After the soil becoming saturated from the bottom of the cup to the surface, the cups were removed from the pan of water and placed in a humid enclosure until drainage was complete. At that time soil cores where were weighed and then placed in an oven at 75°C, till getting completely dry. After that soil samples were cooled in a desiccator and reweighed.

The soil water holding capacity was then calculated as:

$$\textbf{WHC (g g}^{-1}\textbf{)} = \textbf{mass}_{\textbf{water}} /\ \textbf{mass}_{\textbf{dry soil}}$$

where:

$\mathbf{mass_{water}}$= mass of the water contained in the saturated soil= mass of the saturated soil - mass of the oven dried soil

$\mathbf{mass_{dry\ soil}}$= mass of the oven dried soil

2.2.1.5 Soil organic matter

5 g of oven-dry sieved soil (2mm mesh) were weighed in small baked clay cups and burned in a muffle furnace at 550°C for 2 hours. After that, ashes were placed in a desiccator and weighed as well.

Soil organic matter content, expressed as percentage, is then given by:

$$\textbf{Organic matter} = ((\mathbf{mass_{dry\ soil}} - \mathbf{mass_{dry\ ash}}) / \mathbf{mass_{dry\ soil}}) \times 100$$

2.2.1.6 Soil temperature

On each sampling date, soil temperature was measured (4 replicates in each plot) down to a depth of 10 cm by means of a thermo-pHmeter (Hanna Instruments).

2.2.2 Actual denitrification rate

Actual denitrification rate was determined through the Acetylene Inhibition Technique (AIT) on intact soil cores (Robertson et al., 1999), without modifying soil chemico-physical characteristics and avoiding disturbance of oxygen gradient, in order to obtain results as representative as possible of the real denitrifying activity occurring in the field.

The method is based on the inhibition of the nitrous oxide reductase by high partial pressure (1-10 kPa) of acetylene (Balderston et al., 1976; Yoshinary and Knowles 1976) (Fig.2-6).

Since the reduction of N_2O to N_2 is inhibited, a quantitative conversion of NO_3^- to N_2O occurs, and it's possible to measure the denitrification rate of undisturbed soil cores sealed in air tight containers as the accumulation of N_2O in the presence of acetylene (10% of the headspace) (Fig.2-

7).

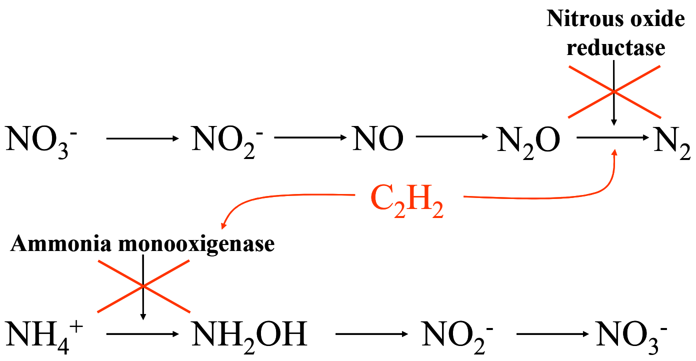


Figure 2-6: N_2O reduction inhibition by high partial pressures of acetylene. The activity of ammonia monooxigenase enzyme is blocked as well.

For this aim it was manufactured a set of 36 PVC container (∅= 5,4 cm, h= 17 cm), with air tight lids equipped with output valves connectible to gas-chromatograph stopcocks and male-luers (Fig.2-8).

On each sampling date, the intact soil cores collected in the field were sealed inside the PVC containers and C_2H_2 was added to at least 10% of the volume of the headspace. Incubation always started within 24 hours.

Since acetylene diffusion inside the core may be limited in fine-textured and/or at very wet soil (Ryden et al., 1979; Parkin et al., 1984), the air space of the soil core inside the containers was repeatedly mixed by means of 60 ml syringes (2 minutes and 5 minutes mixing for sandy and clay textured soils respectively). Moreover, during heavy rainfall periods in winter time, all samplings took place few days after the rainfall events (from 2 days up to 7 days after, depending on rainfall intensity).

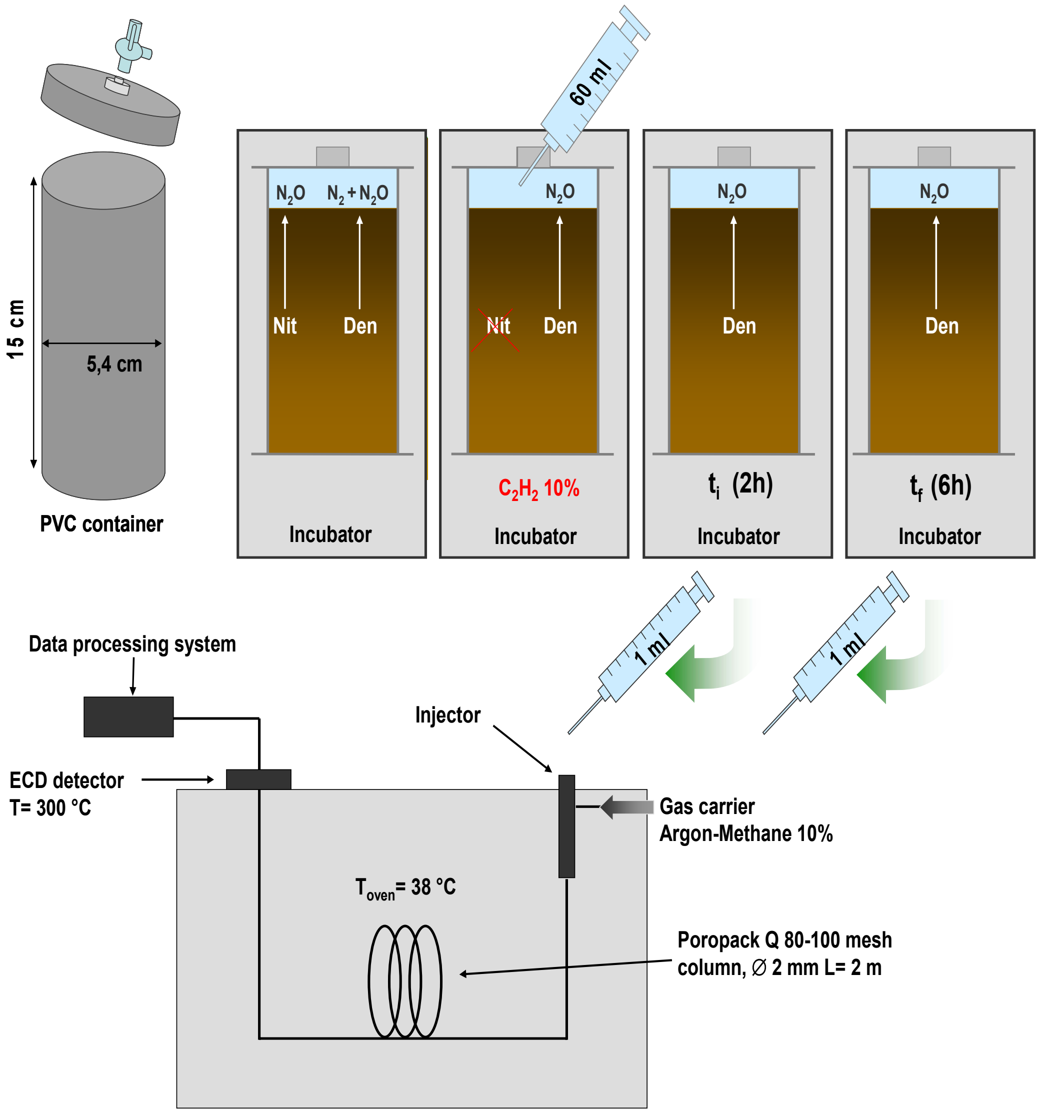


Figure 2-7: Illustrative scheme of the Acetilene Inhibition Technique (AIT) applied on intac soil cores. After adding acetylene the air space was repeatedly mixed by a 60 ml syringe; the same kind of syringe was used to mix the headspace by repeated pumping prior to each sampling.

Figure 2-8: PVC containers for AIT.

After introducing acelylene, containers with intact soil cores inside were incubated at constant field temperature (recorded in the field at 12:00 am on each sampling date).

Gas samples were removed after 3 and 6 hours and analysed on the gas-cromatograph with an electron capture detector (GC 8000, Fison Instruments) (Fig.2-7) and the rate of production between the initial and the final sampling time was taken as the rate of denitrification.

Preliminary experiments were performed on coarse and fine textured soil samples to check if the rate of gas production between initial and final times were linear (Fig.2-9).

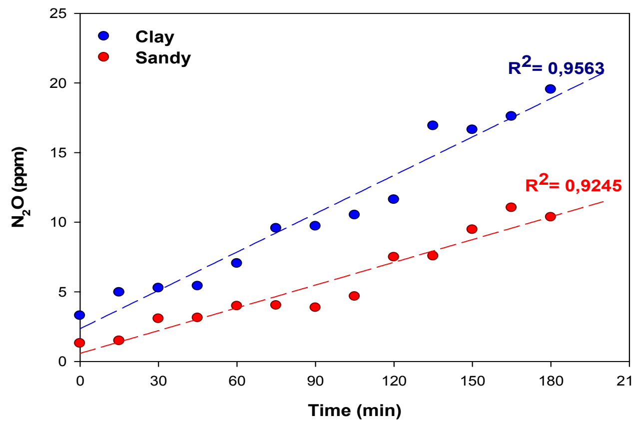


Figure 2-9: Time corse experiment to check linear gas production between initial and final sampling times for fine and coarse textured soils.

Actual denitrification rate was then calculate by the following equation:

$$\mathbf{r_{den}= ((C_f \times H) - (C_i \times H))/(A \times \Delta t)}$$

where

C_i= N_2O concentration at the initial sampling time (μg N_2O-N ml^{-1})

C_f= N_2O concentration at the final sampling time (μg N_2O-N ml^{-1})

H= Headspace volume (accounting for the internal headspace volume of each soil core)

A= Core surface area

A= Core surface area

Δt= time between initial and final sampling time

The total headspace volumes were determined by calculating the volume of the empty containers and subtracting the volume of the soil cores inside, taking into account for their porespace and water content. The accuracy of this calculation was checked by measuring the volume of water required to fill the containers (with soil cores inside) completely.

The amount of N_2O accumulated inside the PVC containers was then corrected for gas dissolved in the liquid phase using Bunsen coefficients to predict the amount of N_2O dissolved in the liquid phase from the concentration in the gas phase (Moraghan and Buresh 1977, Wilhelm et al., 1977):

$$\mathbf{N_2O_{tot} = N_2O_g\,(V_g + V_l \times \beta)}$$

where

N_2O_{tot}= total amount of N_2O in water plus gas phase

N_2O_g= N_2O concentration in the gas phase (μg N_2O-N ml^{-1})

V_g= volume of the gas phase

V_l= volume of the liquid phase

β= Bunsen coefficient (1.06 at 5°C; 0.882 at 10°C; 0.743 at 15°C; 0.632 at 20°C; 0,544 at 25°C; 0.472 at 30°C)

The two-hour lag period before the initial sampling is necessary to allow acetylene completely diffuse inside the soil core while time between initial and final samplings should long enough to detect low rate of activity, at the same time preventing acetylene effect on soil carbon metabolism (Yeomonas and Beauchamp, 1982; Terry and Duxbury, 1985; Flather and Beauchamp, 1992) and avoiding deplection of soil O_2 level or soil NO_3^- pool. Of course convenience is a critic factor as well.

Some more major problems with the acetylene method are the inhibition of NO_3^- production via nitrification (Haynes and Knowles, 1978; Walter et al., 1979; Mosier 1990), causing a deplection of soil NO_3^- pool during incubation, and the failure of nitrous oxide-reductase inhibition at low NO_3^- concentration. Anyway they can be considered not critical in agricultural ecosystems characterized by high N-input through manuring and mineral/organic chemical fertilization practices.

2.2.3 Net nitrification rate

Net nitrification rate (**r_{nit}**) was determined by the Buried-bag incubation method (Hart et al.,1994), measuring the change of NO_3^- concentration in intact soil cores incubated *in-situ* for approximately 30 days, sealed inside 30 μm thick polyethylene bags which allow gas diffusion and prevent any exchange with soil solution (Fig.2-10).

In detail on each sampling date two sets of undisturbed soil cores were collected in the field close to each other: the former was carried to the laboratory and processed to determine the initial soil NO_3^-concentration (the mean value from all the cores sampled in one day) while the latter was left in the field for 3-4 weeks and finally processed for NO_3^- concentration as well.

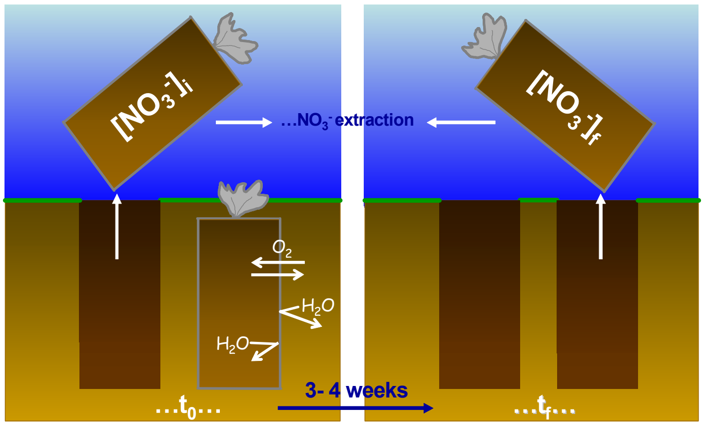


Figure 2-10: Illustrative scheme of the Buried-bag incubation method.

Net nitrification rate is then equal to:

$$\mathbf{r_{nit} = (C_f - C_i)/ \Delta t}$$

where

C_i= NO_3^- concentration in soil at the initial time (the mean value from all the cores sampled in one day, expressed as μg NO_3^--N g_{dm}^{-1})

C_f= NO_3^- concentration in soil at the final time (μg NO_3^--N g_{dm}^{-1})

Δt= time between initial and final sampling time

This method cannot account for changes in NO_3^- pool of soil coming from microbial uptake and denitrification, anyway it is designed to exclude plant uptake and leaching interference.

A more critical problem is related to incubation moisture. In fact if field moisture at time of sampling is quite different from the soil moisture dynamic over the incubation period, net nitrification rates derived could be not representative of the real nitrifying activity in the field, being whether overestimated or underestimated depending on the specific situation considered. Anyway they may still be valuable for nearby treatment comparison if moisture dynamics are similar among treatments (Robertson et al., 1999).

2.2.4 N_2O fluxes from soil

N_2O fluxes from soil were measured *in-situ* by cylindrical PVC no automated static chambers (Fig.2-11).

Figure 2-11: Cylindrical PVC static chambers for N_2O fluxes assessment (∅= 20 cm, h= 15 cm).

The chambers, supplied with butyl rubber septa on their air tight lids, were inserted 5 cm dept into the soil. After that, gas samples were collected by a 60 ml syringe (soon after closing the chambers, at 15 minutes and at 30 minutes) and stored in 6ml evacuated air-tight storage glass vials (Fig.2-12).

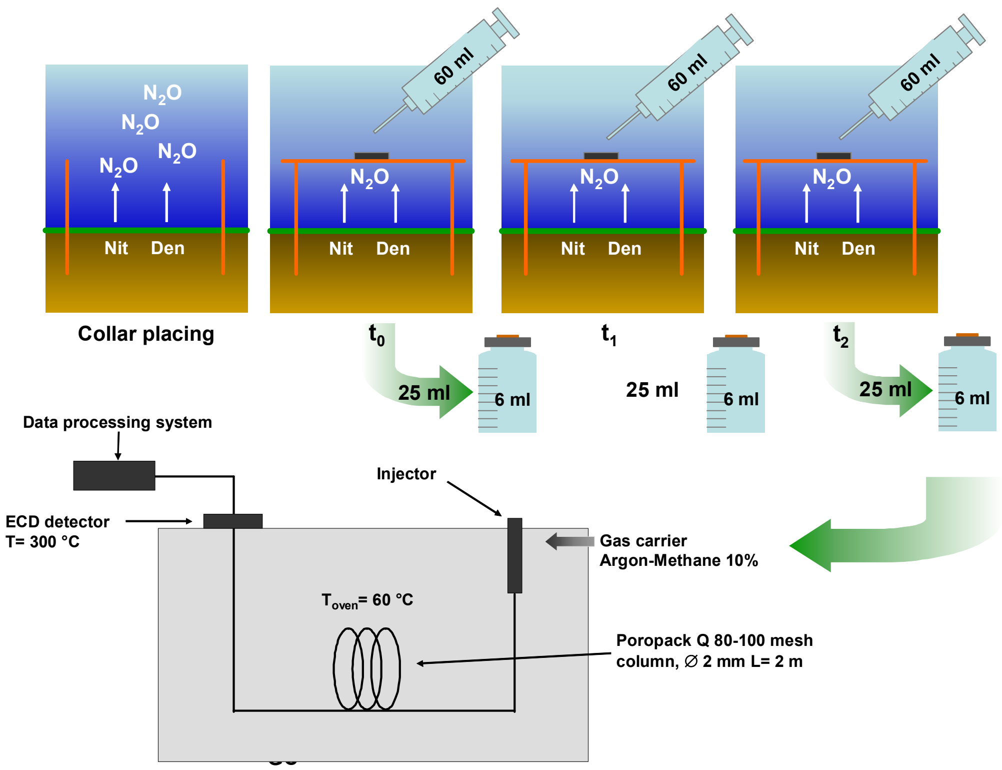


Figure 2-12: Illustrative scheme of N_2O fluxes measurements by the static chamber method.

Gas samples were analysed on the gas-cromatograph with an electron capture detector (GC 8000, Fison Instruments) and N_2O fluxes from soil were calculated by the following equation:

$$N_2O \text{ fluxes} = a/S$$

where

a= direction coefficient of the regression line of N_2O concentration with time

S= surface area of soil inside the chamber

As far as concern measurements of N_2O emission from soil during *Zea mays* crop in 2006, headspace was sampled only twice: soon after chamber closing and at 30 minutes. In that case, the preceding equation was replaced as follow:

$$\mathbf{N_2O\ fluxes= (C_f - Ci)/(S \times \Delta t)}$$

where

$\mathbf{C_i}$**=** N_2O concentration at the initial sampling time (μg N_2O-N ml^{-1})

$\mathbf{C_f}$**=** N_2O concentration at the final sampling time (μg N_2O-N ml^{-1})

Δt= time between initial and final sampling time

S= surface area of soil inside the chamber

2.2.5 Relative nitrifier and denitrifier contributions to N_2O fluxes from soil

The different contribution of nitrifying and denitrifying to N_2O emission from soil was determined by the method of short exposure to high partial pressure of acetylene (Kester et al., 1996), adapted for intact cores (Kester et al., 1997).

This method is based on the different recovery time of denitrifier and nitrifier activity after exposure to acetylene. It's well known (Haynes and Knowles, 1978; Walter et al., 1979; Mosier , 1980) acetylene is able to affect nitrification as well, inhibiting ammonia monooxigenase enzyme right from low partial pressures (1-10 Pa) (Fig.2-13).

As far as concern the inhibition of nitrous oxide reduction in denitrifying bacteria, the nitrous oxide reductase enzyme is non-competitively inhibited by acetylene and easily recovers after all the acetylene is evaporated out of the soil, since acetylene simply reduce efficiency of nitrate as an electron acceptor (Baldertson et al., 1976; Erich, 1980; Kristjansson and Hollocher, 1980; Terry and Duxbury, 1985; Kester et al., 1996, 1997).

Nitrification recovery is slower than denitrification recovery after acetylene exposure, since acetylene is a suicide substrate for the ammonia monooxigenase enzyme, and inhibition is irreversible (Walter et al., 1979; Haynes and Knowles, 1982; Hyman and Wood, 1985; Hyman and Harp, 1992).

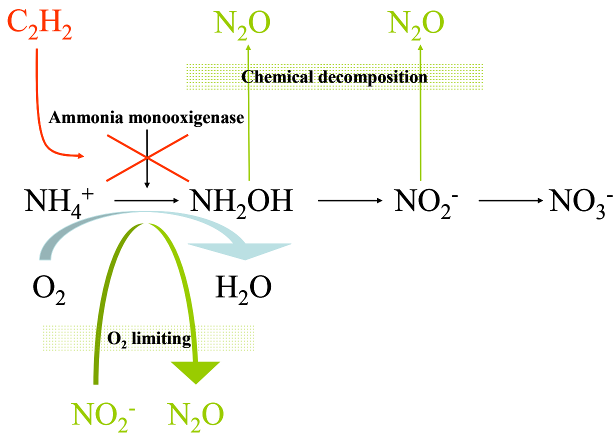


Figure 2-13: Ammonia monooxigenase inhibition by acetilene. All pathways for N_2O production by nitrification are blocked as well.

On each sampling date two separate set of intact soil cores were collected close to each other in the field. One set was kept as control (no acetylene addition) and used to determine N_2O emission from both nitrifying and denitrifying bacteria; the other one was processed to estimate the denitrifier contribution to N_2O fluxes from soil.

Nitrification contribute was excluded by exposing the soil cores for 1 hour to high partial pressure of acetylene (10% of headspace volume) (Fig.2-14).

Differently from the AIT for denitrification rate assessment, the air space inside the container was not mixed by large syringe; in this way acetylene was allowed to reach the nitrifier sites readily accessible to diffusing gas, while contact with denitrifier community was reduced. Aftyerwards top lids were removed and acetylene was allowed to evaporate. At 30 hours soil cores were sealed inside the containers again and N_2O accumulation at constant field temperature was measured.

The difference of N_2O production rate per sample (the mean values from all the cores collected on one day) between the control set and the nitrification inhibition set, was used to calculate the contribution of nitrifier bacteria to N_2O evolved from soil, expressed as a percentage:

$$\mathbf{N_2O_{nit}= N_2O_{con} - N_2O_{den}}$$

and

$$\mathbf{N_2O_{nit}\%= ((N_2O_{con} - N_2O_{den})/ N_2O_{con}) \times 100}$$

where:

N_2O_{con}= N_2O fluxes from control cores (µg N_2O-N m^{-2} h^{-1})

N_2O_{den}= N_2O fluxes from nitrification inhibited cores (µg N_2O-N m^{-2} h^{-1})

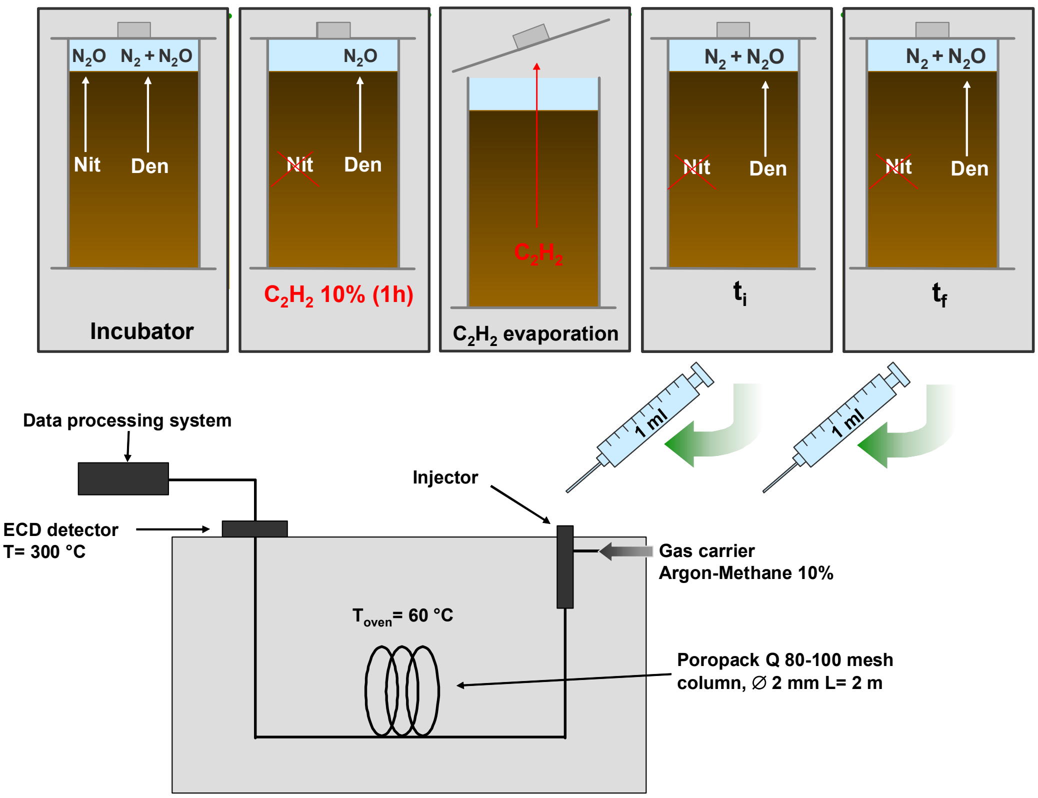


Figure 2-14: Illustrative scheme of the Short exposure to acetylene method, to distinguish between nitrifier and denitrifier N_2O production.

The method of short exposure to high partial pressure of acetylene was chosen instead of the more widespread PPM method (Klemedsson et al., 1988 a), since collateral inhibition of nitrous oxide reduction with 1 to 10 Pa acetylene (a major drawback of the PPM method causing the underestimation of nitrifier N_2O production) may be serious above all in soil characterized by high levels of denitrifying activity (Klemedtsson and Hansson, 1990).

Considering that the experimental site is an agricultural field subjected to no-stop inputs of fertilizer N and therefore exhibiting high potential for N losses through denitrification, the short exposure to high acetylene partial pressure method was chosen to avoid the problem of nitrifier N_2O production underestimation, above all as far as concern the fine textured soils.

Anyway the method didn't work for clay soils because of the slow and incomplete evaporation of acetylene out of the cores.

2.2.6 Statistical analyses

The statistical analyses were performed using SigmaStat 9.0.

All mean values were calculated as arithmetic mean and bars in the graphs represent standard error ot the mean.

Significant differences between two sample populations were tested by the Mann Whitney-Test ($P<0,05$), except as regard the assessment of the relative nitrifier and denitrifier contributions to N_2O emission from soil, when significant differences between control and nitrification inhibited soil cores were checked by the Mann Whitney-Test, set for unequal variance ($P<0,05$).

Multiple comparisons where performed by the One Way ANOVA Holm-Sidak-Test ($P<0,05$).

Simple correlation (Pearson product-moment Test, $P<0,05$) and regression analyses were performed to assess significant relationships and dependences between parameters, respectively.

Normal distribution of data was always checked before running correlation analyses (Kolmogorov-Smirnov Test, $P<0,05$) and if necessary data were log-transformed.

All data for regression analyses, both linear and non linear, passed the Durbin-Watson Statistic Test, Normality Test and Constant Variance Test

3 MONITORING OF DENITRIFYING ACTIVITIES AND N_2O FLUXES FROM THE CLAY TEXTURED SOIL.

3.1 INTRODUCTION

In this study denitrification rates and N_2O fluxes from soil were monitored in the part of the agricultural field characterized by clay soil profile (relating to most of the total cropped surface area inside the farm).

Denitrification is considered the main source for N_2O emissions from soils, above all in fine textured soils, characterized on the whole by high colloids content and water retention capacity (McKeeney et al., 1980; Webster and Dowdell, 1982; Matson et al., 1990; Skiba et al., 1992; Granli and Bockman, 1994).

Anyway, in soil with very high clay content, reduction of N_2O to N_2 can be favoured under pronounced anaerobic conditions and a relevant amount of N_2O gas can be retained into the soil profile, since dissolved in the soil aqueous phase or because of physical barriers limiting diffusion to the atmosphere (Arah et al., 1991; Granli and Bockman, 1994).

In effect, it's not simple to predict the interaction of the various factors controlling denitrification in soil, and denitrifying activity can show a great variability not only depending on different soil textures but also inside the same soil. It is in fact among the soil processes exhibiting the highest values of spatial variability as usually confined to *hotspots* (i.e. soil aggregates characterized by anoxic microsite, high nitrate availability and organic carbon content), not homogeneously distributed along the soil profile (Parkin, 1987; Parkin et al., 1987; Rice et al., 1988; Paul e Beauchamp, 1989; Parkin, 1990; Parson et al., 1991; Petersen et al., 1991; De Klein e Van Longtestijn, 1995; Nielsen et al., 1996; Nielsen e Revsbech, 1997; Kester et al., 1997; Abbasi e Adams, 2000; Simek et al., 2004).

Several studies investigated the enhancing effects on denitrifying activity of irrigation (Freney et al., 1985; Ryden and Lund 1980; Mahmood et al., 1998) and both mineral N fertilizers (Simek et al., 2000; Dambreville et al., 2006) and zootecnical slurry application (Arcara et al, 1999; Rochette et al., 2000; Simek et al., 2000; Henault et al., 2001; Dambreville et al., 2006).

Anyway only few study have been performed up to the present in irrigated crops under

Mediterranean climate conditions (Teira-Esmatges et al., 1998; Sanchez et al., 2001; Vallejo et al, 2004) notwithstanding they might be potentially high N_2O emitters via denitrification as high moisture content due to irrigation coincide with high soil temperatures, favouring the process as well (Maag and Vinther, 1999).

For istance Vallejo (Vallejo et al., 2004) pointed out relevant N losses by denitrification (up to 4 g N m^{-2}) during the irrigation period in a Mediterranean irrigated maize crop in central Spain (mean annual rainfall 460 mm and daily mean temperature ranging from 13.5 °C to 30 °C during the maize cropping cycle), much higher than those ones detected by Arcara (Arcara et al., 1999) from a non-irrigated maize crop in North Italy (0,397 g N m^{-2}) under less Mediterranean conditions (mean annual rainfall 920 mm and daily mean temperature ranging from 10 °C to 27 °C during the maize cropping cycle).

3.2 EXPERIMENTAL SET-UP

Measurements of actual denitrification rate (r_{den}) and N_2O fluxes from soil surface were performed inside the part of the field characterized by clay profile during the *Lolium italicum* (Sep'04 – Apr'05) and the *Zea mays* (Jun'05 – Aug'05, Jun '06 – Sep '06) crops (Fig.3-1).

Figure 3-1: Aerophotogram of the agricultural field. Dark blue squares show the experimental plots (15 m x 15 m) for monitoring activities related to the *Lolium italicum* crop and the *Zea mays* crop in 2005 (May '05 – Aug '05); the light blue square show the experimental plot (15 m x 15 m) for monitoring activities during the *Zea mays* crop in 2006 (Jun '06 – Sep '06).

As far as concern *Lolium italicum*, the analysis of actual denitrification rate and N_2O fluxes from soil started about 50 days after sowing, while, during the *Zea mays* crop in 2005, they were performed throughout the growing period, from corn sowing to mowing (Table 3-1).

Table 3-1: Analyses performed at the clay sites during the *Lolium italicum* and *Zea mays* crops. The numbers specify field replicates for each kind of measurements on each sampling day.

	Sampling date	r_{den}	N_2O fluxes	pH	WFPS	NO_3^--N	NH_4^+-N	OM %
Lolium italicum	20/10/04		8	8	8	8		8
	17/11/04	8	8	8	8	8		8
	15/12/04	8	8	8	8	8		8
	01/02/05	12	8	8	12	8		8
	03/03/05	12	8	8	12	8		8
	17/03/05	12	8	8	12	8		8
	06/04/05	12	8	8	12	8		8
Zea mays 2005	23/05/05	12	8	8	12	8		8
	26/05/05	12	8	8	12	8		8
	30/05/05	12	8	8	12	8		8
	08/06/05	12	8	8	12	8		8
	29/06/05	12	8	8	12	8		8
	20/07/05	12	8	8	12	8		8
	28/07/05	12	8	8	12	8		8
Zea mays 2006	04/07/06	4	4	4	4	4	4	4
	11/07/06	4	4	4	4	4	4	4
	12/07/06 pre-irr	4	4	4	4	4	4	4
	12/07/06 post-irr	4	4	4	4	4	4	4
	17/07/06	8	8	8	8	8	8	8
	24/07/06	4	4	4	4	4	4	4

On each sampling date intact soil cores (∅= 5 cm, h= 15 cm) for actual denitrification rate assessment were collected close to the cover box collars placed in the field; after $\mathbf{r}_{den}$ calculation the same cores were processed for WFPS determination. Moreover 4 separate cores were sampled in each plot as well, for soil physico-chemical characterization.

Further measurements of actual denitrification rate and N_2O fluxes from soil with clay texture were performed during the maize growing season in 2006 at the late fertilization (Fig.3-1 and Table 3-1).

At that time, to study in more detail spatial variability of denitrification and N_2O fluxes from soil, and analyse at what extent it can be attributed to spatial variability of driving parameter such as soil NO_3^- concentration and WFPS, on each sampling date intact soil cores (∅= 5 cm, h= 15 cm) for $\mathbf{r}_{den}$ calculation were sampled from soil surface inside the cover box collars placed in the field; part of the undisturbed soil cores was then gently removed by a sharp knife before starting $\mathbf{r}_{den}$ measurements, and processed for soil physico-chemical characterization: soil temperature, pH, gravimetric water content, organic matter and N-mineral concentration (NO_3^- + NH_4^+). One further undisturbed soil core was collected on each sampling date for bulk density assessment.

3.3 Results and discussion

3.3.1 Soil temperature, pH and organic matter

Soil temperature showed a seasonal pattern with higher values in summer time (Fig.3-2).

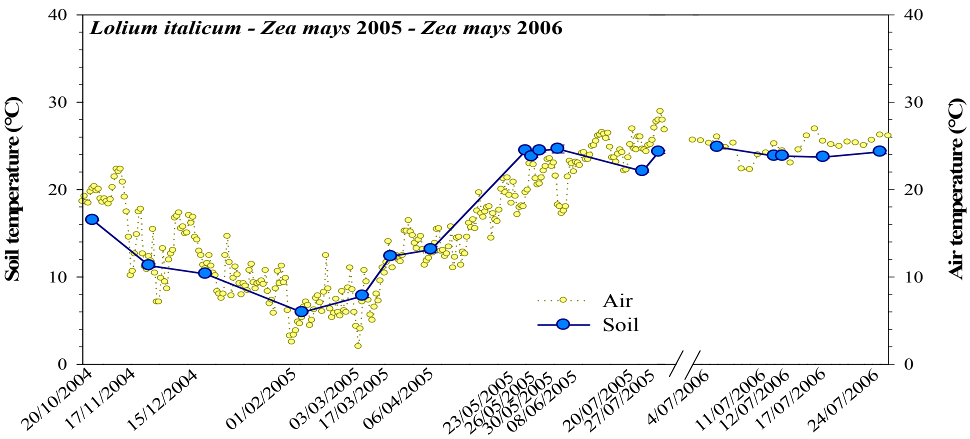


Figure 3-2: Soil temperature at the experimental site during the observation period; air temperature is shown as well.

Soil pH was subalkaline on average (Table 3-2), in agreement with the high presence of carbonates in soil. Values closer to neutrality were detected on sampling dates after winter rains and irrigation events in summer period.

Organic matter content in soil was high (>5%) and didn't show significant variations in time (Table 3-2).

Table 3-2: Mean values and standard errors of soil pH and organic matter content in the course of the *Lolium italicum* and the *Zea mays* growths.

	Sampling date	pH	OM%s
Lolium italicum	17/11/04	7.43±0.02	8.0±0.7
	15/12/04	7.07±0.02	6.8±0.5
	01/02/05	7.33±0.07	7.6±0.2
	03/03/05	7.21±0.03	8.70±0.5
	17/03/05	7.55±0.02	6.91±0.6
	06/04/05	7.81±0.04	6.97±0.2
Zea mays 2005	23/05/05	7.96±0.02	8.2±0.2
	26/05/05	7.39±0.09	7.4±0.8
	30/05/05	7.09±0.03	8.2±0.5
	08/06/05	7.12±0.08	8.4±0.2
	29/06/05	6.79±0.10	9.0±0.3
	20/07/05	8.52±0.02	8.7±0.2
	28/07/05	8.41±0.06	8.2±0.5
Zea mays 2006	04/07/06	7.12±0.02	8.1±0.2
	11/07/06	7.39±0.04	8.3±0.4
	12/07/06 pre-irr	7.35±0.04	8.0±0.5
	12/07/06 post-irr	7.07±0.02	8.5±0.2
	17/07/06	7.31±0.04	8.2±0.6
	24/07/06	7.81±0.03	8.7±0.4

3.3.2 Soil moisture and WFPS

The trends observed for both soil moisture and WFPS throughout the observation period can be related to winter rainfalls and irrigation events, needed to support crop growth and development in summer period (Figg.3-3, 3-4, 3-5).

In detail, as far as concern *Lolium italicum* grow, high values were recorded in winter time after heavy rains while a decrease was noticed starting from March 2005 (Fig.3-3).

Much lower values were detected during maize crop both in 2005 and 2006, with the highest ones being recorded on sampling dates soon after irrigation events (Figg.3-4, 3-5).

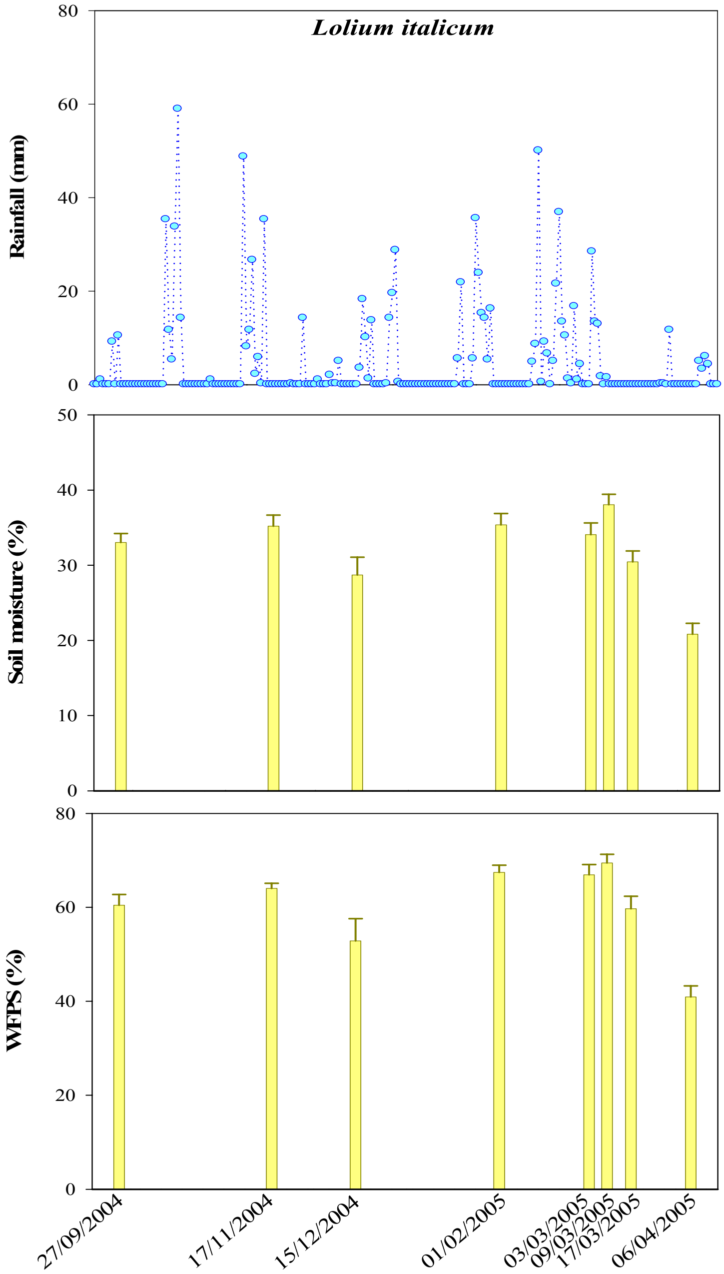


Figure 3-3: Mean values and standard errors for soil moisture and WFPS in the course of the *Lolium italicum* crop; rainfalls are showed as well.

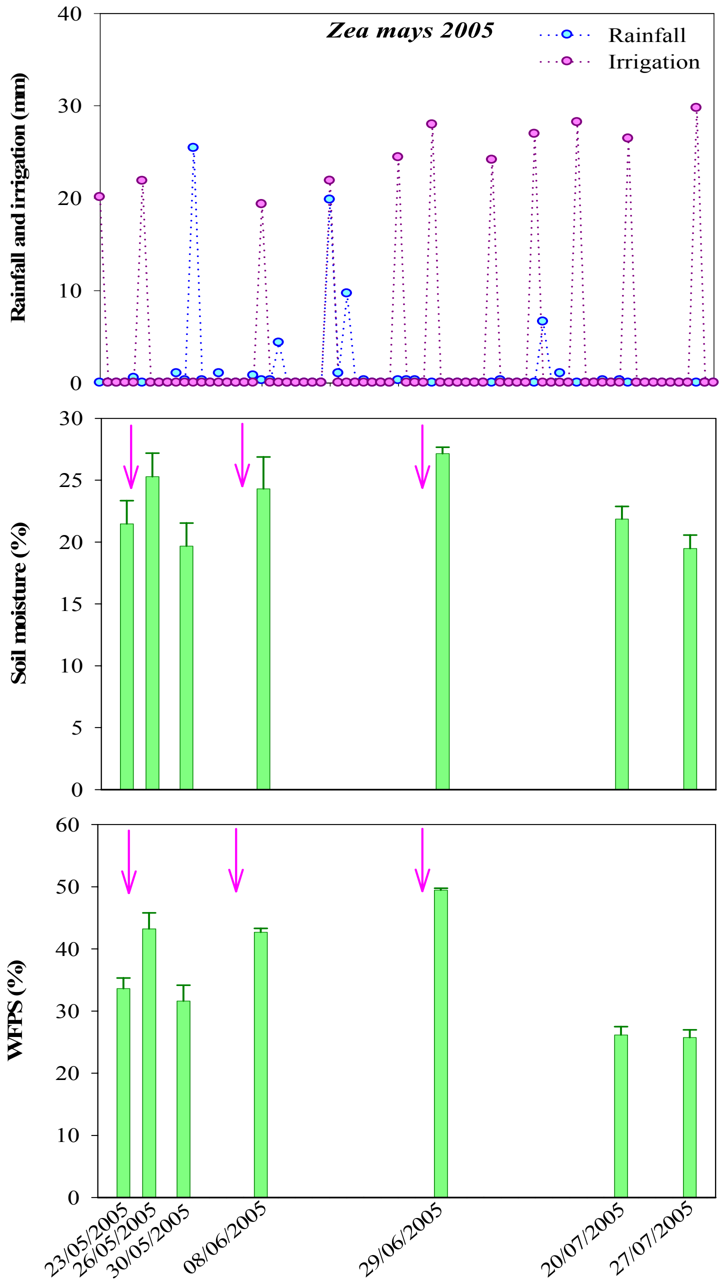


Figure 3-4: Mean values and standard errors for soil moisture and WFPS in the course of the *Zea mays* crop in 2005; rainfall and irrigation events are showed as well. The pink arrows indicate irrigation events.

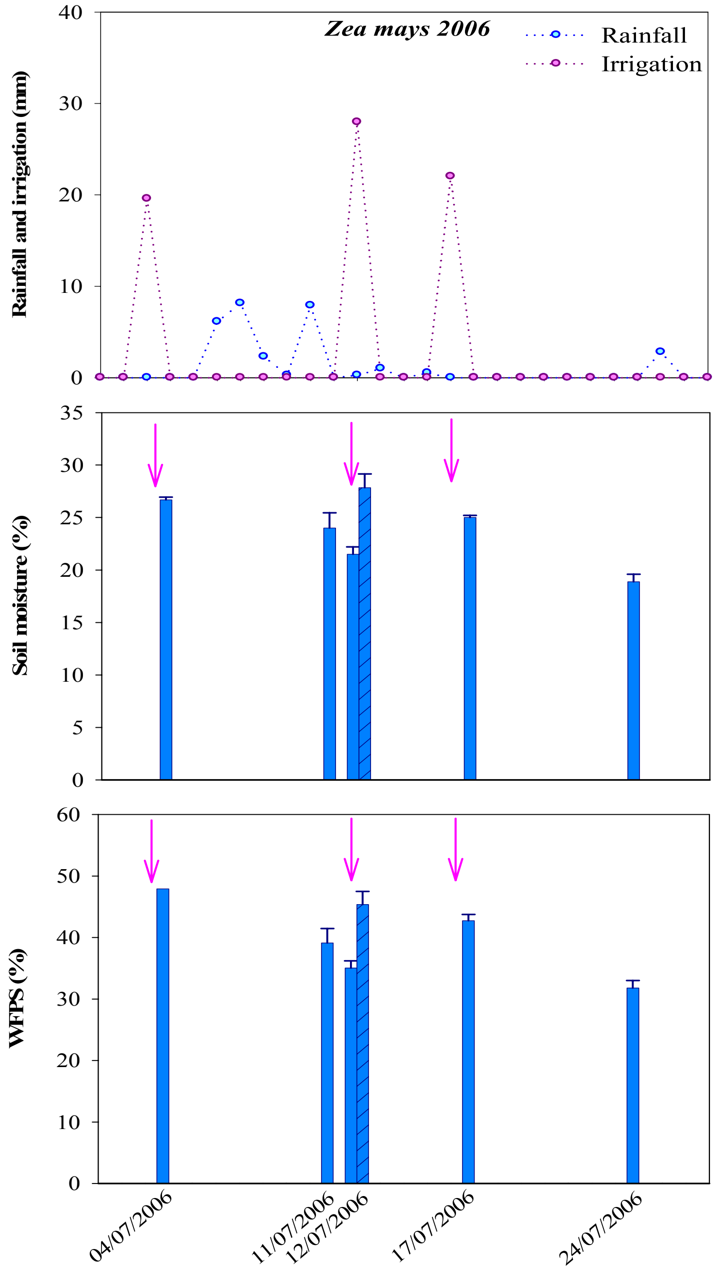


Figure 3-5: Mean values and standard errors for soil moisture and WFPS in the course of the *Zea mays* crop in 2006; rainfall and irrigation events are showed as well. On 12/07/2006 date the dotted bar represents post-irrigation sampling time. The pink arrows indicate irrigation events.

3.3.3 Soil NO_3^- and NH_4^+ concentration

Huge variations of soil nitrate concentration were found throughout the observation period, depending on N inputs as fertilizer and processes depleting soil NO_3^--N pool such as bacterial denitrifying activities, plant uptake and leaching.

For istance the steep decrease of nitrate concentration detected two months after *Lolium italicum* sowing (Fig.3-6) is probably the resultant of radical absorption (Pandey et al., 2000), microbial immobilization (Nielsen e Revsbech, 1997) and leaching through autumnal and winter rains, as demonstrated by the higher nitrate concentration detected in the deeper soil layers (10-20 cm and 20-30 cm) than in the top ones (0-10 cm) during winter period (Fig.3-7).

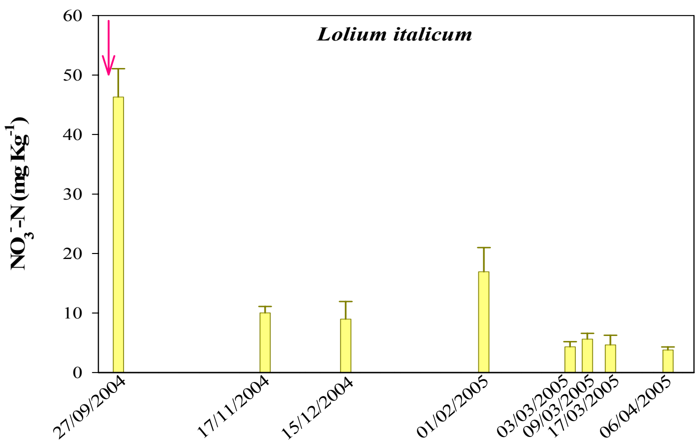


Figure 3-6: Mean values and standard errors for soil NO_3^--N concentration in the course of the *Lolium italicum* crop. The pink arrow indicates the sowing mineral fertilization.

During *Zea mays* crop in 2005, soil nitrates had a concentration peak soon after fertilizer N supply at the sowing and late fertilization times (Fig.3-8). After that, they decreased fast starting from the first irrigation events following fertilizations. This step-down is probably due to the combined effects of plant uptake and consumption through bacterial activities, such as denitrification, after soil rewetting. It might be argued leaching by irrigation is probably not a critical factor in nitrate loss from the system since, because of the high temperatures, water movement along the soil profile during summer time in Mediterranean regions, is usually from the bottom to the top.

Anyway this general trend can temporarily reverse following rain or irrigation events, therefore leading to a movement of soil nitrates towards deeper soil layer (with percolation outside the root-zone only in case of unusual heavy rain and/or overirrigation events).

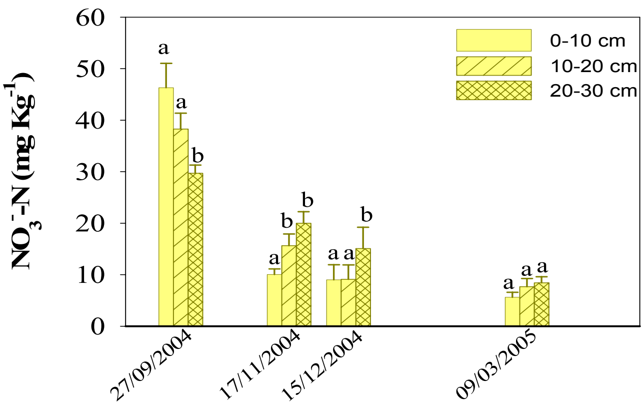


Figure 3-7: Mean values and standard errors for soil NO_3^--N concentration at different depth along the soil profile in the course of the *Lolium italicum* crop. Different letters point out significant differences between soil layers on each sampling date (One Way ANOVA Holm-Sidak Test, P<0,05).

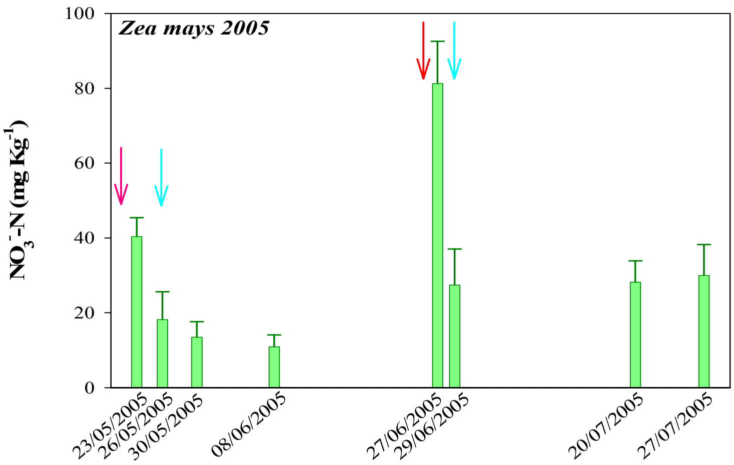


Figure 3-8: Mean values and standard errors for soil NO_3^--N concentration during *Zea mays* crop in 2005. The pink, red and cyan arrows indicate the sowing mineral fertilization, the late fertilization and irrigation events respectively.

Also during *Zea mays* crop in 2006, nitrate availability in soil increased soon after the late fertilization, anyway much higher concentration on average were detected in soil compared with similar crop growing stages in 2005 (Fig.3-9).

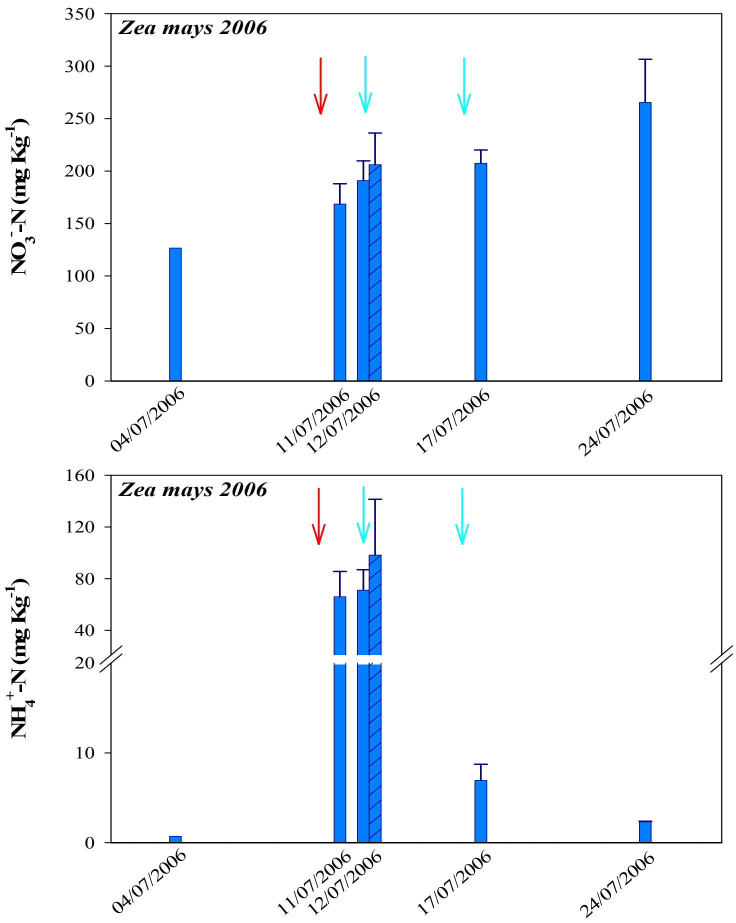


Figure 3-9: Mean values and standard errors for soil NO_3^- and NH_4^+ concentration in the course of the *Zea mays* crop in 2006. On 12/07/2006 date the dotted bar represents post-irrigation sampling time while the red and cyan arrows indicate the late fertilization and the irrigation events respectively.

These higher soil nitrate concentrations can be explained considering the different crops preceding *Zea mays* and the different kind and amount of fertilizes N applied in 2005 and 2006 (see section 2.1.3).

Differently from *Zea mays* in 2005, grown after a *Lolium italicum* grass crop, *Zea mays* in 2006 was preceded by a mixed cultivation of *Lolium italicum* and *Trifolium Alessandrinum* (legume).

Leguminuos species are able to fix the N they need from the atmosphere and when grown in a rotation preceding an N-requiring crop they may result in a high level of residual N in the soil that can be utilized by the following crop, up to 40 kg N ha^{-1} for leguminous monocultures (CDPA, 1999).

Moreover, before sowing, *Zea mays* in 2006 received an amount of buffalo slurry sewage 4-5 times as big as maize crop in 2005 and, at the late fertilization time, part of the urea fertilizer spread on the field (50% of the total amount applied) did not contain the 3.4 DMPP nitrification inhibitor, evidently causing a faster nitrification of soil NH_4^+ pool (Fig. 3-9).

3.3.4 Actual denitrification rate

Denitrification rate showed great variability, with coefficient of variation (CV) ranging from 10% up to 185,8% (mean value at about 90%), in agreements with the large variation often found for this parameter in field studies (Nielsen et al., 1996; Nielsen e Revsbech, 1997; Kester et al., 1997; Abbasi e Adams, 2000; Simek et al., 2004)

Denitrification trend throughout the observation period (Fig.3-10) can be explained on the basis of the combined effect of changing NO_3^- concentration and WFPS in soil.

As far as concern the winter grass crop (Fig.3-11), the highest values of $\boldsymbol{r_{den}}$ were detected after heavy rains in February, at high values of WFPS (about 70%) and not limiting soil NO_3^- concentration.

At the beginning of March, at still high values of WFPS, denitrifying activity showed a steep decrease evidently caused by soil NO_3^- reduction through leaching and plant uptake; afterwards slight values of $\boldsymbol{r_{den}}$ were measured as a consequence of both decreasing soil moisture and low soil nitrate concentration.

On the whole a positive correlation was found between $\boldsymbol{r_{den}}$ and soil NO_3^- concentration (Fig.3-12) while no significant relation was detected between $\boldsymbol{r_{den}}$ and WFPS, since nitrates were probably limiting for the most of the observation period.

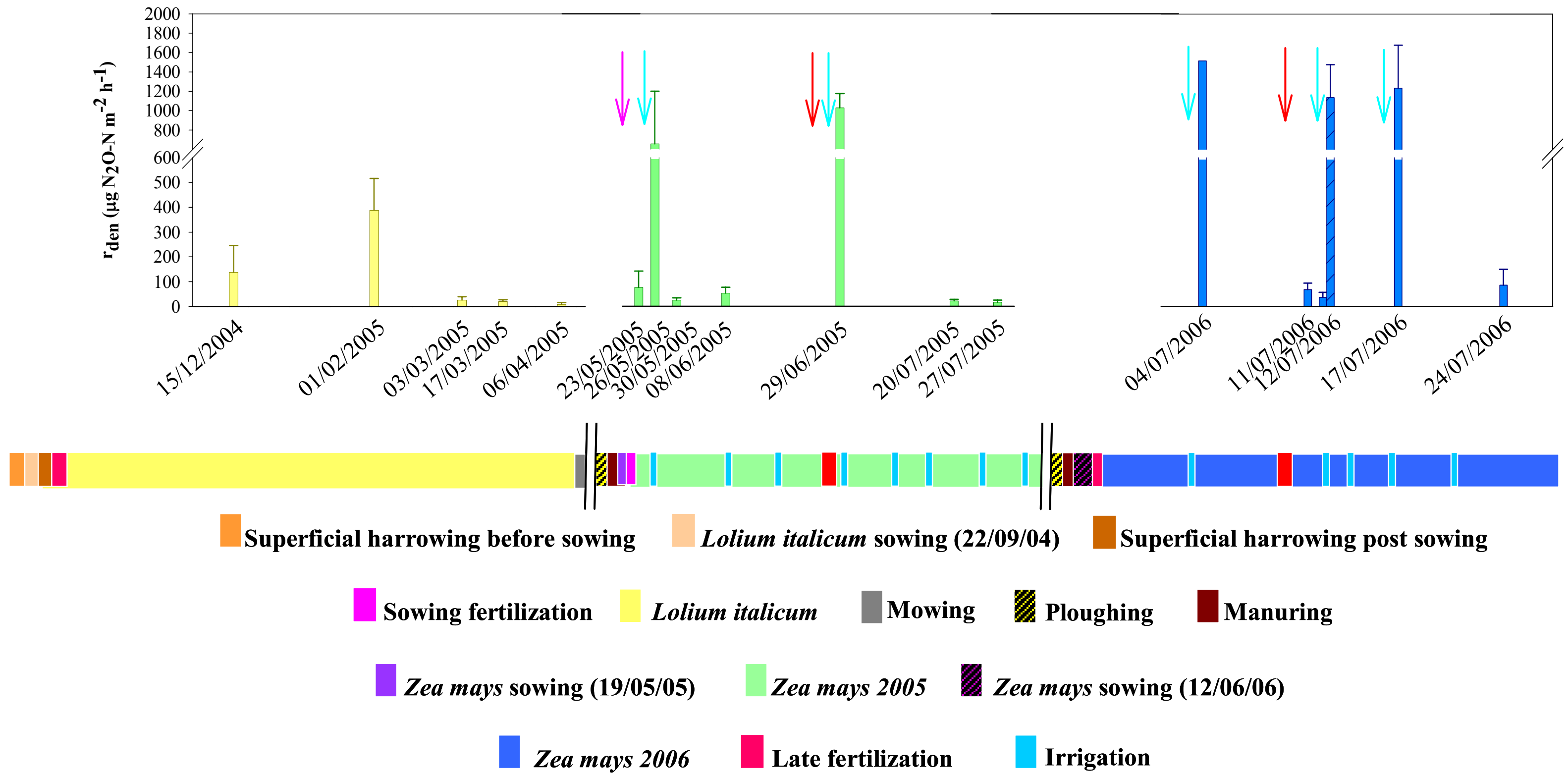


Figure 3-10: Mean values and standard errors for actual denitrification rate (r_{den}) throughout the observation period. The pink, red and cyan arrows indicate the sowing mineral fertilization, the late fertilization and the irrigation events respectively.

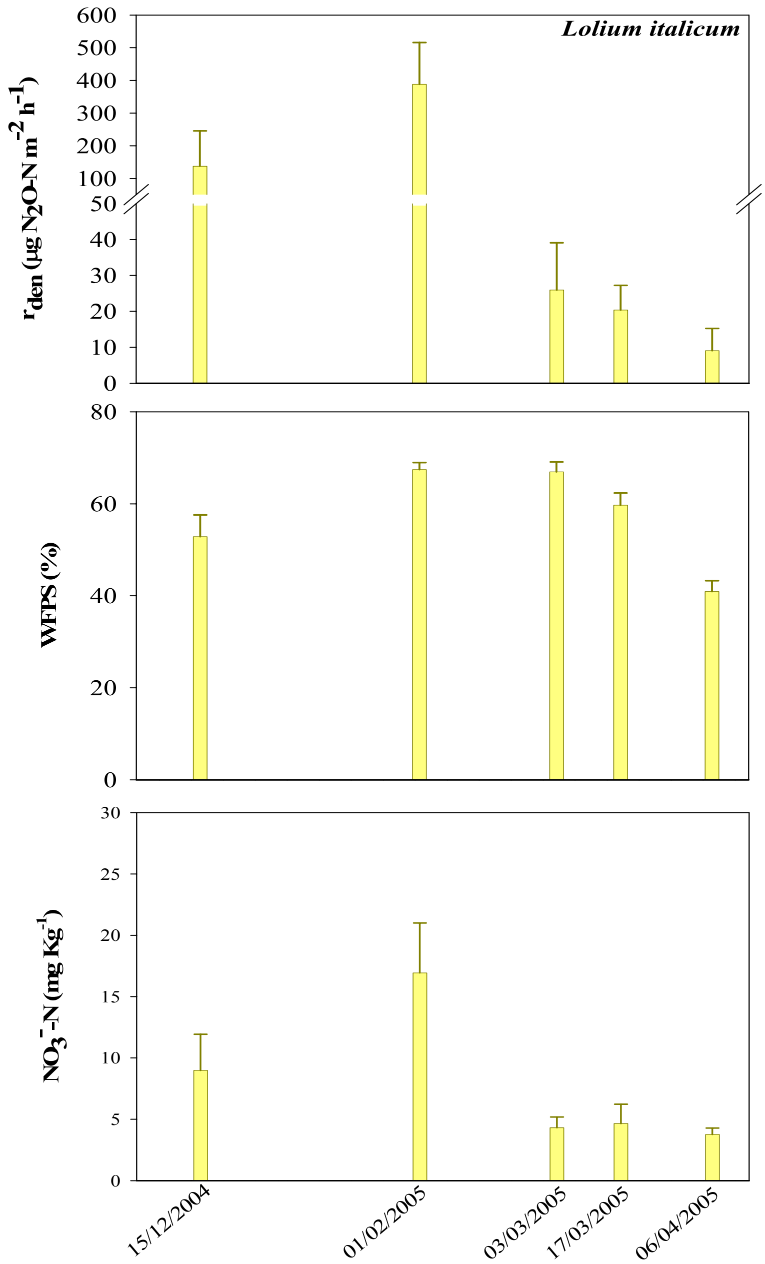


Figure 3-11: Mean values and standard errors for actual denitrification rate (r_{den}), soil NO_3^- and WFPS in the course of the *Lolium italicum* growth period.

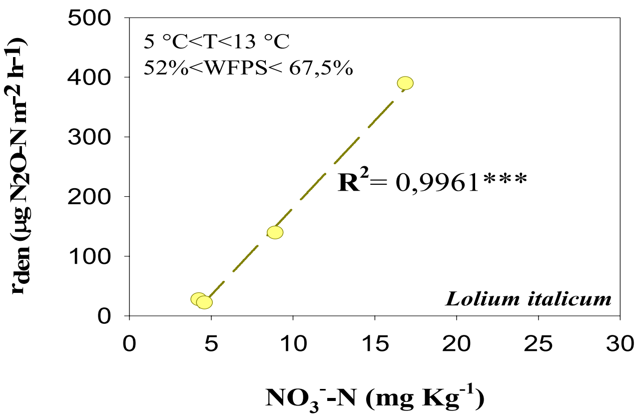


Figure 3-12: Actual denitrification rate (r_{den}) v.s. soil nitrate concentration (mean values from each sampling date) on the course of the *Lolium italicum* growth period (Pearson product-moment Test: * P< 0,05, ** P< 0,01, *** P< 0,001).

In the course of the maize growth period in 2005, peaks of denitrifying activity were recorded soon after the first irrigation events following both the mineral sowing fertilization and the late fertilization (Fig.3-13).

The activation of denitrification process was clearly due to the increased WFPS following irrigation, at high soil NO_3^- concentration and temperature (Arcara 1999; Maag and Winther, 1999; Vallejo et al., 2003; Vallejo et al., 2004)

Otherwise, when soil NO_3^- concentration and/or WFPS were limiting, denitrifying activities were quite slight. For istance, no peak of denitrification rate was detected soon after the mineral-N supply at the sowing time, probably because of limited microbial activity at low WFPS (Mahmood et al., 1998; Strong e Fillery, 2002); similarly slight values of r_{den} were found after the second irrigation event following the sowing fertilization, probably as a consequence of the decreased NO_3^- concentration in soil (Fig.3-13).

Anyway a significant correlation was found only between r_{den} and WFPS at NO_3^--N concentrations above 15 µg g^{-1} (Fig.3-14), while actual denitrification rate showed no significant relation with soil nitrate, in consequence of the slight number of data at not limiting WFPS values.

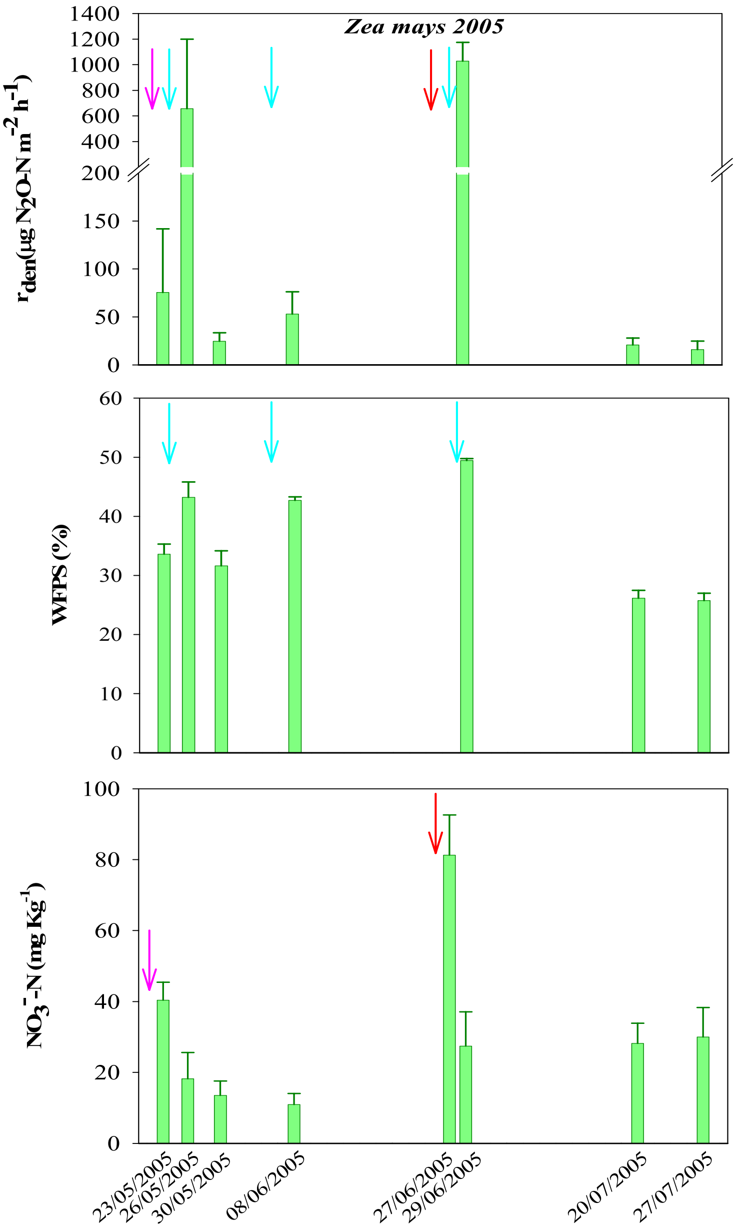


Figure 3-13: Mean values and standard errors for actual denitrification rate (r_{den}), soil NO_3^- and WFPS in the course of the *Zea mays* growth in 2005. The pink, red and cyan arrows indicate the sowing mineral fertilization, the late fertilization and the irrigation events respectively.

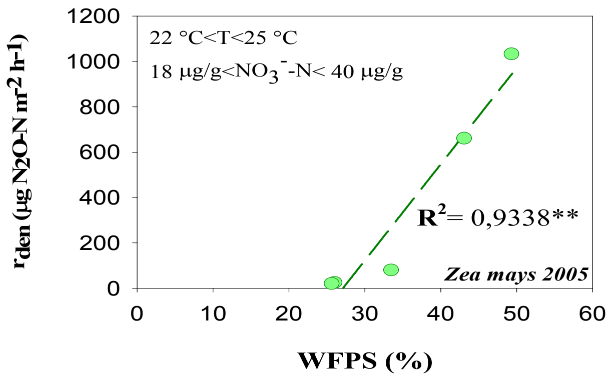


Figure 3-14: Actual denitrification rate (*r_{den}*) v.s. soil WFPS (mean values from each sampling date) on the course of the *Zea mays* growth period in 2005 (Pearson product-moment Test: * P< 0,05, ** P< 0,01, *** P< 0,001).

Finally during *Zea mays* crop in 2006, given the high soil nitrate availability, WFPS appeared the only limiting factor, with peak of denitrifying activity being detected at WFPS above 40% (Figg.3-15, 3-16). Moreover, at similar values of WFPS, despite the much higher soil nitrate concentrations, denitrification appeared to peak up to values as high as those ones detected in the course of the maize crop in 2005, suggesting NO_3^- concentrations in soil were probably so high to be not limiting any more.

It's noteworthy that the maximum values of denitrification rate recorded through the observation period (on average about 1500 µg N_2O-N m^{-2} h^{-1}), every time soil nitrates and WFPS favoured the process, were consistently higher than the maximum values reported by Arcara (Arcara et al., 1999) on intact soil cores from the silty clay soil of a Mediterranean non-irrigated maize crop in Modena (Northen Italy), under both urea and pig slurry fertilization (on average about 287 µg N_2O-N m^{-2} h^{-1} with a single peak up to 690 µg N_2O-N m^{-2} h^{-1}).

It might be argued this is a consequence of the less dry climate during the maize cropping season in Modena, with soil WFPS kept quite high by frequent rainfall events. In fact it has often been found that denitrification peaks at higher values when soils are going through wetting/drying cycles than when soil water content is constantly high (Smith and Patrik, 1983; Granli and Bockman, 1994).

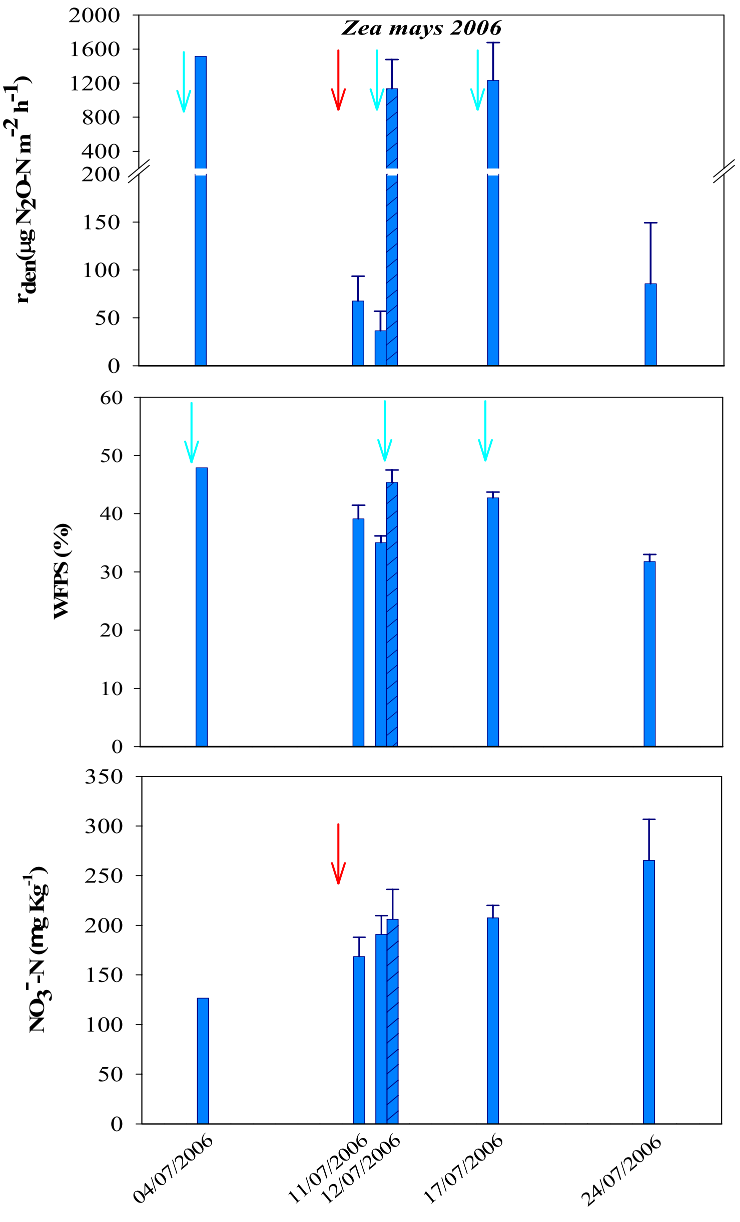


Figure 3-15: Mean values and standard errors for actual denitrification rate (rden), soil NO_3^- and WFPS in the course of the *Zea mays* growth period in 2006. On 12/07/2006 date the dotted bar represents post-irrigation sampling time while the red and cyan arrows indicate the late fertilization and the irrigation events respectively.

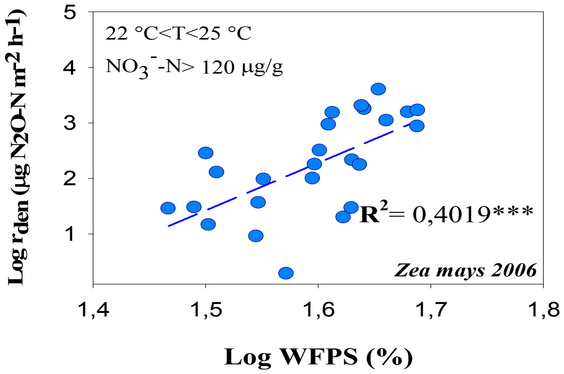


Figure 3-16: Actual denitrification rate (r_{den}) v.s. soil WFPS (from each intact soil core) in the course of the *Zea mays* growth period in 2006 (Pearson product-moment Test: * P< 0,05, ** P< 0,01, *** P< 0,001).

On the other hand, maximum denitrification rates detected in this study were much lower than denitrifying peaks recorded by Vallejo (Vallejo et al., 2003) on intact soil cores from a sandy loam soil of a Mediterranean irrigated maize crop in central Spain, fertilized with different organic N fertilizers (about 2050 μg N_2O-N m^{-2} h^{-1}; 3690 μg N_2O-N m^{-2} h^{-1}; 6150 μg N_2O-N m^{-2} h^{-1} and 8487 μg N_2O-N m^{-2} h^{-1} for urea, surface applied pig slurry, incorporated pig slurry and sheep manure treatments, respectively).

Anyway, beyond comparable soil NO_3^- concentration, at the experimental field less water was supplied by the means of irrigation practice (about from 20 mm to 30 mm weekly) than at the Spanish site (about from 40 mm to 60 mm weekly) and lower WFPS were detected throughout the maize cropping seasons despite the higher soil WHC (for the Spanish sandy loam soil WFPS is 62% for field capacity).

3.3.5 N_2O fluxes from soil

N_2O fluxes from soil (Fig.3-17) showed a very high spatial variability as well (mean value of CV at 85,3%), as often reported for trace gas fluxes (Ambus and Christensen, 1995; Velthof and Oenema, 1995; Kester et al., 1997; Abbasi e Adams, 2000; Simek et al., 2004).

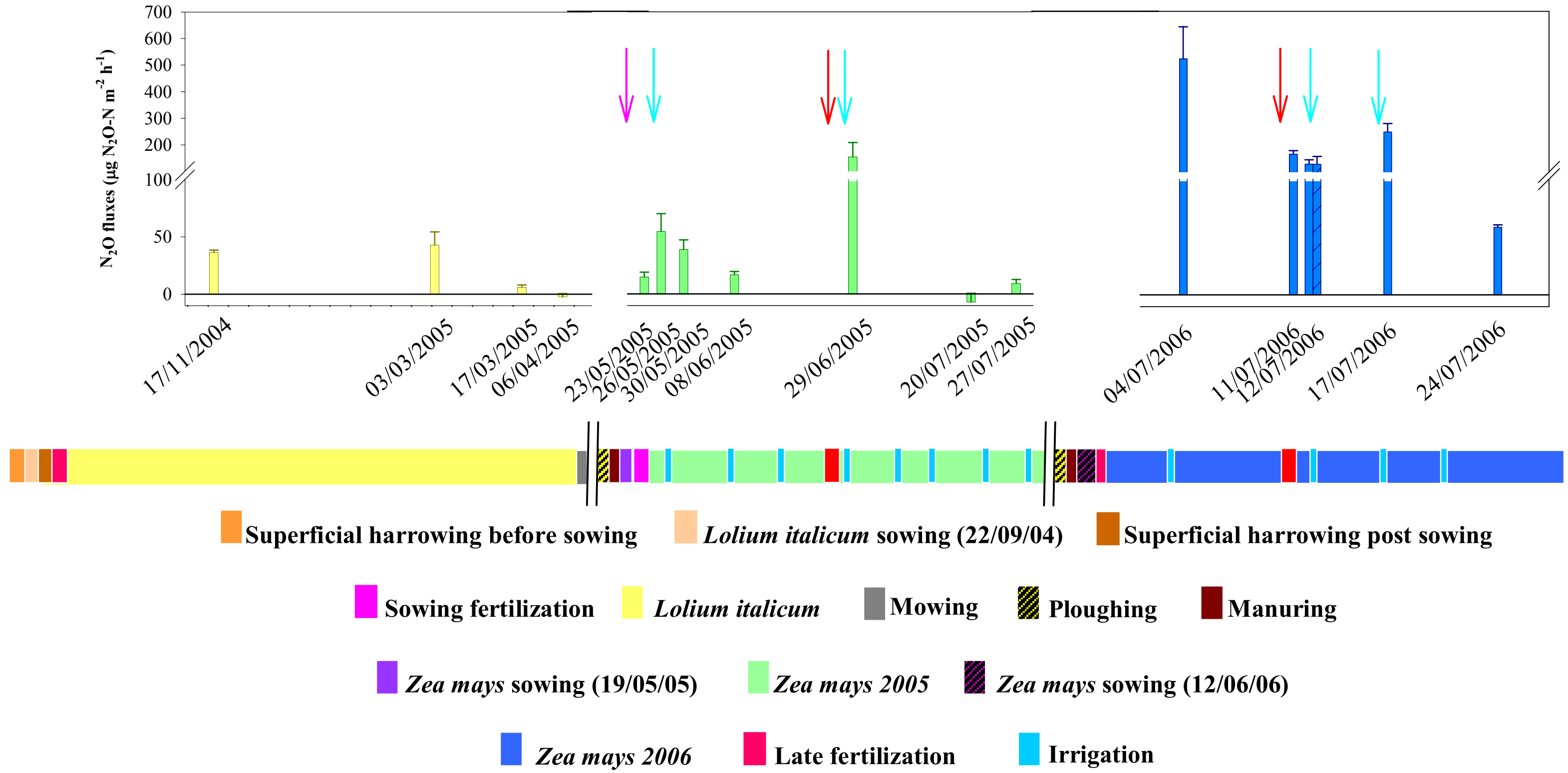


Figure 3-17: Mean values and standard errors for N_2O fluxes throughout the observation period. The pink, red and cyan arrows indicate the sowing mineral fertilization, the late fertilization and the irrigation events respectively.

On the whole, they were in the ranges of low- medium values, with peaks being detected after irrigation events during the maize cropping cycles, on sampling dates when denitrification rates were intense. As already pointed out for actual denitrification rate, also variation of N_2O emissions throughout the observation period can be explained on the basis of the combined effect of mineral-N supply and WFPS in soil.

During the *Lolium italicum* growing season, the highest values of N_2O fluxes from soil were detected before the complete loss of soil nitrates through leaching and plant uptake. Afterwards a steep reduction of both denitrifying activity and N_2O evolution from soil occurred (Fig.3-18).

In the course of the maize growth in 2005 (Fig.3-19), remarkable N_2O fluxes were detected together with the peaks of denitrifying activity, as already pointed out (section 3.3.4) in consequence of the favouring combined effects of high soil nitrate concentration, WFPS and temperature (Arcara 1999; Maag and Winther, 1999; Vallejo et al., 2003; Vallejo et al., 2004).

Anyway, even if N_2O fluxes revealed to raise at increasing nitrates, no significant relation was found with soil NO_3^- concentration, because of the scanty data available at not limiting WFPS values. Differently, positive correlations were detected between N_2O fluxes and both WFPS and actual denitrification rate (Figg.3-20 B, 3-21).

Higher N_2O fluxes were detected on average during the maize grow in 2006 at the late fertilization time (Figg.3-17 and 3-22) compared with similar crop growing stages in 2005, probably as a consequence of the higher soil nitrate concentrations enhancing the N_2O/N_2 product ratio of bacterial denitrification (Nomik, 1956; Blakmer and Bremner, 1978; Bremner, 1978; Fireston et al., 1980; Vinther , 1984; Christensen, 1985; Ottow et al., 1985, Kroeze et al, 1989).

The highest values were detected on sampling dates following irrigation events, except on 12/07/2006, when, contrary to the high denitrification rates, only slight N_2O fluxes were recorded in situ from soil surface. Since on that date N_2O emissions from soil were measured soon after the pivot passage above the crop, it can be assumed not enough time had passed for N_2O to be released from soil surface after being produced by microbial denitrification.

A positive correlation was found between N_2O fluxes and soil nitrates at WFPS> 40%, while at WFPS< 40% the amount of N_2O produced appeared to increase at increasing values of soil NH_4^+ concentration (Fig.3-23, A and B).

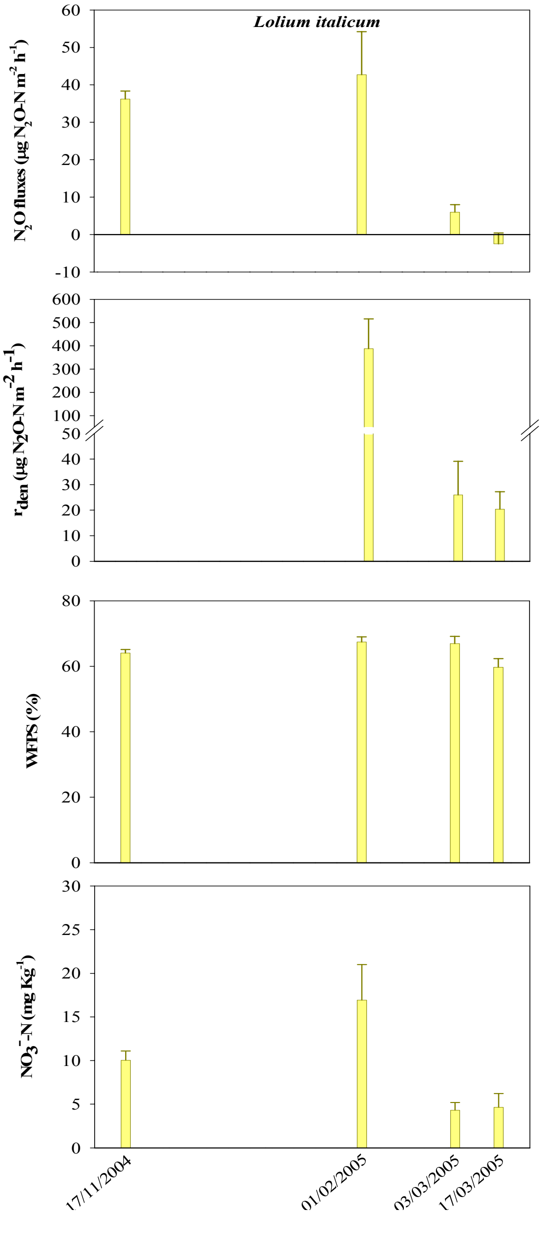


Figure 3-18: Mean values and standard errors for N_2O fluxes, actual denitrification rate (rden), soil NO_3^- and WFPS in the course of the *Lolium italicum* growth period.

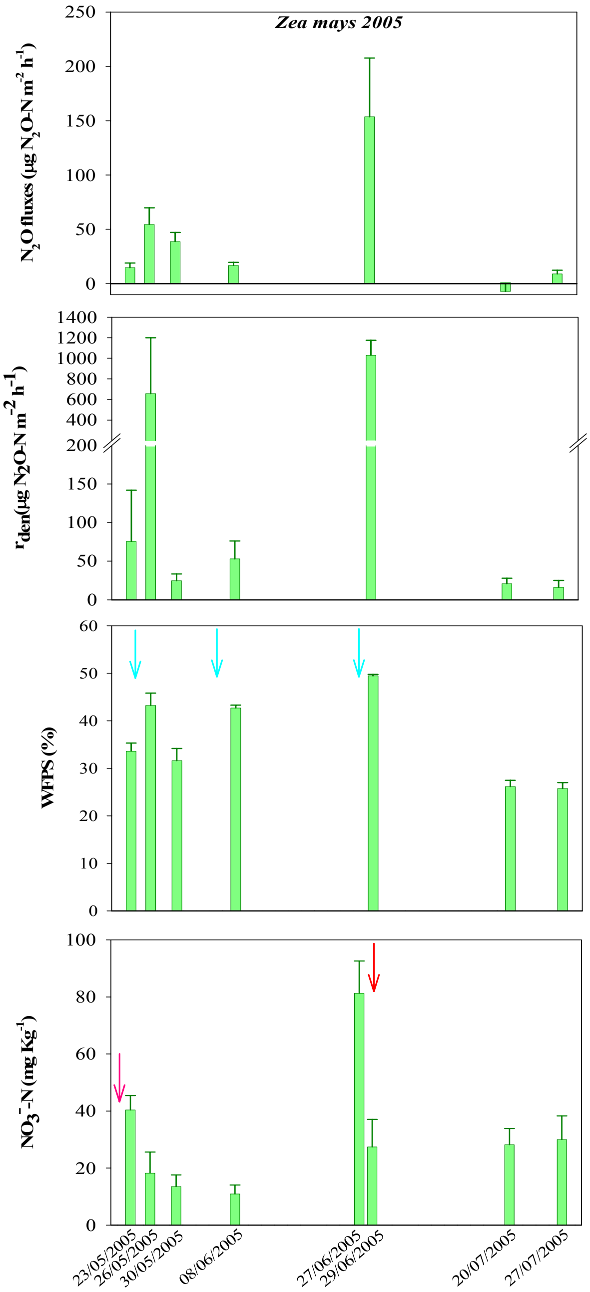


Figure 3-19: Mean values and standard errors for N_2O fluxes, actual denitrification rate (*rden*), soil NO_3^- and WFPS in the course of the *Zea mays* growth period in 2005. The pink, red and cyan arrows indicate the sowing mineral fertilization, the late fertilization and the irrigation events respectively.

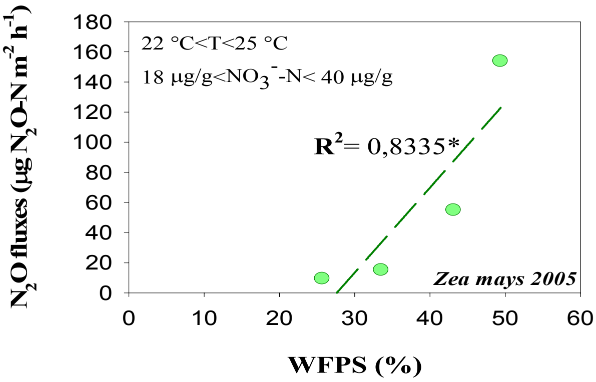


Figure 3-20: N_2O fluxes v.s. soil WFPS (mean values from each sampling date) on the course of the *Zea mays* growth period in 2005 (Pearson product-moment Test: * P< 0,05, ** P< 0,01, *** P< 0,001).

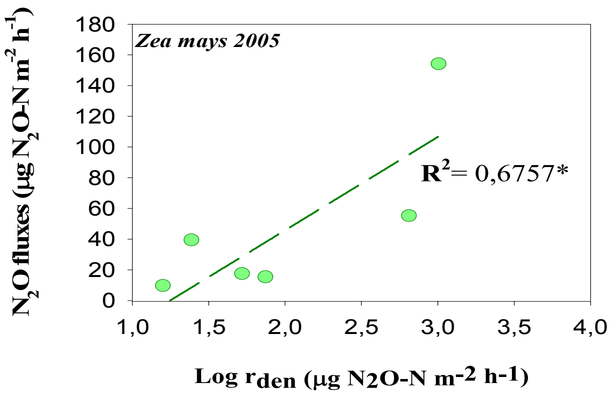


Figure 3-21: N_2O fluxes v.s. r_{den} (mean values from each sampling date) on the course of the *Zea mays* growth period in 2005 (Pearson product-moment Test: * P< 0,05, ** P< 0,01, *** P< 0,001).

Moreover significant correlations were detected between N_2O fluxes, WFPS and actual denitrification rate Fig.3-23, C and D).

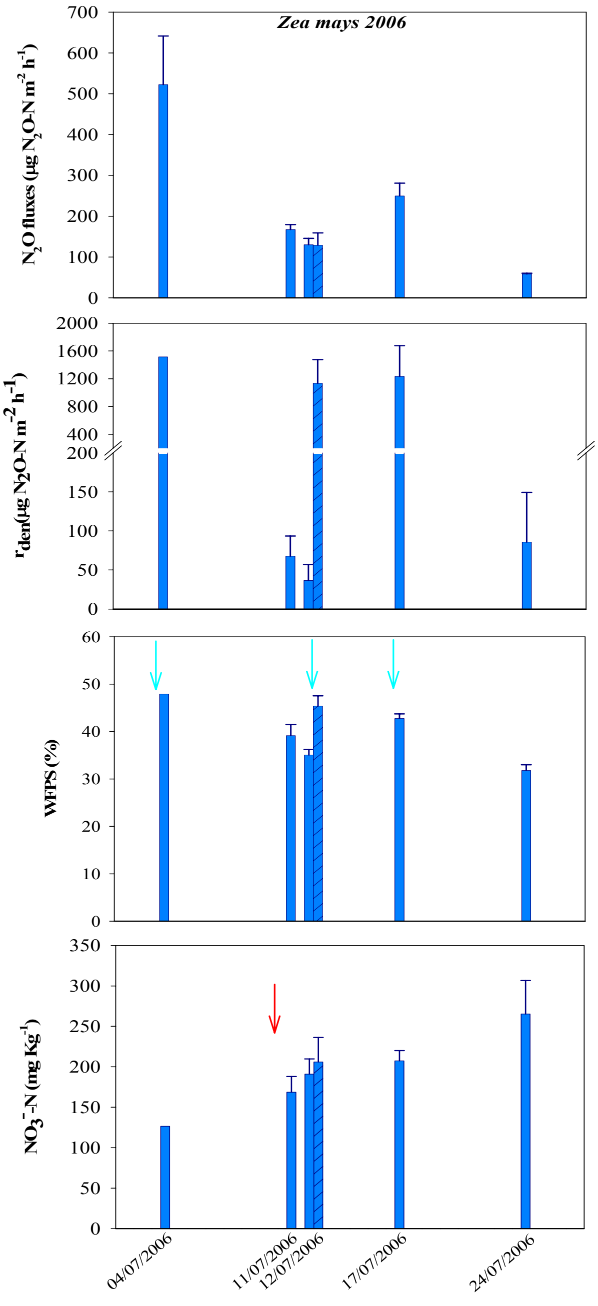


Figure 3-22: Mean values and standard errors for N_2O fluxes, actual denitrification rate (r_{den}), soil NO_3^- and WFPS in the course of the *Zea mays* growth period in 2006. The pink, red and cyan arrows indicate the sowing mineral fertilization, the late fertilization and the irrigation events respectively.

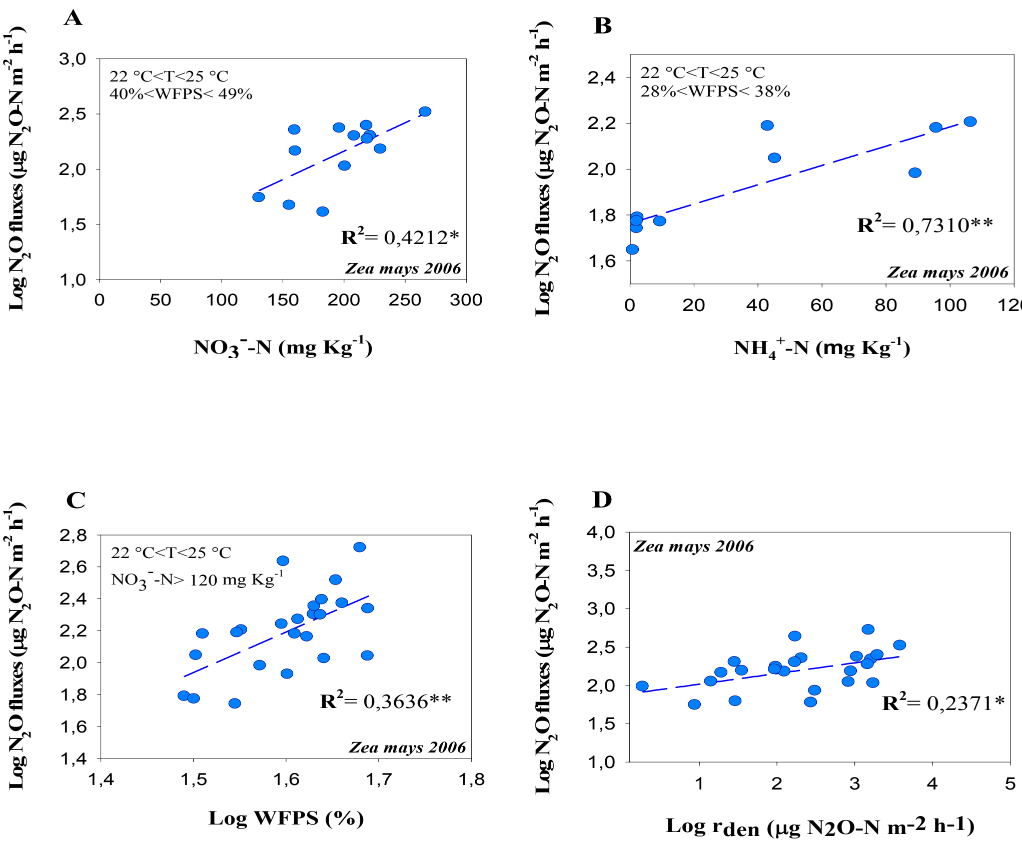


Figure 3-23: N_2O fluxes v.s.A) soil NO_3^- concentration, B) soil NH_4^+ concentration, C) soil WFPS and D) actual denitrification rate (from each intact soil core) on the course of the *Zea mays* growth period in 2006 (Pearson product-moment Test: * P< 0,05, ** P< 0,01, *** P< 0,001).

Anyway even if significant correlations were found on the whole between variables, both r_{den} and N_2O emissions exhibited a wide range of values at given soil nitrates and WFPS, showing evidently it's not always possible to go beyond analytical variability of an intact soil core.

The positive correlation between N_2O fluxes and r_{den} , detected in the course of the maize growth both in 2005 and 2006, show denitrifying activity is probably as a key factor determining the amount of N_2O evolved from this kind of fine textured soil characterized by high values of water retention capacity and organic colloids.

Anyway the positive correlation found between soil NH_4^+ concentration and N_2O emissions at low values of WFPS (<40%) suggest also nitrification can be resposible for N_2O-N losses from clay soil under aerobic conditions.

3.3.5.1 N_2O uptake

Slight negative N_2O fluxes from soil were detected by the end of the growing season of both *Lolium italicum* and *Zea mays* crops in 2005, at lower soil nitrates in comparison with the first stages of the plants growth (Figg.3-17, 3-18, 3-19).

Up to recent times studies about N_2O emission from soil have considered only unidirectional exchanges of N_2O from soil to atmosphere, without taking into account the possibility that soil may act as a sink for atmospheric N_2O as well.

Nowdays it's widely accepted that N_2O consumption may occur in soil, anyway, the role of probable key parameters such as soil NO_3^- availability and microbial diversity has not been investigated in detail yet, and some contradictions exist about the specific soil conditions favouring this phenomenon, since in soils characterized by the highest potential for N_2O uptake (i.e. fine textured soil reducing most N_2O to N_2 via denitrification) N_2O diffusion from the atmosphere might be severely hindered (IPCC, 1997).

As far as concern this study, N_2O fluxes were measured only by static chambers at the soil surface, while no determinations of N_2O concentration in the gaseous phase of soil were carried out along the soil profile, therefore it can't be excluded that they might have been the consequence of some kind of mistake such as an inappropriate closing of the manual chamber.

Anyway results supporting the idea of a real consumptive process causing N_2O atmospheric uptake in the field, comes from experiments performed by Vieten et al. (2005) on clay soil samples collected at the experimental site.

Vieten et al. (2005) analysed net bidirectional exchanges of N_2O between fresh soil aggregates from intact soil cores and atmosphere, performing a flow-through incubation experiment by manipulating the gas composition (N_2O and O_2) at the inlet of the incubation vessels.

It's noteworthy they noticed that at O_2 concentrations in the range between 20% and 2% soil exhibited a low net N_2O production, while a further decrease of O_2 supply (O_2 0.2%), led to a net uptake of N_2O, raising at increasing N_2O inlet concentration and with uptake rates differing between soil samples. Otherwise soon after restoring O_2 concentration to 2%, N_2O consumption disappeared.

The finding of N_2O uptake induced in soil by low O_2 concentration, even if not as inconfutable proof, suggests denitrification may be the process responsible for N_2O consumption in the soil cores analysed and, in a wider sense, of atmospheric N_2O uptake in the field.

Of course measurements of the N_2O concentration in the soil atmosphere, for instance by the recently developed approach of permeable membrane tube systems (Gut et al., 1998; Neftel et al., 2000), could furnish more details about N_2O flux profile in the soil, thus providing the necessary information to validate N_2O fluxes detected at the soil surface.

3.4 CONCLUSIONS

Both denitrification rate and N_2O fluxes from soil showed a great spatial variability and their variations in time through the observation period could be explained with the evolution of both NO_3^- concentration and WFPS in soil. In fact on the whole they were positively related to both NO_3^- and WFPS at not limiting values of soil water content (WFPS>40%) and nitrate availability (15 mg NO_3^--N Kg^{-1}), respectively.

Soil NO_3^- appeared limiting for denitrifying activity during most of winter period, mainly because of leaching through winter rains. Differently soil water content appeared the main factor affecting denitrifier bacteria in the course of the maize cropping cycles, with peaks of denitrification rates being detected soon after irrigation events following fertilizer N applications, clearly as a result of the combined enhancing effect of high soil temperatures and not limiting soil nitrates and WFPS's.

This study showed considerable denitrification rates in the clay soil of an irrigated maize crop in South Italy (on average maximum values close to 1500 μg N_2O-N m^{-2} h^{-1}), more marked than those one detected from a silty clay soil of a non-irrigated maize crop in North Italy (Arcara et al., 1999), under less Mediterranean conditions (on average maximum values close to 287 μg N_2O-N m^{-2} h^{-1} with a single peak up to 690 μg N_2O-N m^{-2} h^{-1}). On the other hand denitrification peaks did not reach the high values detected in an irrigated maize crop in centrale Spain (Vallejo et al., 2003), where despites the lower potential for N losses via denitrification (a sandy loam soil), higher denitrifier activity were promoted (from 2050 μg N_2O-N m^{-2} $h^{-}1$ up to 8487 μg N_2O-N m^{-2} h^{-1}, depending on the kind of organic N fertilizer applied), probably as a consequence of the greater masses of irrigation water applied.

The trend observed for N_2O fluxes from soil largely reflected denitrification rates variation in time, and as a matter of fact a significant positive correlation was found between N_2O emission and denitrification rate, showing denitrifying activity is probably a main process regulating the amount of N_2O evolved from this kind of fine textured soil. Anyway a positive correlation found between soil NH_4^+ concentration and N_2O emissions at low values of WFPS (<40%) seems to suggest also nitrification can be resposible for N_2O-N losses from clay soil under more aerobic conditions.

4 DIFFERENCES OF DENITRIFYING AND NITRIFYING ACTIVITIES AND ASSOCIATED N_2O EMISSIONS, BETWEEN FINE AND COARSE TEXURED SOILS.

4.1 INTRODUCTION

Denitrifying activity is often reported as the main source of N_2O in soils and traditionally N_2O production via autothrophic nitrification is considered to be minor as compared to N_2O evolution through denitrification.

Anyway nitrification can be favoured in well areated soils, and several investigation about factors affecting N_2O emissions via this process in soil, have showed that the amount of N_2O produced increases with the increase of soil pH, temperature, organic matter, easily nitrifiable fertilzer N and with water content raising from air dry to field capacity (Bremner and Blackmer, 1980, 1981; Minami and Fukushi, 1986; Sahrawat and Keeney, 1986).

Moreover since soil is a very heterogeneous system, nitrification and denitrification can occur simultaneously in adiacent microsites and the balance between nitrification and denitrification as the prevailing process determining N_2O emission from soil can swicht rapidly mainly depending on soil WFPS (Klemedtsson et al., 1988; Tortoso e Hutchinson, 1990; Davidson, 199; Davidson, 1992; Skiba et al., 1993; Skiba e al., 2001) and O_2 concentration variations through consumption by microbial respiration (Wolf e Russow, 2000).

Many studies in microbial cultures (Goreau et al., 1980; Bollmann and Conrad, 1998) and both natural (Davidson, 1991; Davidson, et al., 1993; Kester et al., 1997; Khalil et al., 2004; Bateman and Baggs, 2005) and agricultural soils (Lind and Doran, 1984; Klemedtsson et al., 1988; Tortoso e Hutchinson, 1990; Davidson, 1992; Skiba et al., 1993; Abassi and Adams, 2000; Skiba e al., 2001) pointed out that under oxic conditions nitrification is the main source for N_2O emissions, generally in the range between 30% up to 60% WFPS, while the amount of N_2O evolved by denitrification seems to show a marked increase at WFPS exceeding 60%, or after prolonged wet periods.

Anyway, as already pointed out in section 1.3.2, most experiments were performed on soil samples handled to some extent before measurements and therefore probably not fully representative of the real phenomenons naturally occurring in soil.

Since at the experimental site both clay and sandy profiles are present inside the same agricultural field, the objective of the present study was to evaluate possible differences between fine and coarse textured soils regarding both denitrifying-nitrifying activities and their relative contribution to the total amount of N_2O evolved in the field.

As variations of denitrification rate and N_2O fluxes from soil depending on soil nitrates and WFPS patterns, have been already pointed out for clay soil in Chapter 3, in the following sections to avoid repetitions no further explanations will be furnished about changes in time of soil physico-chemical and biological parameters, except for new variables analysed and characteristic trends of the sandy textured soil.

4.2 EXPERIMENTAL SET-UP

In order to take into account the different soil texture at the experimental site, three sampling plots were defined: two plots were located inside the part of the field characterized by clay texture and one inside the area with sandy soil profile (Fig.4-1).

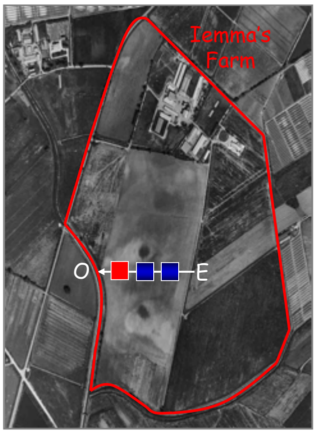


Figure 4-1: Experimental plots (15 m x 15 m) along an E-W transect inside the agricultural field for monitoring activities related to clay and sandy sites (shown by blu and red squares respectively*).*

During the course of the *Lolium italicum* (Sep'04 – Apr'05) and the *Zea mays* (Jun'05 – Aug'05) growths, measurements of actual denitrification rate $\boldsymbol{r}_{den}$, net nitrification rate $\boldsymbol{r}_{nit}$ and N_2O fluxes from soil surface were performed in each plot (Table 4-1, Table 4-2).

Table 4-1: Analyses performed at the clay and sandy sites in the course of the *Lolium italicum* and the *Zea mays* growth periods. The numbers specify field replicates for each kind of measurements on each sampling day.

	Sampling date	Soil profile	r_{den}	N_2O fluxes	$N_2O_{nit\%}$ $N_2O_{den\%}$	pH	WFPS	NO_3^--N	OM %
Lolium italicum	20/10/04	Clay		8		8	8	8	8
		Sandy		4		4	4	4	4
	17/11/04	Clay	8	8		8	8	8	8
		Sandy	4	4	4	4	4	4	4
	15/12/04	Clay	8	8		8	8	8	8
		Sandy	4	4	4	4	4	4	4
	01/02/05	Clay	12	8		8	12	8	8
		Sandy	12	4	8	4	12	4	4
	03/03/05	Clay	12	8		8	12	8	8
		Sandy	12	4	12	4	12	4	4
	17/03/05	Clay	12	8		8	12	8	8
		Sandy	12	4	12	4	12	4	4
	06/04/05	Clay	12	8		8	12	8	8
		Sandy	12	4	12	4	12	4	4
***Zea mays* 2005**	23/05/05	Clay	12	8		8	12	8	8
		Sandy	12	4	12	4	12	4	4
	26/05/05	Clay	12	8		8	12	8	8
		Sandy	12	4		4	12	4	4
	30/05/05	Clay	12	8		8	12	8	8
		Sandy	12	4	12	4	12	4	4
	08/06/05	Clay	12	8		8	12	8	8
		Sandy	12	4		4	12	4	4
	29/06/05	Clay	12	8		8	12	8	8
		Sandy	12	4		4	12	4	4
	20/07/05	Clay	12	8		8	12	8	8
		Sandy	12	4		4	12	4	4
	28/07/05	Clay	12	8		8	12	8	8
		Sandy	12	4		4	12	4	4

Moreover at the sandy site, during the *Lolium italicum* crop up to the first stages of the maize growing season, the relative contribution of nitrifying ($\mathbf{N_2O_{nit\%}}$) and denitrifying bacteria ($\mathbf{N_2O_{den\%}}$) to N_2O fluxes from soil was investigated as well (Table 4-1).

Actual denitrification rate and its relative contribution to N_2O emission from soil were determined on 15 cm dept intact soil cores (∅= 5 cm, h= 15 cm) collected close to the cover box collars placed in the field, while net nitrification rate during Lolium cultivation was studied at different depths along the soil profile (0-10 cm, 10-20 cm and 20-30 cm) (Table 4-2).

After $\mathbf{r}_{den}$ calculation the undisturbed soil cores were processed for WFPS determination, moreover on sampling date for actual denitrification rate and N_2O fluxes assessment, 4 further separate cores were sampled in each plot to determine the following ancillary parameters: soil temperature, pH, organic matter and NO_3^- concentration.

Table 4-2: Incubation periods and number of field replicates for net nitrification rate at different depth along the soil profile in the course of the *Lolium italicum* growth.

	Incubation period	Soil profile	Depth	r_{nit}
Lolium italicum	27/09/04 – 20/10/04	Clay	0 - 10 cm	8
			10 - 20 cm	8
			20 - 30 cm	8
		Sandy	0 - 10 cm	4
			10 - 20 cm	4
			20 - 30 cm	4
	17/11/04 – 15/12/04	Clay	0 - 10 cm	8
			10 - 20 cm	8
			20 - 30 cm	8
		Sandy	0 - 10 cm	4
			10 - 20 cm	4
			20 - 30 cm	4
	15/12/04 – 09/03/05	Clay	0 - 10 cm	8
			10 - 20 cm	8
			20 - 30 cm	8
		Sandy	0 - 10 cm	4
			10 - 20 cm	4
			20 - 30 cm	4
	09/03/05 – 06/04/05	Clay	0 - 10 cm	8
			10 - 20 cm	8
			20 - 30 cm	8
		Sandy	0 - 10 cm	4
			10 - 20 cm	4
			20 - 30 cm	4

4.3 RESULTS AND DISCUSSION

4.3.1 Soil temperature, pH and organic matter

On the whole no differences for soil temperature and pH were found between the sandy and clay soils (Fig.4-2 and Table 4-3).

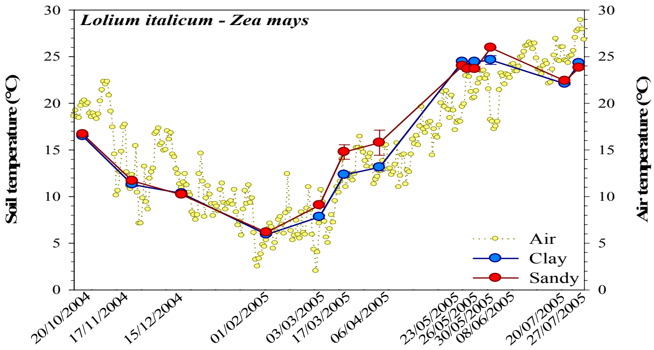
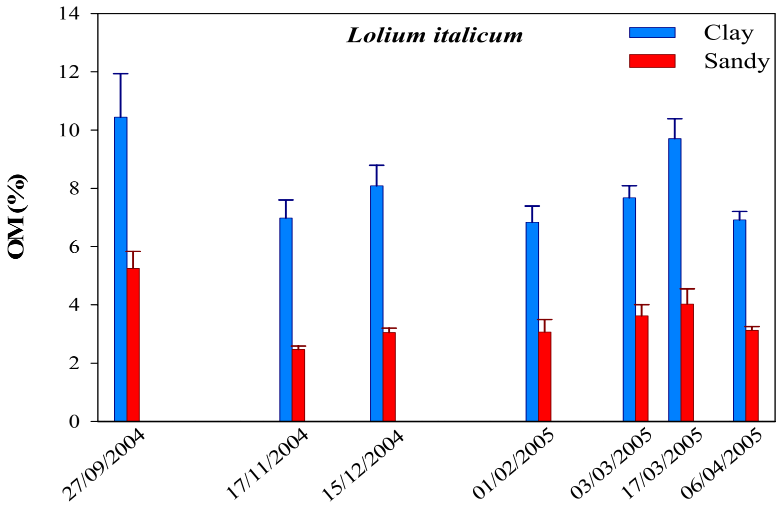


Figure 4-2: Soil temperature for clay and sandy soils at the experimental site during the observation period; air temperature is shown as well.

Table 4-3: Mean values and standard errors of soil pH clay and sandy soils in the course of the *Lolium italicum* and the *Zea mays* growths. Different letters point out significant differences between sites on each sampling date (Mann Whitney-Test, P<0,05).

		pH	
	Sampling date	Sandy	Clay
Lolium italicum	17/11/04	7.90±0.01 a	7.43±0.02 b
	15/12/04	7.51±0.01 a	7.07±0.02 b
	01/02/05	6.85±0.08 a	7.33±0.07 b
	03/03/05	7.31±0.00 a	7.21±0.03 a
	17/03/05	7.10±0.07 a	7.55±0.02 b
	06/04/05	7.26±0.08 a	7.81±0.04 b
Zea mays 2005	23/05/05	8.17±0.02 a	8.2±0.2 a
	26/05/05	8.44±0.09 a	7.4±0.8 b
	30/05/05	8.51±0.03 a	7.2±0.5 b
	08/06/05	7.12±0.08 a	7.4±0.2 a
	29/06/05	6.79±0.10 a	7.71±0.3 b
	20/07/05	8.32±0.02 a	8.7±0.2 a
	28/07/05	8.21±0.06 a	8.2±0.5 a

Differently significant higher values of soil organic matter content were detected at the clay site throughout the course of the maize and Lolium crops (Fig.4-3), also along the soil profile (Fig.4-4).



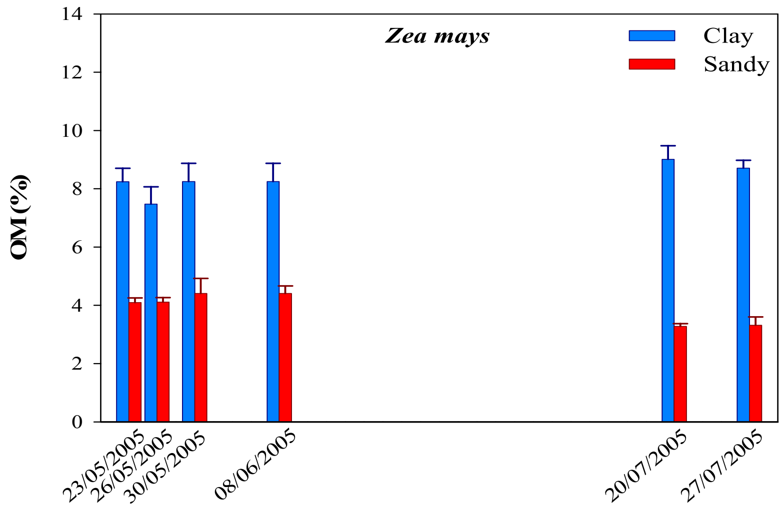


Figure 4-3: Mean values and standard errors for soil organic matter at the clay and sandy sites in the course of the *Lolium italicum* and the *Zea mays* growth periods. Higher values of OM (%) were detected in the fine textured soil throughout the observation period (Mann Whitney-Test, P<0,05).

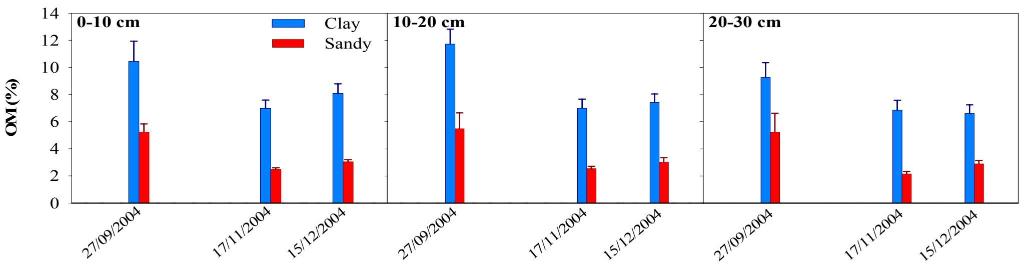


Figure 4-4: Mean values and standard errors for soil organic matter along the soil profile at the clay and sandy sites in the course of the *Lolium italicum* crop. Soil OM (%) showed higher values in the fine textured soil throughout the observation period (Mann Whitney-Test, P<0,05).

4.3.2 Soil moisture and WFPS

Higher values of soil moisture and WFPS were detected at the clay site throughout the observation period, in agreement with the different soil water retention capacities characterizing fine and coarse textured soils (see Table 2-2 in section 2.1.1).

The differences between soil sandy and clay profiles were more marked (Fig.4-5) in the course of the winter grass crop, while during the maize growth, because of summer drought, they were less evident (Fig.4-6).

Differences of soil moisture between sites were found along the soil profile also, down to a depth of 30 cm (Fig.4-7).

Both in clay and sandy soils, as already pointed out in Chapter 3 (see section 3.3.2), the trends observed for soil moisture and WFPS can be related to winter rainfalls and irrigation events during summer period.

4.3.3 Soil nitrate concentration

Soil nitrate concentration showed higher values in fine textured soils throughout the winter grass crop (Mann Whitney-Test, P<0,05) (Fig.4-8). During maize grow no significant difference was found between sites, anyway on the whole higher values of NO_3^--N were always detected at the clay site and the differences were significant on few sampling dates (Fig.4-9).

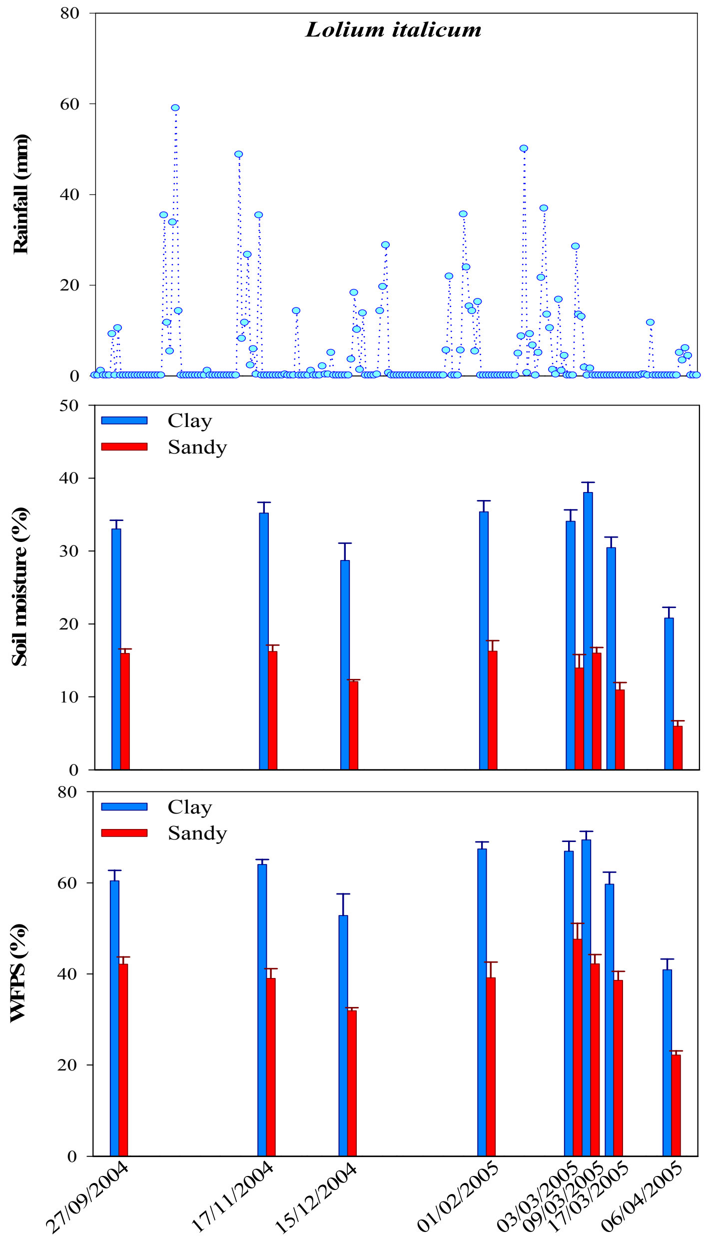


Figure 4-5: Mean values and standard errors for soil moisture and WFPS at the clay and sandy sites in the course of the *Lolium italicum* growth; rainfalls are showed as well. Both soil moisture and WFPS showed higher values in the fine textured soil throughout the observation period (Mann Whitney-Test, P<0,05).

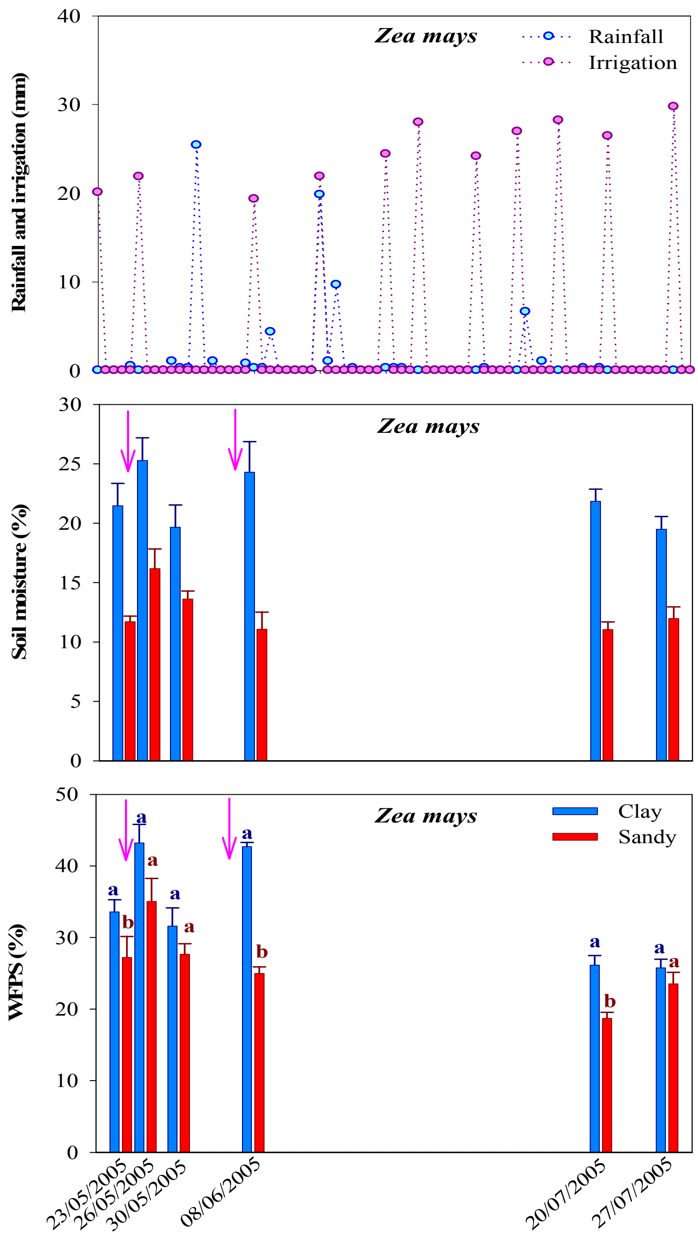


Figure 4-6 Mean values and standard errors for soil moisture and WFPS at the clay and sandy sites in the course of the *Zea mays* growth; rainfall and irrigation events are showed as well. Soil moisture showed higher values in fine textured soils throughout the observation period (Mann Whitney-Test, P<0,05), while in relation to WFPS plot, different letters point out significant differences between sites on each sampling date (Mann Whitney-Test, P<0,05).

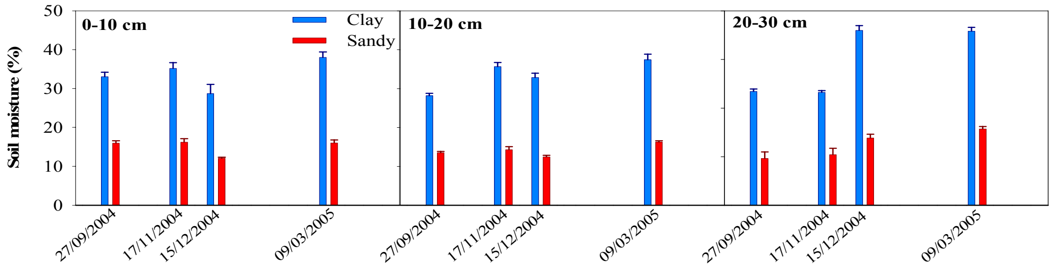


Figure 4-7: Mean values and standard errors for soil moisture along the soil profile at the clay and sandy sites in the course of the *Lolium italicum* crop. Soil moisture showed higher values in the fine textured soil throughout the observation period (Mann Whitney-Test, P<0,05).

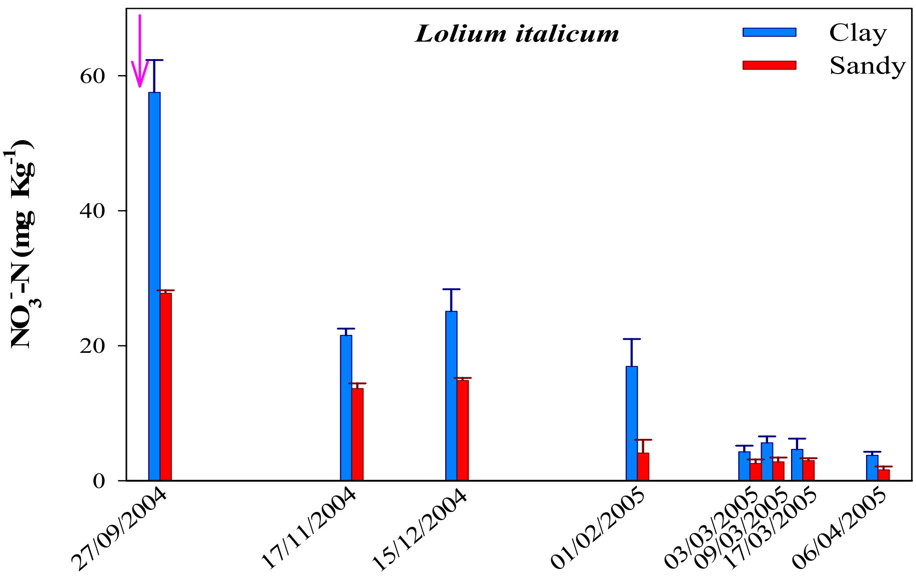


Figure 4-8: Mean values and standard errors for soil NO_3^--N concentration at the clay and sandy sites in the course of the *Lolium italicum* growth period. NO_3^--N concentration showed higher values in the fine textured soil throughout *Lolium italicum* crop (Mann Whitney-Test, P<0,05) The pink arrow indicates the sowing mineral fertilization.

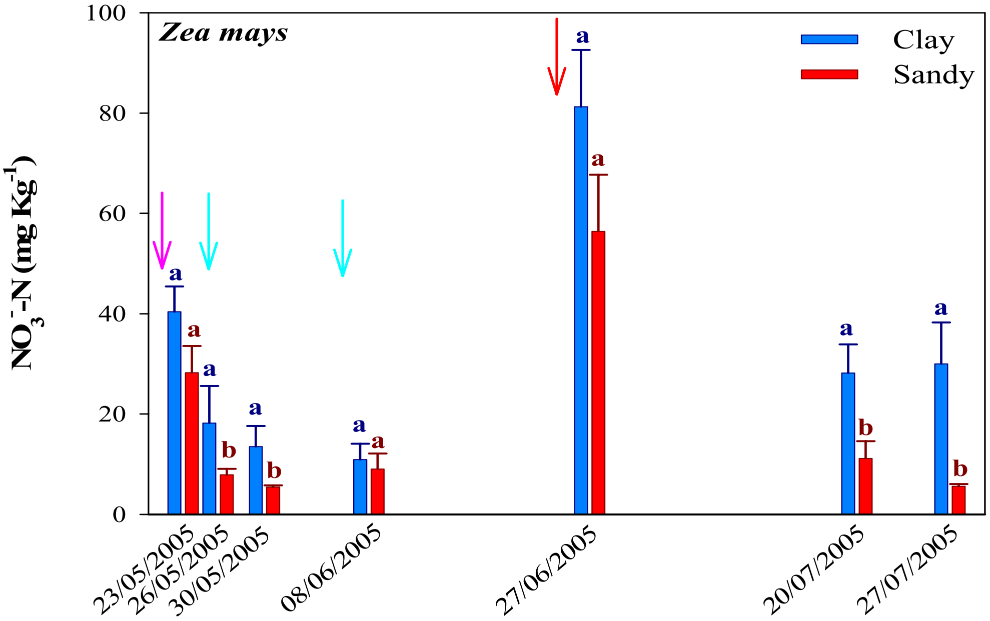


Figure 4-9: Mean values and standard errors for soil NO_3^--N concentration at the clay and sandy sites in the course of the *Zea mays* growth period. Different letters point out significant differences between sites on each sampling date (Mann Whitney-Test, P<0,05). The pink, red and cyan arrows indicate the sowing mineral fertilization, the late fertilization and irrigation events respectively.

For both clay and sandy profiles changes of soil nitrate concentrations can be explained considering the time of mineral fertilizers spreading and the depletion of soil NO_3^--N pool by denitrifying activities, plant uptake and leaching through rains (see section 3.3.3 in Chapter 3).

4.3.4 Actual denitrification rate

As already pointed out for denitrifying activity at the clay site (see section 3.3.4 in Chapter 3), also in the coarse textured soil actual denitrification rate showed a great spatial variability (Fig.4-10), with values of coeffiecient of variation up to 126,6%.

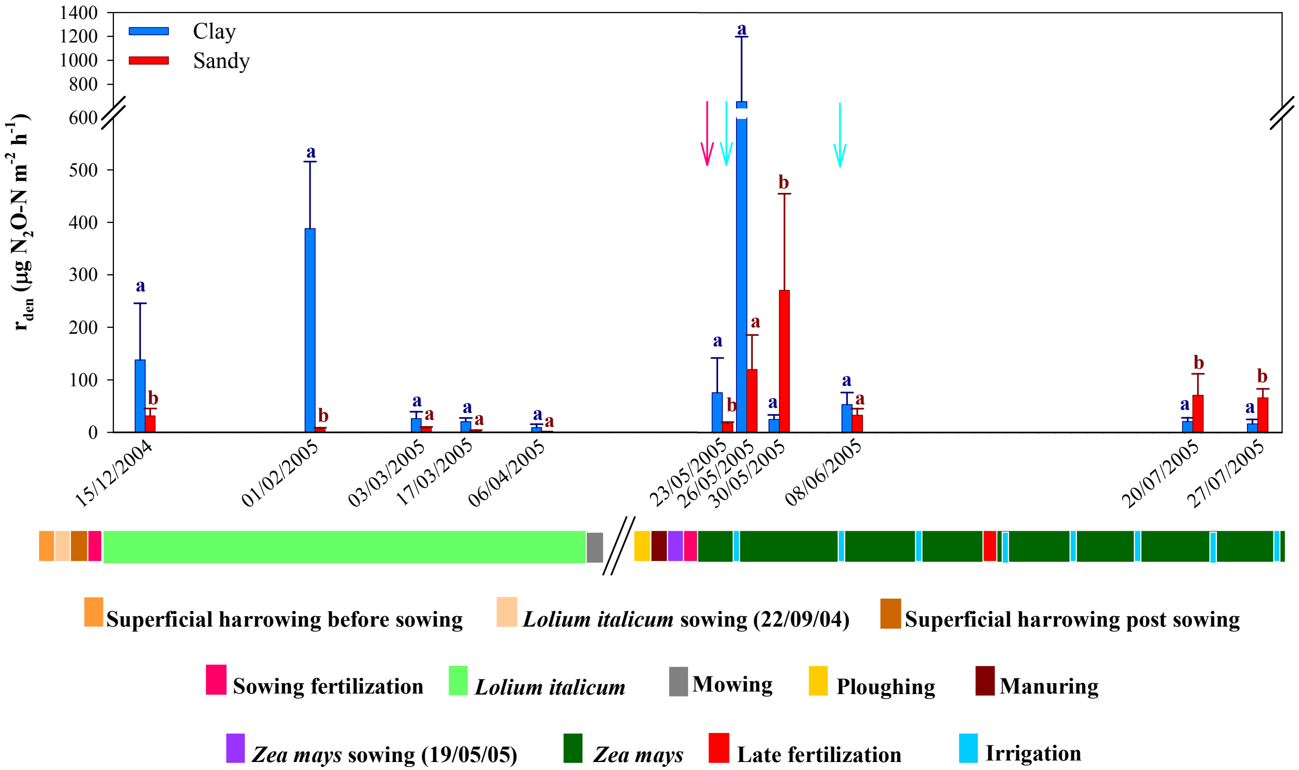


Figure 4-10: Mean values and standard errors for actual denitrification rate (r_{den}) at the clay and sandy sites in course of the *Lolium italicum* and *the Zea mays growths*. Different letters point out significant differences between sites on each sampling date (Mann Whitney-Test, P<0,05).The pink, red and cyan arrows indicate the sowing mineral fertilization, the late fertilization and the irrigation events respectively

On the whole no significant difference was found for $\boldsymbol{r_{den}}$ between the sandy and clay profiles (also as a consequence of the large differences in variance), anyway it's noteworthy higher values of actual denitrification rate were recorded at the clay site throughout the most of the observation period (Fig.4-10).

These differences between the two soil profile appeared to be more pronounced on sampling date when both soil NO_3^- concentration and WFPS were not limiting and are fully in agreement with the higher values of soil nitrate concentration, WFPS and organic material characterizing the fine textured soil (Bowman e Focht, 1974; Ryden et al., 1979; Ryden 1983; Aulakh et al., 1983; Burton e Beauchamp, 1985; Mosier et al., 1986; Parkin et al., 1987; Ellis et al., 1995; Mahmood et al., 1998; Parton et al., 1996; Parry et al., 1999; Abbasi e Adams, 2000; Cai et al., 2001; Strong e Fillery, 2002).

In the course of the Lolium italicum growth, denitrification in sandy soil was invariably low probably in consequence of the slight soil nitrate concentration detected at the site (Fig.4-11).

Differently, during the maize crop a peak of denitrifying activity was measured after the first irrigation event following the sowing mineral fertilization as a consequence of the combined favouring effect of not limiting nitrate availability and WFPS at high soil temperature (Fig.4-12).

Anyway the rise in denitrification rate in sandy soil was slighter than at the clay site and lower as well, suggesting the activation of nitrification may require more time than the activation of denitrification after fertilization and soil rewetting.

4.3.5 Net nitrification rate

Net nitrification rate (Fig.4-13) in both sandy and clay sites was subject to high spatial variability, with CV up to 100% and 80% in coarse and fine textured soils respectively.

In the top soil layer (0-10 cm) the highest net nitrification rates in both clay and sandy soils were detected in the first month following Lolium sowing, probably as consequence of the increased soil NH_4^+ concentration through mineral-N supply at the sowing time.

Moreover, even if with no statistical significance, at the sandy site $\boldsymbol{r_{nit}}$ was higher than in the clay soil, in agreement with the better degree of oxygenation characterizing coarse textured soils (Focht and Verstraete, 1977).

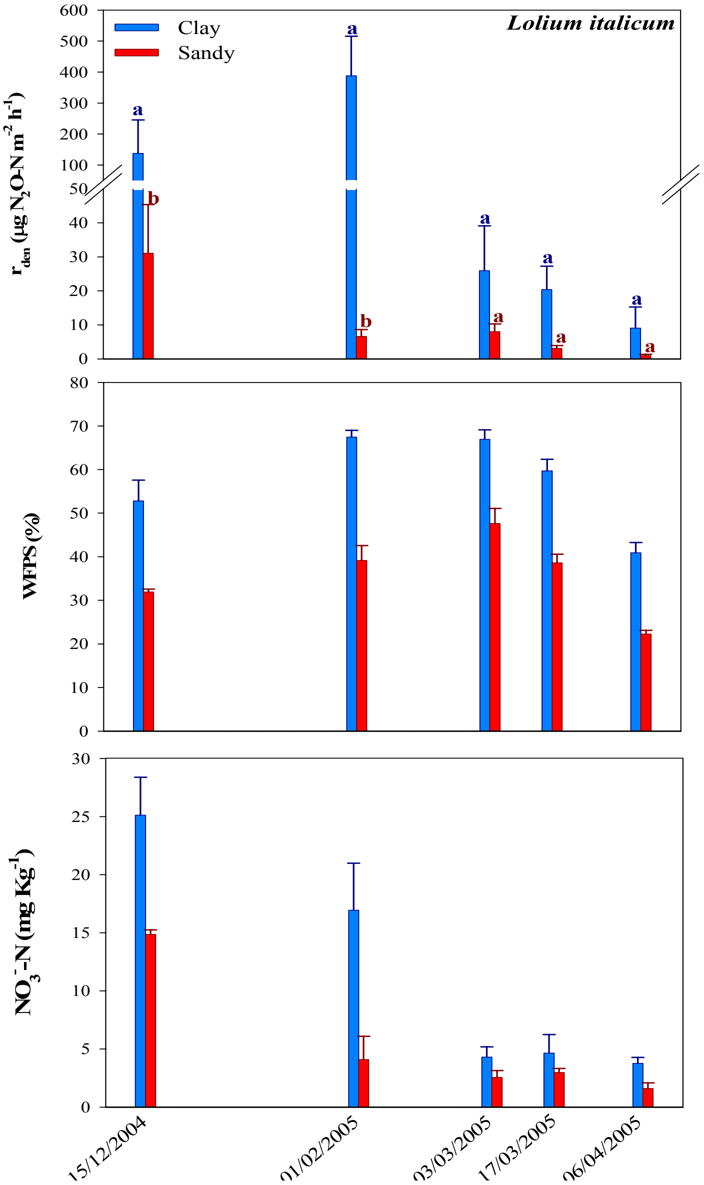


Figure 4-11: Mean values and standard errors for actual denitrification rate (r_{den}), soil NO_3^- and WFPS at the clay and sandy sites in the course of the *Lolium italicum* growth period. Different letters point out significant differences between sites on each sampling date (Mann Whitney-Test, P<0,05).

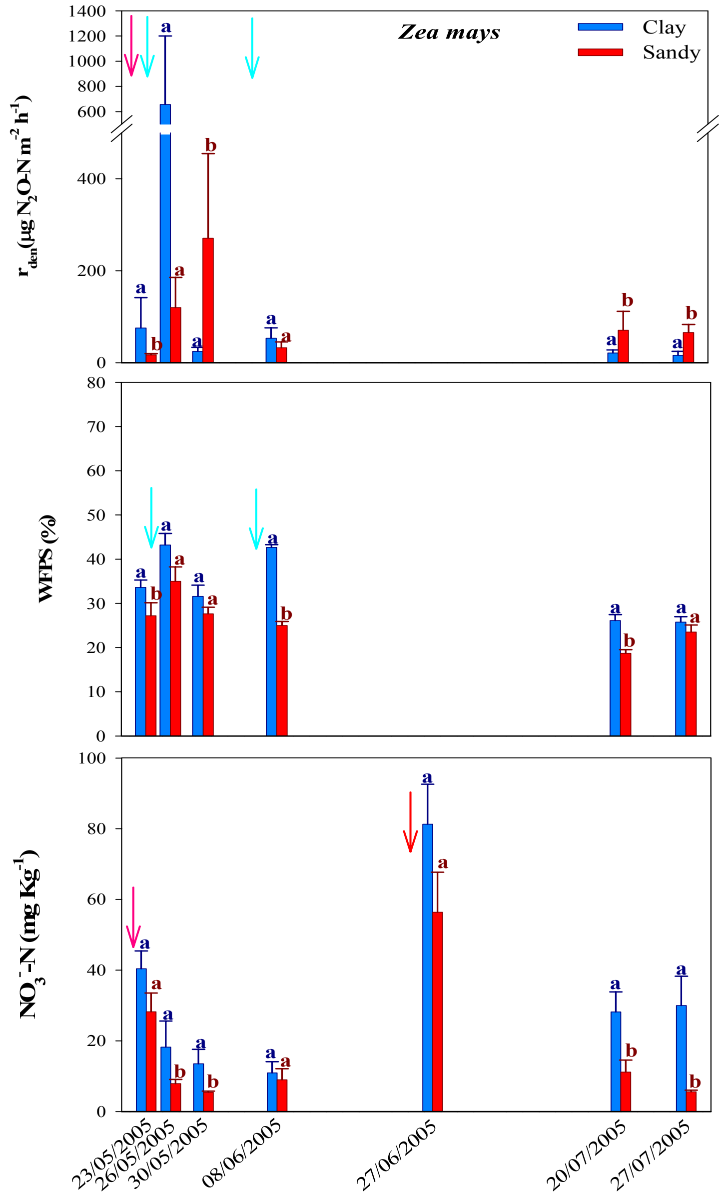


Figure 4-12: Mean values and standard errors for actual denitrification rate (r_{den}), soil NO_3^- and WFPS at the clay and sandy sites in the course of the *Zea mays* growth period. Different letters point out significant differences between sites on each sampling date (Mann Whitney-Test, P<0,05). The pink, red and cyan arrows indicate the sowing mineral fertilization, the late fertilization and the irrigation events respectively.

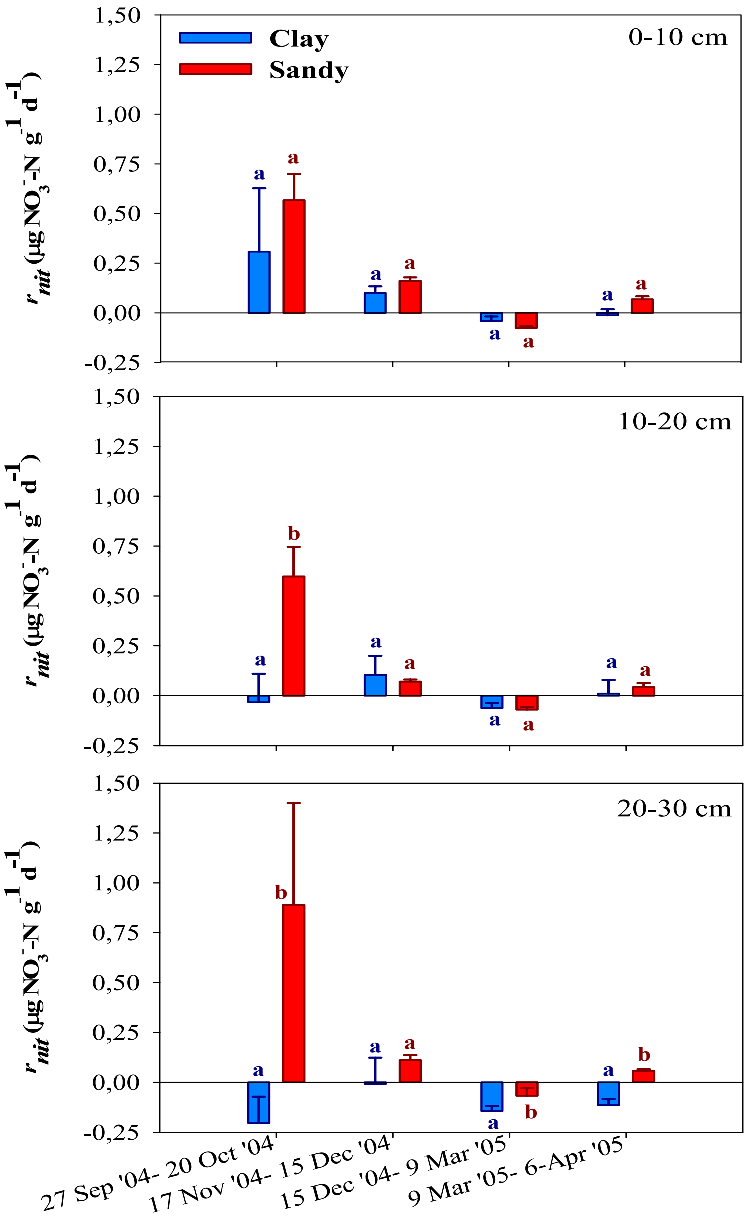


Figure 4-13: Mean values and standard errors for net nitrification rate (r_{nit}) at different depth along the soil profile during *Lolium italicum* crop. Different letters point out significant differences between soil layers on each sampling date (Mann Whitney-Test, P<0,05).

Starting from November, throughout the *Lolium italicum* growing season, a steep slow down of net nitrification was observed in the top layer of both soil profiles, probably due to a decrease of soil NH_4^+ content through plant uptake (Firestone e Davidson, 1989).

Net nitrification rate at the sandy site showed the same pattern in the different layers along the soil profile, while at the clay site, during the first month after Lolium sowing, r_{nit} in the top layer differed greatly from the values recorded in 10-20 cm and 20-30 cm deep soil cores.

The higher values of net nitrification rate at clay soil surface may be a consequence of the improved oxygen gradiend through the superficial harrowing performed pre and post Lolium sowing. Differently in deeper soil cores, with no disturbance of the natural oxygen gradient, r_{nit} showed negative values as often detected for the clay soil during the observation period, in agreement with the higher WFPS and denitrification rates recorded at the fine textured soil.

Anyway it's proper to point out net nitrification rate can't be explained in detail since data concerning soil NH_4^+ concentration, a key factor regulating the process, are not available. That's why it would be more appropriate in this study considering r_{nit} data as ancillary data useful to expound and validate results concerning actual denitrification rate and N_2O fluxes from soil.

4.3.6 N_2O fluxes from soil

Besides high spatial variability (CV up to 84,2%), N_2O fluxes at the sandy site showed a different trend in the course of both lolium and maize crops, compared with the clay site (Fig.4-14).

During most of the winter grass crop, N_2O fluxes from sandy soil were lower than at the clay site (Fig.4-15). Even if with no statistical evidence, they appeared to decrease at increasing values of soil WFPS, moreover they were no related at all with soil nitrate concentration, differently from the clay soil showing a fall in N_2O emission at decreasing soil nitrates (section 3.3.6 in Chapter 3).

In the course of the maize growth, a peak of N_2O emission from the sandy site was detected after the mineral fertilization and irrigation events following corn sowing, when denitrifying activities where more intense as well (Fig.4-16).

As already pointed out for actual denitrification rate, this increase in N_2O evolved from soil appeared slower and less intense than at the clay site.

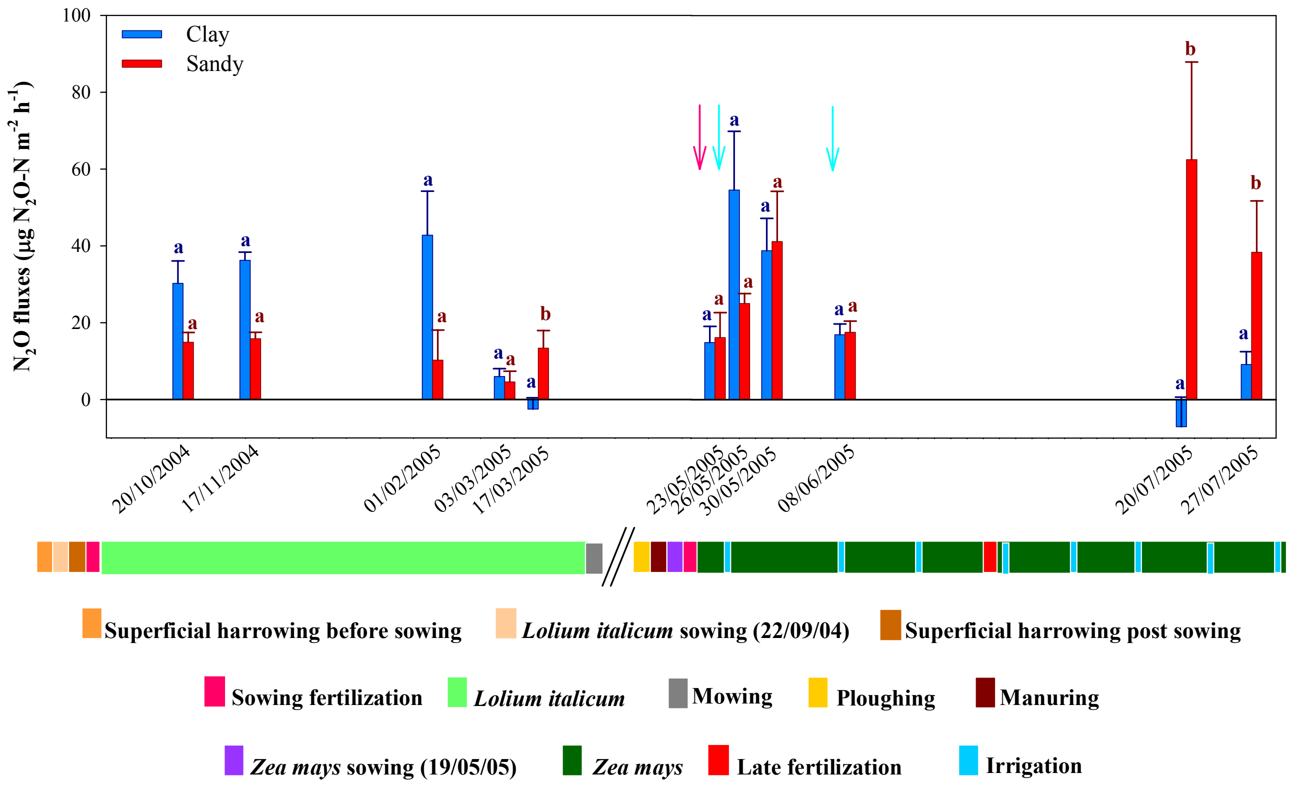


Figure 4-14: Mean values and standard errors for N_2O fluxes from clay and sandy soils in course of the *Lolium italicum* and the *Zea mays* growths. Different letters point out significant differences between sites on each sampling date (Mann Whitney-Test, P<0,05).The pink, red and cyan arrows indicate the sowing mineral fertilization, the late fertilization and the irrigation events respectively.

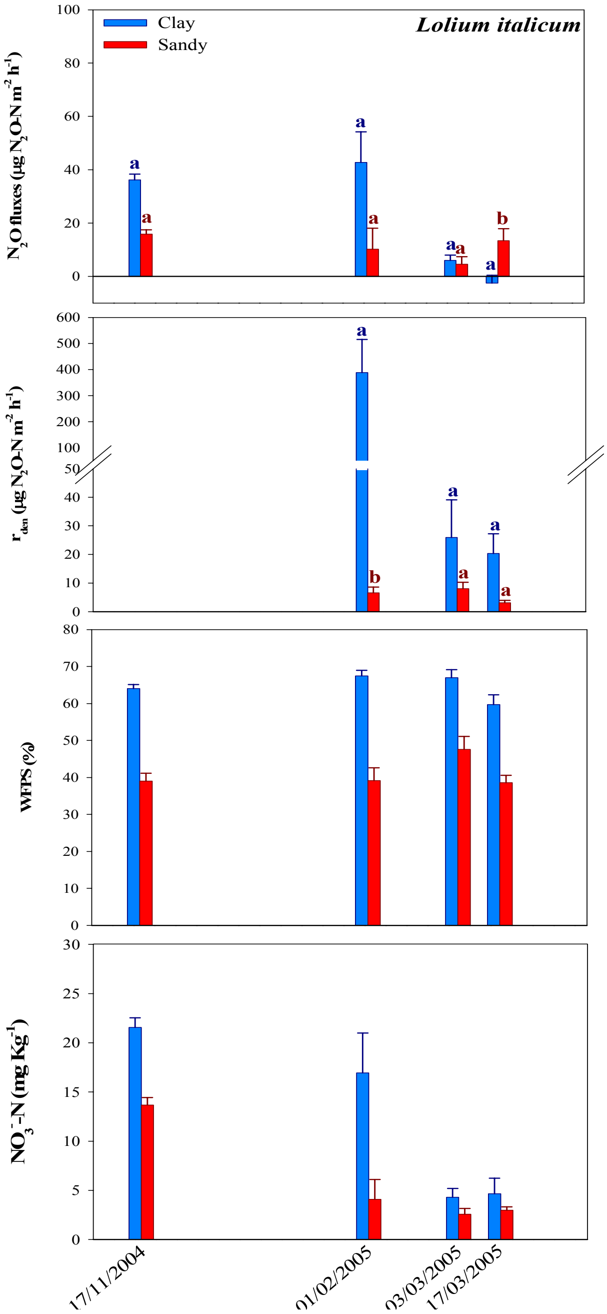


Figure 4-15: Mean values and standard errors for N_2O fluxes, actual denitrification rate (r_{den}), soil NO_3^- and WFPS at the clay and sandy sites in the course of the *Lolium italicum* growth period. Different letters point out significant differences between sites on each sampling date (Mann Whitney-Test, P<0,05).

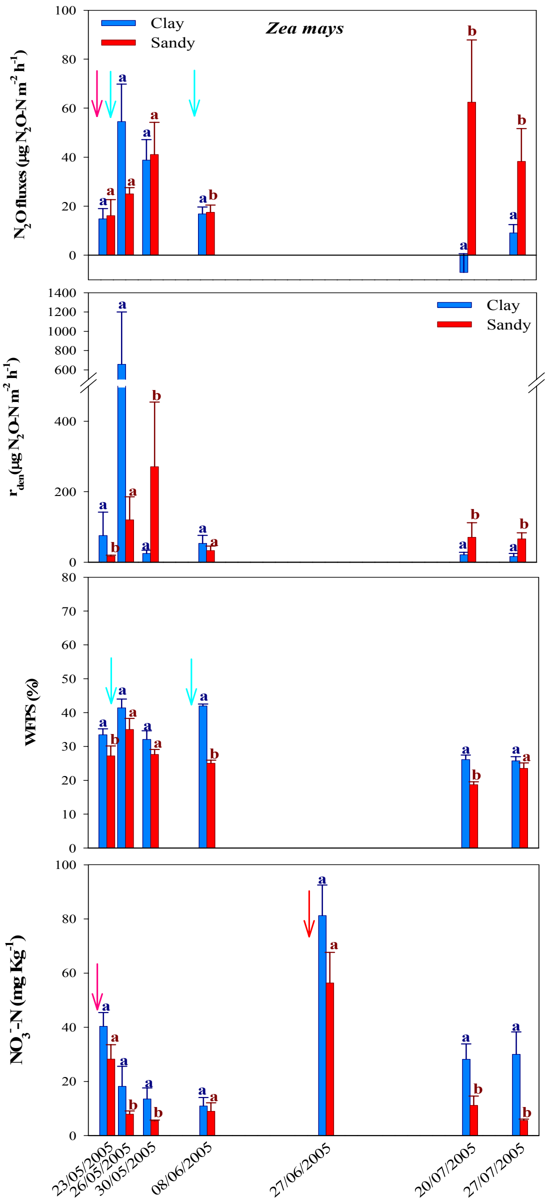


Figure 4-16: Mean values and standard errors for N_2O fluxes, actual denitrification rate (r_{den}), soil NO_3^- and WFPS at the clay and sandy sites in the course of the *Zea mays* growth period. Different letters point out significant differences between sites on each sampling date (Mann Whitney-Test, P<0,05). The pink, red and cyan arrows indicate the sowing mineral fertilization, the late fertilization and the irrigation events respectively.

Anyway remarkable N_2O fluxes from sandy soil were noticed at very late stages of the corn growing season also, when both soil NO_3^- concentration and denitrifying activity were very low; moreover once again the amount of N_2O evolved from soil appeared to decrease at increasing values of soil WFPS.

As a matter of fact, while on the whole at the clay site N_2O fluxes seemed to raise at increasing values of soil NO_3^- concentration and WFPS (see Chapter 3 for a more detailed description of correlations between N_2O fluxes, soil NO_3^- concentration and WFPS) and showed a significant positive correlation with actual denitrification rate (Fig.4-17), at the sandy site N_2O fluxes were related with neither soil nitrates nor r_{den}, suggesting denitrifying activity is not the main process determining the amount of N_2O gas emitted from this kind of coarse textured soil.

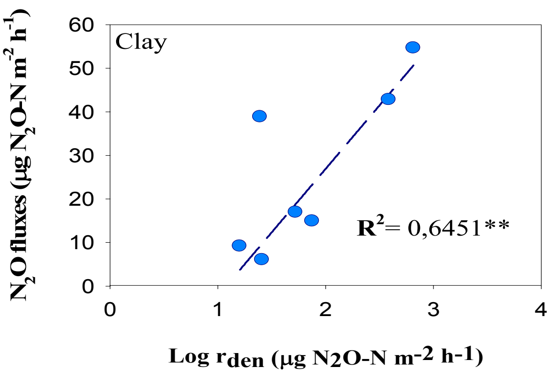


Figure 4-17: N_2O fluxes v.s. r_{den} (mean values from each sampling date) on the course of the *Lolium italicum and Zea mays* growths period. Pearson product-moment Test: * P< 0,05, ** P< 0,01, *** P< 0,001.

4.3.7 Relative contribution of denitrifying and nitrifying activities to N_2O fluxes from the sandy soil

As shown in Figure 4-18, the relative nitrifier contribution to N_2O emission from soil showed to not depend in a simple manner on soil WFPS detected at the sampling time, being probably at a great extent influenced by the environmental conditions characterizing the period preceding the measurements as well.

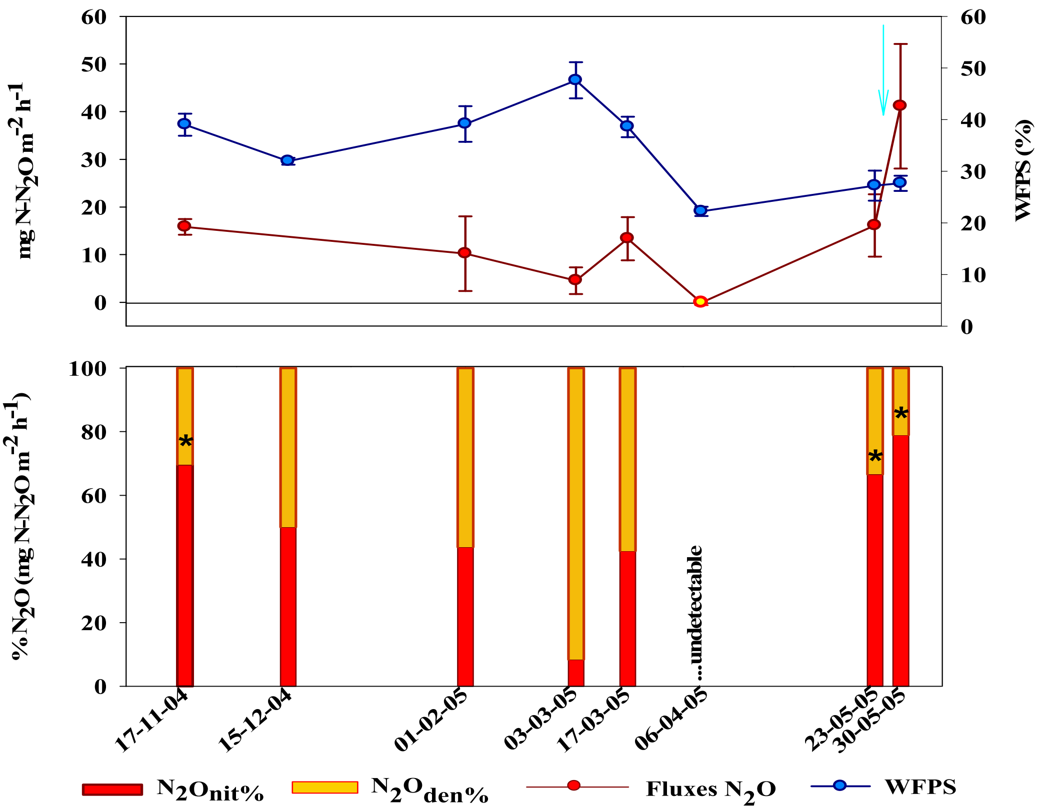


Figure 4-18: A) Mean values of N_2O fluxes, WFPS and B) relative contribution of nitrification ($N_2Onit\%$) and denitrification ($N_2Oden\%$) to N^2O emission from soil at the sandy site. The asterisks mark the relative nitrification contributions calculated from significant decrease of N_2O production after inhibition of nitrification (Mann Whitney-Test for unequal variance, P<0,05). The data reported by the yellow edged circle was derived by measurements on intact soil cores. The cyan arrow indicates the first irrigation event following the sowing mineral fertilization.

In November, at WFPS slightly above 40%, close to field capacity, most of the N_2O evolved from soil was due to nitrification, while on sampling dates in December and February, despite of similar water contents, a marked decrease of N_2O via nitrification was noticed. It might be argued this finding is the result of the prolonged wet period through winter rains, probably favouring denitrifier activity and, as matter of fact, at the beginning of March, when the highest value of WFPS was detected (above WHC), denitrifying activity showed to be responsible for the most (about 90%) of N_2O emissions from soil.

Afterwards, with decreasing soil WFPS in the course of spring, the relative contribution of nitrification raised again and, following the first irrigation event, on 30/05/05 sampling date, nitrifying bacteria appeared to be responsible for 80% of the marked N_2O emission recorded from soil in the field.

At values of WFPS close to 20%, very low N_2O flux (close to zero) was detected from soil probably as a consequence of both a generalized slowdown of bacterial metabolism and a complete oxidation of NH_4^+ to NO_3^- by nitrification at optimum O_2 supply (Poth e Focht, 1985; Davidson, 1991; Granli and Bokman, 1994). Differences between nitrifier and denitrifier contribution to such a low amount of N_2O evolved from soil couldn't de detected as well.

Similarly Kester (Kester et al., 1997), in his study about NO and N_2O emissions from soils of natural and anthropic ecosystems, could not found any significant correlation between soil WFPS and the relative nitrifier and denitrifier contributions to N_2O fluxes from intact soil cores, even if on the whole the more important role of denitrification in the N_2O production was detected in autumn, when soil showed the higher moisture content.

It's notewhorty an interesting significant correlation was found between N_2O fluxes and N_2Onit% (Fig.4-19) suggesting nitrifier bacteria and associated N_2O emissions are probably favoured in the sandy soil analysed.

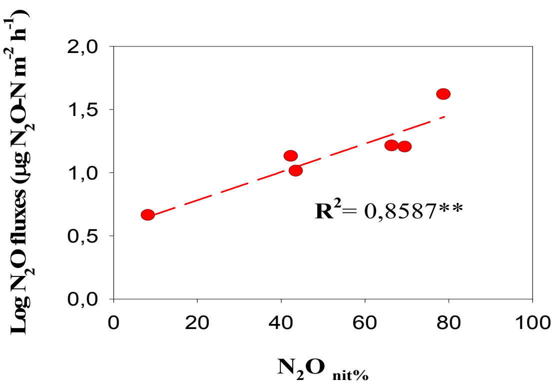


Figure 4-19: N_2O fluxes from soil v.s. the relative contribution of nitrification ($N_2O_{nit\%}$) at the sandy site (mean values from each sampling date). Pearson product-moment Test: * P< 0,05, ** P< 0,01, *** P< 0,001.

Of course further studies focused on characterization of microbial diversity (for instance by modern molecular techniques based on the identification of the key functional genes involved in nitrification and denitrification processes) could be helpful for a more detailed understanding of variations of nitrifier contribution in time at the sandy site and of differences detected between the clay and sandy soils as well.

Finally it's proper to underline only on three sampling dates the relative nitrification contributions could be calculated from a significant decrease of N_2O production after nitrification inhibition (Figg.4-17, 4-20).

This lack of statistical significance is very likely due to the high spatial variability exhibited by both nitrification and denitrification processes, as already pointed out by Kester (Kester et al, 1997) who found a significant difference between control and nitrification inhibited soil cores only in 54% of sampling dates.

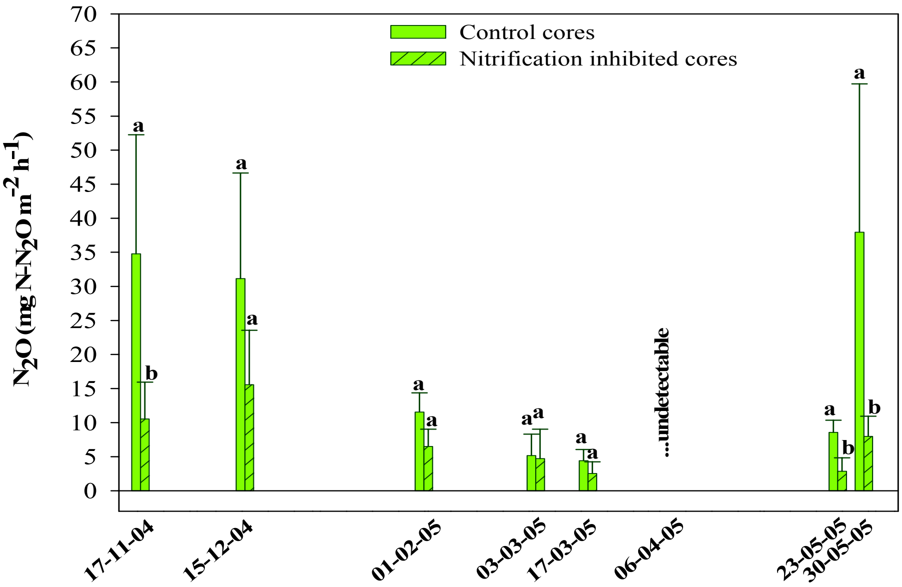


Figure 4-20: Mean values and standard errors of N_2O production from control and nitrification inhibited soil cores. Different letters point out significant differences between sites on each sampling date (Mann Whitney-Test for unequal variance, P<0,05).

4.4 CONCLUSIONS

N_2O fluxes, denitrification and nitrification rates showed different patterns between clay and sandy soils, according to their different physico-chemical characteristics.

At the clay sites, characterized by higher soil NO_3^- concentrations, organic matter content and WFPS, denitrification activities showed the highest values and appeared a foundamental process determining N_2O emissions from soil, as suggested by the significant correlation found between actual denitrification rate and the amount of N_2O evolved from the fine textured soil.

In the coarse textured soil, with lower NO_3^- concentrations, organic matter content and WFPS, nitrification activities and related N_2O emissions appeared to be promoted.

Higher net nitrification rates were in fact detected at the sandy site (along the soil profile down to a depth of 30 cm) in the first month following the winter grass sowing, before the general steep slow down noticed at both sites likely as consequence of the combined effect of a decrease in soil mineral N through plant uptake and the development of more anaerobic conditions through winter rains.

Moreover up to WFPS values close to field capacity, nitrification showed to be a main potential source for N_2O emissions from soil, and an interesting significant correlation was found between N_2O fluxes and N_2Onit%, suggesting nitrifier bacteria and N_2O emissions via this process are probably favoured in the sandy soil analysed.

Anyway the relative nitrifier contribution to the overall amount of N_2O evolved from soil showed to not depend in a simple manner on soil WFPS detected at the sampling time since, after prolonged wet periods, a marked decrease of N_2O via nitrification was noticed also on sampling date when WFPS was close or slightly under field capacity.

5 EFFECTS OF DIFFERENT UREA-N SUPPLY ON DENITRIFYING ACTIVITY AND N_2O EMISSIONS FROM SOIL AT THE CLAY SITE

5.1 INTRODUCTION

Denitrification is often considered detrimental to agriculture since it can interfere on soil-plant relationship because of competitive demand for N-mineral source, therefore representing a main pathway for N losses from the system both as N_2O and N_2 (Hunphreys et al., 1990; Freney et al., 1995; Mosier et al., 2002; Galloway et al., 2004).

Anyway, since plants are better competitors for soil N than N_2O-producing bacteria are, when amount and time of application of fertilizer N match crop needs, it is assumed N_2O fluxes are relatively low until plant N demand decreases resulting in a greater N availability for bacterial processes (McSwiney and Robertson, 2005).

Moreover an appropriate use of fertilizers N can also effectively prevent from N losses by NO_3^- leaching, dramatically increasing when N fertilizer is supplied at a rate exceeding the level at which crop yields no longer increase (Steinhilber and Meisinger, 1995; Andrasky et al, 2000; Power et al., 2000).

Maize cultivations require big amounts of fertilizer N so that farmers should be careful with the time of application, trying to spread the most of it on the very moment it is expected to contribute significantly to the N needs of the crop, in order to limit nitrogen losses through leaching and microbial consumption.

According to the manual "Manuale di corretta prassi produttiva per il mais" suitable for Mediterranean countries, farmers should not exceed the maximum quantity of 250 kg N ha^{-1} as total fertilizer N applied and, preferably, they should split the input in two moments separated in times: the first (not exceeding 100 kg N ha^{-1} and not as NO_3^- compounds) at the sowing time and the second as late as possible during the maize growing season to result synchronized with the maximum crop demand. Moreover they should take into account for the residual N in the field, properly reducing the total amount of fertilizer N if the maize culture is preceded by manure application (1.2 kg N ha^{-1} less per 1000 Kg of manure) and legumes in the crop rotation (200 kg N ha^{-1} as maximum total amount).

In order to study the efficiency of fertilizer use by the maize crop in relation to eventual N losses by bacterial denitrification from fertilizer N applied on the agricultural field, measurements of actual denitrification rate and N_2O fluxes from soil were performed in experimental plots supplied with different amount of urea fertilizer, where determinations of nitrogen metabolism of maize plants were carried out as well (Arena, pers.comm.; Parisi et al., 2006).

5.2 EXPERIMENTAL SET-UP

In the course of the *Zea mays* growth in 2005, in a marginal area of the agricultural field with clay soil profile, 6 restricted experimental plots (3m × 5m) were defined with different urea-N supply at the late fertilization time (Fig.5-1).

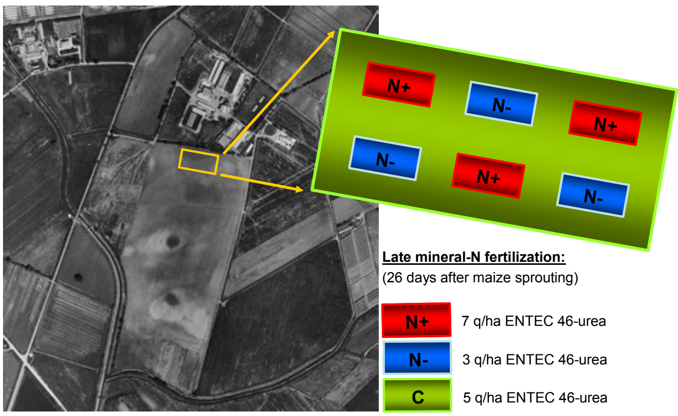


Figure 5-1 Experimental plots receiving higher mineral-N fertilization (N+) and lower mineral-N fertilization (N-) than the whole field considered as control (C).

In detail, 3 plots received lower urea-N fertilization (N-: 138 Kg N ha^{-1}) and 3 plots received higher urea-N fertilization (N+: 322 Kg N ha^{-1}) than the rest of the field (C: 230 Kg N ha^{-1}).

Measurements of actual denitrification rate and N_2O fluxes from soil were performed in each plot at different stages of maize growth: 35 days (29/06/05), 50 days (20/07/05) and 68 days (28/07/05).

Moreover, only for N_2O emission, one sampling more was carried out at the very end of the growing period (93 days), soon before mowing (Table 5-1).

On every sampling date 4 separate soil cores (∅= 5 cm, h= 15 cm) where collected from **N+**, **N-** and **C** treatments to determine soil moisture, nitrate concentration, pH, temperature and organic matter content while soil bulk density analysis for WFPS assessment were performed processing the intact soil cores after r_{den} calculation (Table 5-1).

Table 5-1: Analyses performed in the experimental plot with different urea-N supply in the course of the *Zea mays* crop in 2005. The numbers specify field replicates for each kind of measurements on each sampling day.

	Sampling date	Plot	r_{den}	N_2O fluxes	pH	WFPS	NO_3^--N	OM %
Zea mays 2005	08/06/05	C	3	4	4	3	4	4
	29/06/05	C	6	4	4	4	4	4
		N+	6	4	4	4	4	4
		N-	6	4	4	4	4	4
	20/07/05	C	6	4	4	4	4	4
		N+	6	4	4	4	4	4
		N-	6	4	4	4	4	4
	28/07/05 pre-irr	C	6	4	4	4	4	4
		N+	6	4	4	4	4	4
		N-	6	4	4	4	4	4
	28/07/05 post-irr	C		4		4		
		N+		4		4		
		N-		4		4		
	21/08/05 pre-irr	C	6	4	4	4	4	4
		N+	6	4	4	4	4	4
		N-	6	4	4	4	4	4
	21/08/05 post-irr	C		4		4		
		N+		4		4		
		N-		4		4		

In the same experimental plots, in the course of the maize growth, measurements of plant maximal PS2 photochemical efficiency (Arena et al., pers.comm.) and both soluble protein and total leaf free-amino acid content (Parisi et al, 2006) were carried out as well, to investigate eventual differences in plants nitrogen metabolism between the different urea-N supply treatments.

The maximal PS2 photochemical efficiency is the ratio F_v/F_m of the variable chlorophyll fluorescence (F_v) and the maximal fluorescence level (F_m) giving the potential quantum efficiency of the leaf (Butler and Kitajima, 1975). It is widely used as an indicator of plant health under a wide range of environmental conditions (Björkman and Demmig 1987, Ball et al., 1995). It is assumed healthy terrestrial plants have a dark adapted Fv/Fm value close to 0,8 while a decrease from this threshold indicates both short-term and long-term stress for plants (for instance due to high irradiances and temperatures, limited water shortage and/or mineral-N availability) or the establishment of photoprotective mechanisms related to thermal dissipation processes.

Soluble proteins are markers of leaf N status while total leaf free-amino acid content reflects the whole-plant N status (Hirel et al., 2005).

5.3 RESULTS AND DISCUSSION

5.3.1 Soil temperature, pH and organic matter

Values of soil temperature, pH and organic matter content detected during the manipulation experiment are listed in Table 5-2.

Table 5-2: Mean values and standard errors of soil temperature, pH and organic matter content in the course of the manipulation experiment during the *Zea mays* growth in 2005.

	Sampling date	Plot	T_{soil} (°C)	pH	OM (%)
Zea mays 2005	08/06/05	C	18.7±0.1	7.13±0.10	8.2±0.6
	29/06/05	C	25.4±0.1	6.79±0.08	6.9±0.8
		N+	25.4±0.1	6.84±0.05	7.6±0.4
		N-	25.3±0.1	6.74±0.05	7.9±0.2
	20/07/05	C	24.9±0.1	8.40±0.05	7.6±0.6
		N+	24.8±0.1	8.36±0.00	7.7±0.1
		N-	24.9±0.1	8.34±0.00	7.7±0.1
	28/07/05	C	24.7±0.1	8.11±0.02	6.0±0.3
		N+	24.7±0.1	8.13±0.00	6.7±0.1
		N-	24.8±0.1	8.00±0.01	7.54±0.1
	21/08/05	C	24.7±0.1	8.00±0.01	7.64±0.3
		N+	24.6±0.1	8.10±0.1	7.75±0.2
		N-	24.7±0.1	8.07±0.01	7.8±0.3

On the whole no significant differences were noticed between the experimental plots and, as already found at the clay sites for monitoring activities (Chapter 3, section 3.3.1), soil showed on average subalkaline pH (with values closer to neutrality on sampling dates soon after irrigation events) and a high organic matter content (with no significant variation in time).

5.3.2 Soil moisture and WFPS

At the marginal area of the agricultural field were the experimental plot were defined, irrigation is supplied by means of pumps not able to guarantee uniformity of water application to the soil.

That's why on some sampling dates, differences of soil moisture were detected inside the same kind of treatments (Fig.5-2).

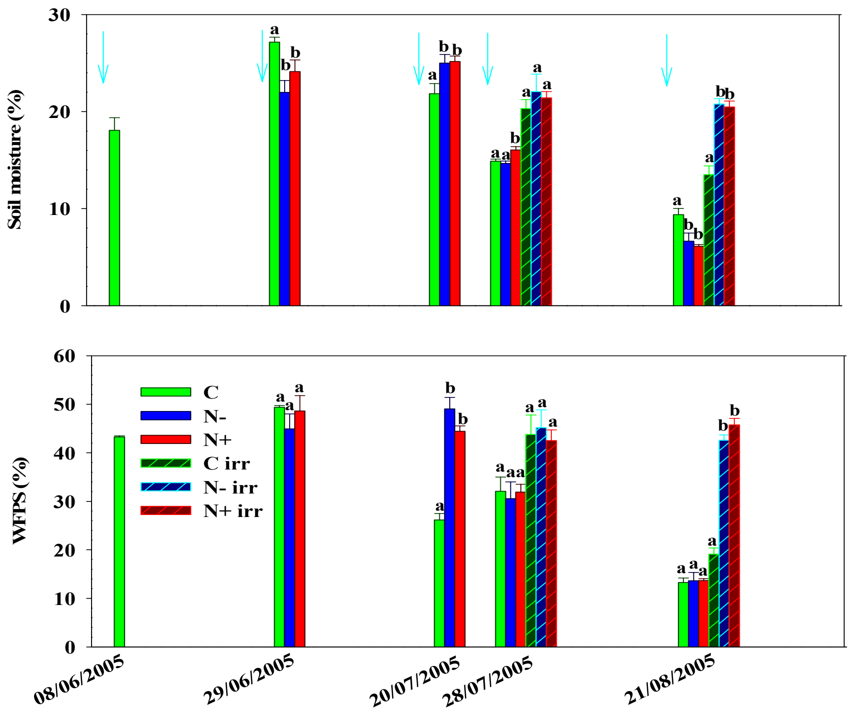


Figure 5-2: Mean values and standard errors for soil moisture and WFPS in the course of the manipulation experiment during the *Zea mays* growth in 2005. The cyan arrows indicate irrigation events while different letters point out significant differences between treatments on each sampling date (One Way ANOVA Holm-Sidak test P<0,05).

In detail, on 20th of July at sampling time, the control has not received irrigation water yet, while on 28th of July and 21th of August the experimental plots appeared like a puzzle of irrigated and not irrigated pieces.

5.3.3 Soil NO_3^- concentration

As shown in Figure 5-3, soil nitrate concentration increased in all the experimental plots after the late fertilization event, with the highest values being detected in **N+** throughout the observation period (Holm-Sidak test, P<0,05). Otherwise no significant differences were noticed between **C** and **N-** plots, even if slightly higher values of NO_3^- were found in the control soils on all sampling dates.

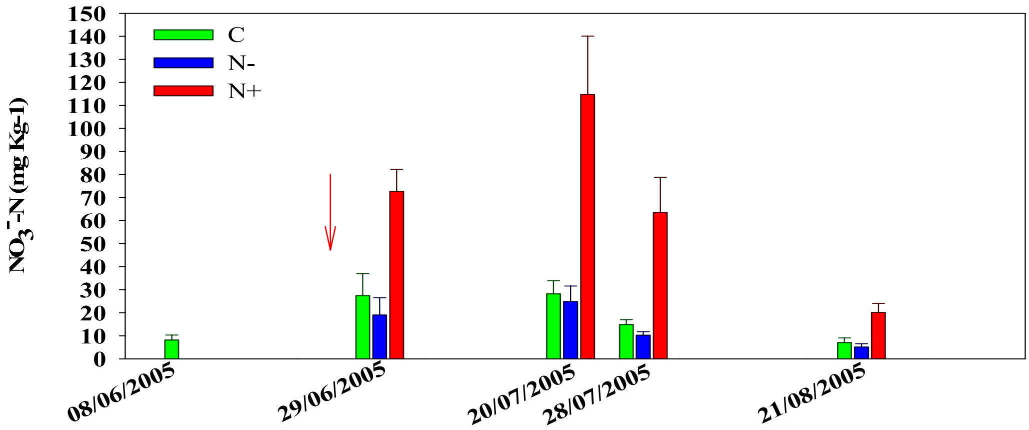


Figure 5-3: Mean values and standard errors for soil NO_3^- concentration in the course of the manipulation experiment during the *Zea mays* growth in 2005. N+ plots showed higher values than C and N- plots throughout the observation period (One Way ANOVA Holm-Sidak test P<0,05). The red arrow indicate the late fertilization (21/06/05).

After 1 month from the fertilization time, soil nitrate were almost the same in all treatments, also showing an increase in **N+** (even if with not statistical significance), probably because of the slow release to the soil of mineral-N from the urea fertilizer applied on the field (Arcara et al., 1999). Afterwards a generalized decrease of nitrate availability was observed, anyway at the high urea-N fertilization site, soil NO_3^- concentration showed not limiting values even at the very end of the maize growing season, just few days before mowing.

5.3.4 Actual denitrification rate

In response to the increased soil nitrate concentration, a peak of denitrifying activity was detected in all the experimental plots following the irrigation event 10 days after the late fertlization (Fig.5-4).

Even if no significant differences were found between treatments, huge differences in mean values were noticed as well, in agreement with soil nitrate concentration and WFPS recorded in each plot. The highest denitrification rate was in fact measured in **N+** while, despite the similar NO_3^- concentrations, an higher denitrification activity was found in **C** as compared to **N-**, according to the higher WFPS in this plot

On the second sampling date, according to the unchanged soil nitrate concentrations, also denitrification rates in **N+** and **N-** did not show significant changes referring to the first sampling, with the highest value being detected in **N+** (even if with no statistical significance). Differently very low denitrifying activity were observed in **C**, evidently attributable to the low WFPS at the sampling time.

Finally on the last sampling day, slight denitrifying activities were detected in all treatments probably as a consequence of the low values of soil WFPS.

As a matter of fact a significant correlation was found between r_{den} and soil nitrate concentration at values of WFPS above 40% (Fig.5-5 A); at the same time when soil NO_3^--N concentration was above 15µg g^-1, bacterial denitrification showed to rise at increasing values of WFPS (Fig.5-5 B).

The high denitrifying activities and N_2O fluxes detected in the different experimental plots 10 days and 30 days after urea-N supply, at non limiting values of soil WFPS, showed soil NO_3^- concentrations were probably enough high in all treatments to cause no competition between microbial community and plant system for N-mineral source demand, suggesting marked N-losses by denitrification might have occurred, even in the less fertilized treatment, up to 1 month after the late fertilization event time, every time soil moisture promoted the process.

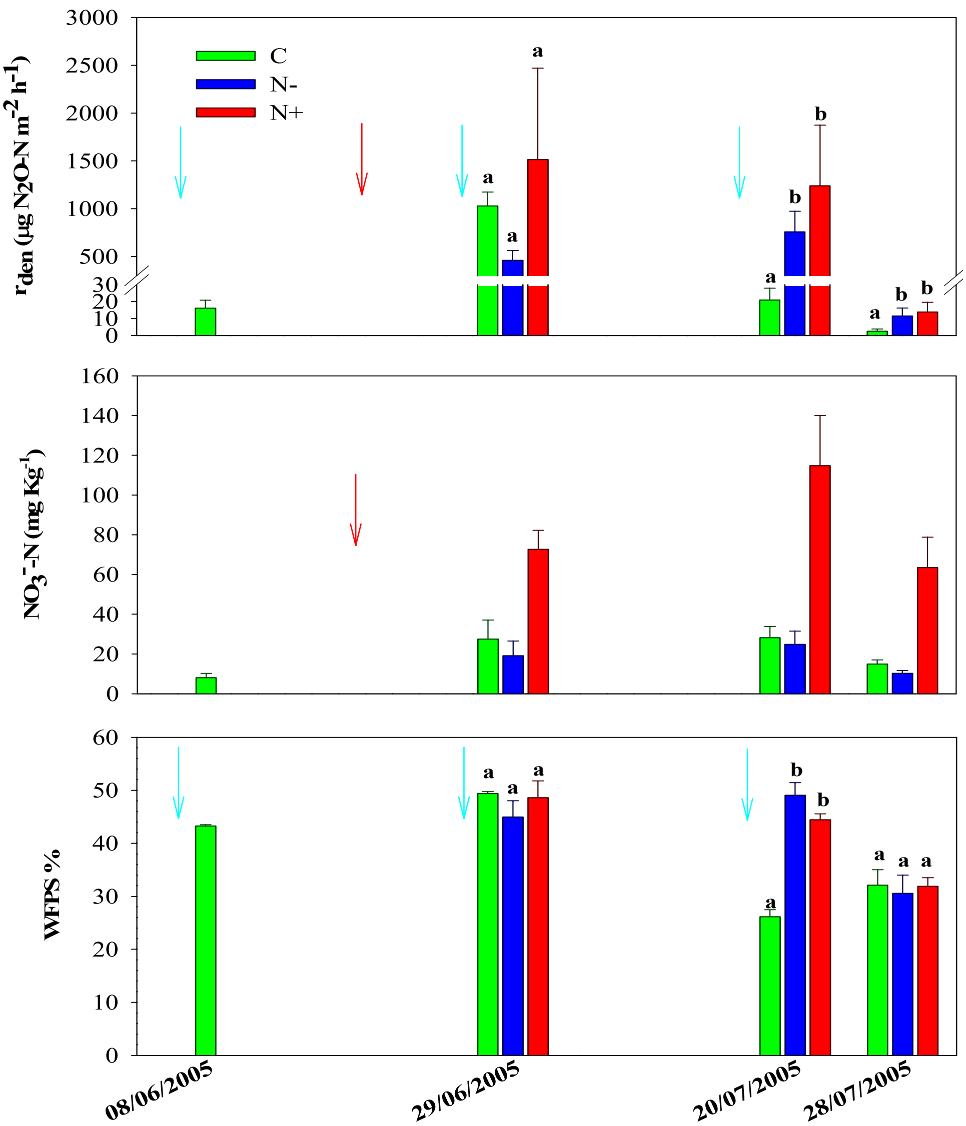


Figure 5-4: Mean values and standard errors for actual denitrification rate (r_{den}), soil NO_3^- and WFPS in the course of the manipulation experiment during the *Zea mays* growth in 2005. Different letters point out significant differences between plots on each sampling date (One Way ANOVA Holm-Sidak test P<0,05). The red and cyan arrows indicate the late fertilization (21/06/05) and the irrigation events, respectively.

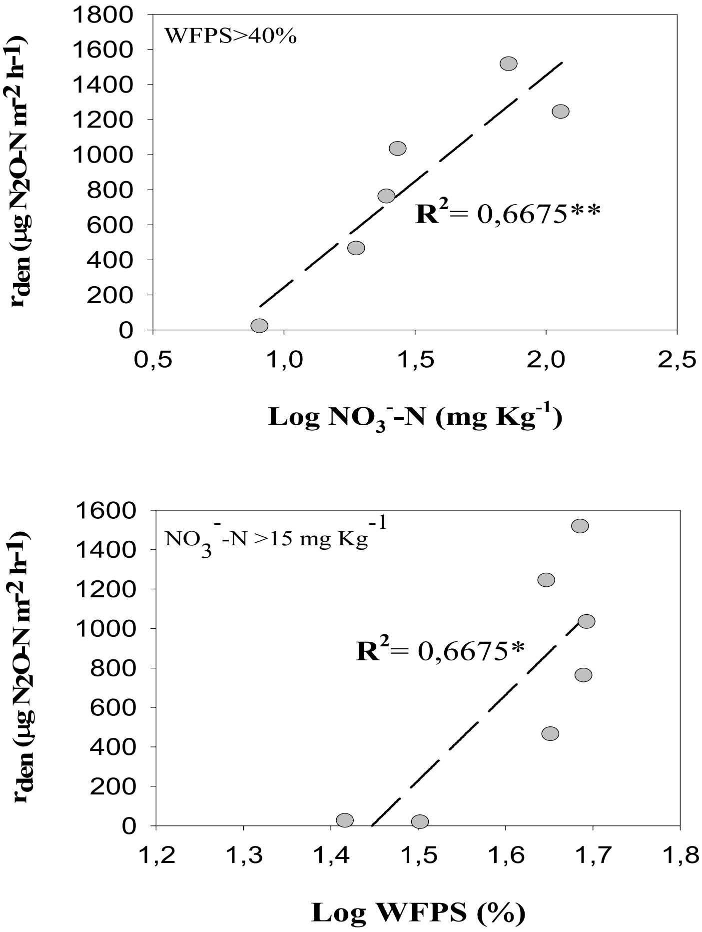


Figure 5-5: Actual denitrification rate (r_{den}) v.s. A) soil nitrate and B) soil WFPS (mean values from each sampling date) on the course of the manipulation experiment during the *Zea mays* growth in 2005 (Pearson product-moment Test: * P< 0,05, ** P< 0,01, *** P< 0,001).

It's noteworthy the results coming from nitrogen metabolism of plants, appeared to suggest soil NO_3^- concentration was not limiting for soil-plant relationships in all treatments as well. In fact all parameters F_v/F_m ratio (Arena, pers. comm.), soluble proteins and total leaf free-amino acid content (Parisi et al., 2006), showed no statistical difference among treatments on all sampling dates, pointing out the different nitrogen fertilizations did not produce any influence on maize performance in the field.

5.3.5 N_2O fluxes from soil

N_2O fluxes from soil exhibited variations similar to denitrifying activity in response to soil NO_3^- and WFPS patterns (Fig.5-6).

In fact, 10 days after the late mineral fertilization, N_2O evolved from soil showed a marked increase following irrigation, with significant higher values in **N+** than in both **N-** and **C** plots, while on the second sampling date, N_2O fluxes from irrigated soil of **N+** and **N-** plots were much higher than in the control plot, not irrigated yet.

On sampling dates when both irrigated and not irrigated areas were present inside each plot, low emission of N_2O were detected from dry soils in all the treatments while somehow higher values were showed by soils supplied with water. In detail pronounced N_2O fluxes were measured from soil of **N+** plots where NO_3^- concentration appeared not to lower below limiting values, whereas the effect of irrigation on the amount of N_2O evolved from the soil of **C** and **N-** treatments was less evident, probably as a consequence of the decreasing soil nitrate concentrations towards values affecting bacterial denitrification.

As shown in Figure 2-7, N_2O fluxes exhibited on the whole a positive correlation with soil NO_3^- concentration and WFPS, at not limiting values of soil moisture (WFPS>40%) and nitrate availability (NO_3^--N>15mg g^{-1}) respectively, moreover they were related to denitrification rate as well, confirming the key role of denitrifying activity in N_2O release from the fine textured soil analysed (see Chapter 3).

The pronounced N_2O fluxes measured after water supply right to the very end of the maize growing season (most likely due to high denitrifying activities) pointed out that for similar values of maize performance (Arena, pers.comm; Parisi et al., 2006), soil nitrate surplus in the N+ treatment caused higher N-losses from the system as compared to C and N- treatments, and it can be assumed a higher amount of nitrate could have been leached through the first autumnal rains as well.

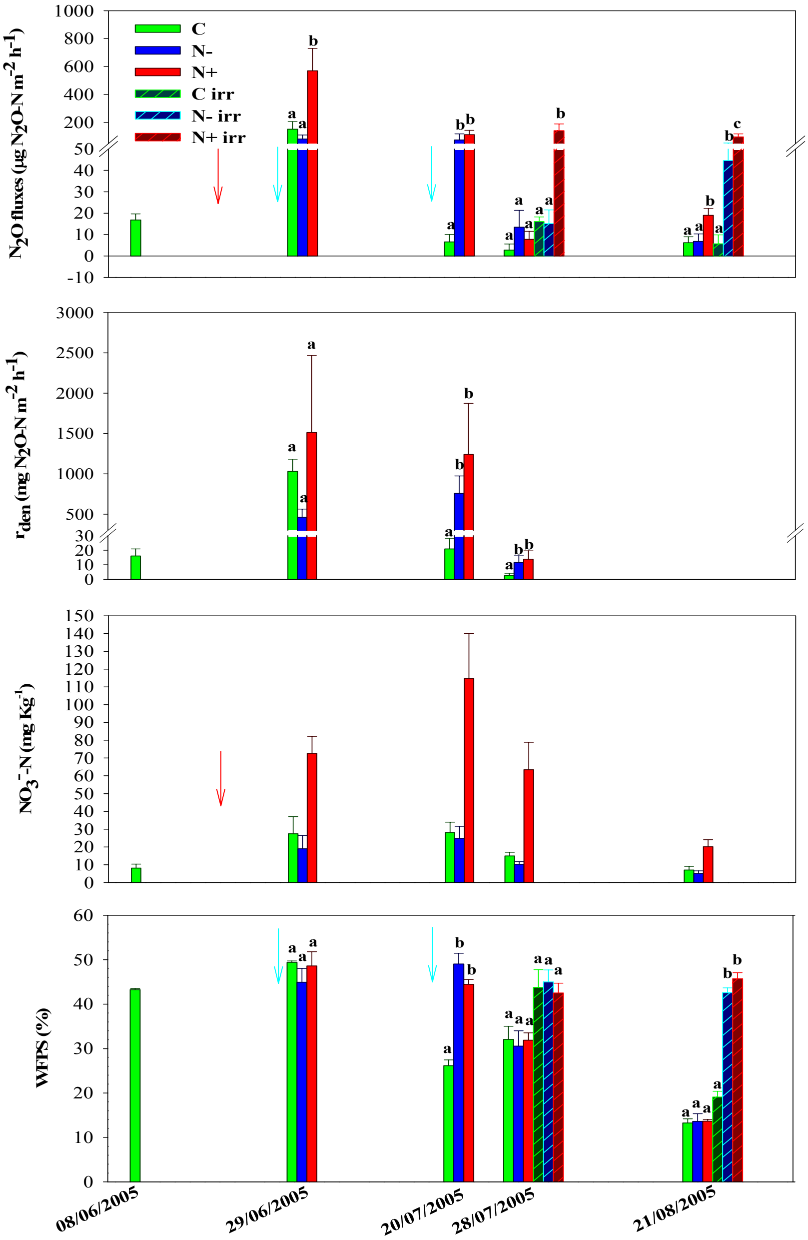


Figure 5-6: Mean values and standard errors for N_2O fluxes, actual denitrification rate (r_{den}), soil NO_3^- and WFPS in the course of the manipulation experiment during the *Zea mays* growth in 2005. Different letters point out significant differences between plots on each sampling date (One Way ANOVA Holm-Sidak test P<0,05).

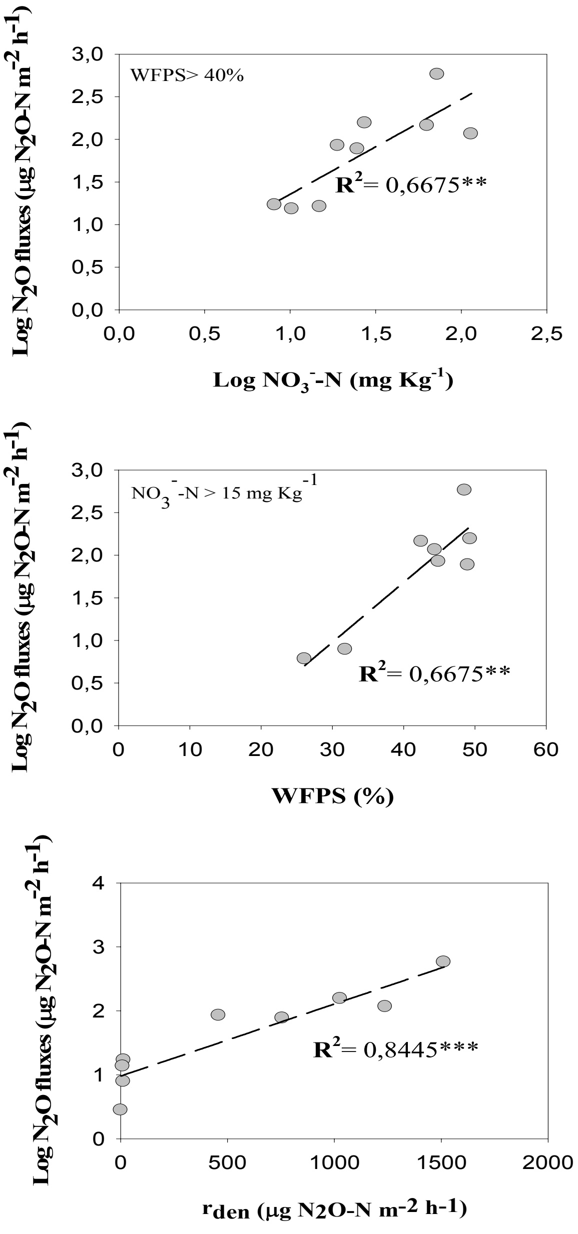


Figure 5-7: N_2O fluxes v.s. A) soil nitrate concentration, B) soil WFPS and C) actual denitrification rate (mean values from each sampling date) on the course of the manipulation experiment during the *Zea mays* growth in 2005 (Pearson product-moment Test: * P< 0,05, ** P< 0,01, *** P< 0,001).

5.4 CONCLUSIONS

Results coming from nitrogen metabolism of plants and denitrifying activity appeared to suggest that at least up to 1 month after the fertilizer N application, soil NO_3^- concentrations were probably enough high in all treatments (even in the less fertilized) to cause no competition between microbial community and plant system for N-mineral source demand.

In fact all parameters F_v/F_m ratio, soluble proteins and total leaf free-amino acid content didn't exhibit significance differences among **C**, **N-** and **N+** experimental plots on all sampling dates, suggesting that the different nitrogen fertilizations did not influence maize performance in the field (Arena, pers.comm; Parisi et al., 2006).

At the same time high denitrifying activities and N_2O fluxes were detected in all the different experimental plots 10 days and 30 day after fertilizer-N supply, at not limiting values of soil WFPS, suggesting through that period marked N-losses by denitrification might have occurred every time soil moisture promoted the process.

Results supporting the idea of N-surpluses at the experimental site come from a recent emergetic analyses of the zootechnic farm also, showing that the system greatly relay on non-stop external inputs of not renewable resources, among which fertilizers N are the main contributing factors (Alfieri, 2005).

Several applications of small amounts of fertilizer N during the growing season might be a more effective mean to supply N for plants growth, anyway multiple applications of fertilizer can't be achieved in a maize crop because of the difficulty of applying fertilizer within a maturing crop canopy.

Even if on the whole no significant differences were found among treatments, the highest values for both denitrification rate and N_2O fluxes from soil were detected in N+, according to the higher NO_3^- concentration recorded. Moreover pronounced N_2O fluxes were measured at the highly fertilized plots right to the very end of the maize growing season, at still relatively high soil NO_3^- concentrations, pointing out that soil nitrate surplus in the **N+** treatment might have caused higher **N-** losses from the system as compared to **C** and **N-** treatments, also enhancing the risk of nitrate leaching through September rains.

Finally once again (see Chapter 3), denitrification rates and N_2O fluxes showed to be positively related between each other and to both NO_3^- and WFPS, at not limiting values of soil water content (WFPS>40%) and nitrate availability (15 mg NO_3^--N Kg^{-1}), respectively.

6 PREDICTING ACTUAL DENITRIFICATION RATE AND N_2O FLUXES AT THE CLAY SITE.

6.1 INTRODUCTION

In order to avoid direct emission measurements a great number of both process models and empirical models have been developed to predict N_2O fluxes from soil.

Process models (Li et al., 1992; Parton et al., 1996; Frolking et al, 1998) are usually quite complex, since they deal with huge sets of input data and calculate N_2O fluxes from their dependence on some relevant soil parameters not directly measured, but in their turn derived from mathematical functions predicting their pattern on the basis of physico-chemical laws. Anyway this kind of general approach, for emission estimates on a large scale, may be not able to furnish accurate predictions of N_2O fluxes at a regional scale, since not able to entirely reproduce characteristic features of local ecosystems (Frolking et al., 1998).

Empirical models (Flessa et al., 1995; Velthof et al., 1996; Conen et al., 2000) are less complex and derive emission estimates directly measuring driving soil parameters such as soil temperature, WFPS and mineral-N availability, so that they can better reflect peculiarity of the system they were derived for. On the other hand, they might be so specific not to be suitable for any other kind of systems, apart that one they were derived for.

It's well known denitrification is a main source of nitrous oxide emission from soil (Williams et al, 1992; Ellis et al, 1996; Vinther et al, 1999; Cavigelli and Robertson, 2001) and several studies tried in vain to identify significant relations between N_2O fluxes, potential denitrifying activity and/or soil characteristics such as soil pH, temperature, water and organic matter content, NH_4^+ and NO_3^- concentrations (Groffman and Tiedje, 1991; Chang et al, 1998; Clements et al, 1999), anyway other authors got opposing positive results (MacKenzie et al., 1998; Simek et al, 2004; Henault et al., 2001).

It's noteworthy, recently Henault et al. (2005) pointed out that models considering soils' capacities to denitrify, to reduce N_2O to N_2 and to emit N_2O during nitrification, can furnish more accurate estimates of N_2O fluxes from soil than models based only on direct measurements of soil physico-chemical key variables. In their paper they presented a new algorithm, Nitrous Oxide Emission (NOE), calculating N_2O fluxes from agricultural soils as the result of production through denitrification and nitrification and reduction through the last step of denitrification. In the model denitrification is in its turn assessed by the simplified model NEMIS (Hénault and Germon, 2000) on the basis of potential denitrification and its regulation through soil temperature, nitrate and water content.

It might be argued actual denitrification rate even could give better results, since it is measured on intact soil cores and consequently is more representative of the real denitrifying activities occurring in the field and their associated N_2O emissions from soil.

For istance Simek (Simek et al, 2004) found a significant correlation between denitrification rate in relatively undisturbed soil cores (AIT on intact soil cores in inert atmosphere with 99,99% He) and N_2O fluxes from the soil of three perennial forage crops systems in Czech Republic, while no similar relation was found for both denitrifying enzyme activity (DEA) and denitrification potential (DP).

Moreover many modelling studies on N cycling deriving denitrification by simplified process models on the basis of potential denitrification, soil nitrate content, degree of water saturation and temperature (NEMIS, SOILN), were not able to predict with accuracy measured actual denitrification rates.

For instance Hénault and Germon (2000) showed that the simple denitrification process model NEMIS worked well for two data sets with parameters specifically derived for, while it appeared not to furnish good estimates for other data sets, suggesting parameters need to be calibrated for different locations depending on characteristic soil and environmental conditions.

On this very subject, Heinen (2006 a) managed to parameterize for different soil types (loamy and sandy) a simplified denitrification model (Johnsson et al., 1987; 1991; Hènault and Germon, 2000, Jansson and Kalberg, 2001; Heinen, 2006 b) by additional data sets of measured actual denitrification rate and concluded many models parameterised for each location may work better than a single one pretending to fit a wide range of conditions by averaged parameters.

As far as concern this study, significant correlations were found for the clay soil between actual denitrification rate, N_2O fluxes, nitrates and water content, in the course of both monitoring and manipulation activities (see Chapers 3 and 5), suggesting denitrification rate might be predictable from soil characteristics, at the same time appearing in its turn as good predictor parameter to estimate emissions, without flux measurements. This section intended to check into detail these hypothesis, performing correlation and regression analyses on the whole set of data regarding the fine textured soil.

6.2 EXPERIMENTAL SET-UP

Correlation and regression analyses between soil physico-chemical and biological characteristics were performed using all the data coming from both the monitoring activity throughout the maize crops (2005 and 2006) and the manipulation experiment in the course of the *Zea mays* growth in 2005.

Data regarding the *Lolium italicum* growth were not considered in this section, since soil NO_3^- concentration exhibited a very narrow range of values, furthermore limiting for most of the observation period, thus preventing from finding useful correlations for this purpose.

As shown in more detail in Table 6-1, possible dependences of both actual denitrification rate and N_2O fluxes on soil nitrate concentration and WFPS were investigated, moreover correlations between actual denitrification rate and N_2O fluxes from soil were analysed as well.

Since soil NO_3^- concentration and WFPS can be limiting to a different extent and at different times in the field, in order to better isolate the dependence of denitrification and N_2O fluxes on each parameter, the whole set of data was divided into more restricted groups characterized by ranges of soil nitrates and WFPS as homogeneous as possible. For istance denitrification rate relation with soil nitrate content was investigated at low (limiting), medium and higher range of soil WFPS, similarly denitrification rate dependence on soil aeration state was studied at low (limiting), medium and higher range of soil nitrates. The same data processing was performed to investigate possible relationships between N_2O fluxes and soil parameters.

Soil nitrate concentration and WFPS were the only driving variables taken into account, since throughout the whole study, no significant relations were found for both denitrification rate and

N_2O fluxes, with soil pH (on average in the optimum range for denitrification) and organic matter content (quite high and therefore probably never limiting); also the relationship between N_2O fluxes and soil NH_4^+ concentration were omitted in this section, since data are available only for the *Zea mays* growth in 2006 and they have been already illustrated in Chapter 3, section 3.3.5.

Table 6-1: Relations analysed between soil characteristics (further explanations inside the text).

Soil parameters related	WFPS range	NO_3^--N range
r_{den} v.s NO_3^--N	WFPS<40% 40%<WFPS<45% 47%<WFPS<50%	
r_{den} v.s WFPS		NO_3^--N<15 mg Kg^{-1} 19 mg g^{-1}<NO_3^--N<29 mg Kg^{-1} NO_3^--N>60 mg Kg^{-1}
N_2O fluxes v.s NO_3^--N	WFPS<40% 40%<WFPS<45% 47%<WFPS<50%	
N_2O fluxes v.s WFPS		NO_3^--N<15 mg Kg^{-1} 19 mg Kg^{-1}<NO_3^--N<29 mg Kg^{-1} NO_3^--N>60 mg Kg^{-1}

Moreover the effect of seasonal variations of temperature on denitrifying activity and N_2O fluxes from soil couldn't be determined as well, since there were only few sampling dates (3 for actual denitrification rate and 2 for N_2O fluxes measurements) coming from winter and summer periods differing only for soil temperature, at similar not limiting soil nitrate concentration and WFPS (for completeness, a short treatment of denitrification rate dependence on seasonal variation of temperature is given, anyway).

Therefore, even if soil temperature is usually included as an influencing parameter in models predicting denitrification (Heinen, 2006 b) and N_2O fluxes from soil (Abassi and Adams, 2000; Conen et al., 2000; Henault and Germond, 2000; Henault et al., 2005), in this study this variable was not taken in account. All the reasonings were in fact referred to the summer maize growing seasons in 2005 an 2006, throughout which not significant variations of both denitrifying activity and N_2O emissions were detected between sampling dates differing only for soil temperature (at similar not limiting soil nitrates and WFPS), evidently as a consequence of the very narrow range of soil temperature involved (23,5 °C<T<25,5 °C).

It is necessary to underline the data processing reported in the following sections is not an attempt to develop an empirical model predicting N_2O fluxes from soil physico-chemical characteristics and actual denitrification rate, but intends to show actual denitrification rate can be an helpful predictor parameter to workout such a kind of model or to improve process models already developed.

In fact, besides the impossibility to assess soil temperature influence, the whole set of correlations found in this study between soil characteristics can't be considered completely satisfactory to work out an empirical model, since they had been developed in the context of a process study not planned with the aim of modelling, and likely less than ideal for this aim.

Samplings were performed in fact on critical days such as before and soon after fertilization and irrigation events, so that they could reveal changes of denitrification rate and N_2O fluxes in consequence of variations of key soil physico-chemical parameters, but they did not document the whole peak and tail of these phenomenons. Moreover interpolating flux values between sampling dates would be inappropriate and might cause remarkable errors since both processes can exhibit high temporal variation, even at the diurnal scale.

That is why the statistical and mathematical reasonings showed in the following sections should be more properly considered as interesting correlations to develop in the future a real empirical model supported by new data from monitoring activities planned for this very purpose.

6.3 RESULTS AND DISCUSSION

6.3.1 Denitrification rate v.s. soil NO_3^- concentration and WFPS

As shown in Figure 6-1 denitrification appeared to be insensitive to changes in soil nitrate concentration at values of WFPS below 40%, corresponding to volumetric moisture contents (θ_d) below 0,215 $cm^3\ cm^{-3}$.

Otherwise, at not limiting values of WFPS, denitrifying activity showed to increase with rising values of nitrate availability (Table 6-2).

Table 6-2: Significant correlations between actual denitrification rate (r_{den}) and both soil nitrate and WFPS. Pearson product-moment Test: * P< 0,05, ** P< 0,01, *** P< 0,001.

	r_{den} v.s NO_3^- (40%<WFPS< 34%)	r_{den} v.s WFPS (19 mg Kg^{-1}<NO_3^--N<29 mg Kg^{-1})	r_{den} v.s WFPS (NO_3^--N>60 mg Kg^{-1})
r^2	0,7465*	0,8930**	0,8538***

In detail the relationship between denitrifier bacterial activity and nitrate can be described by a Michaelis-Menten kinetic: at low and medium nitrate content denitrification increased via a first order equation, while at high nitrate content nitrate was not limiting and the process approached a zero-order equation (Fig.6-1).

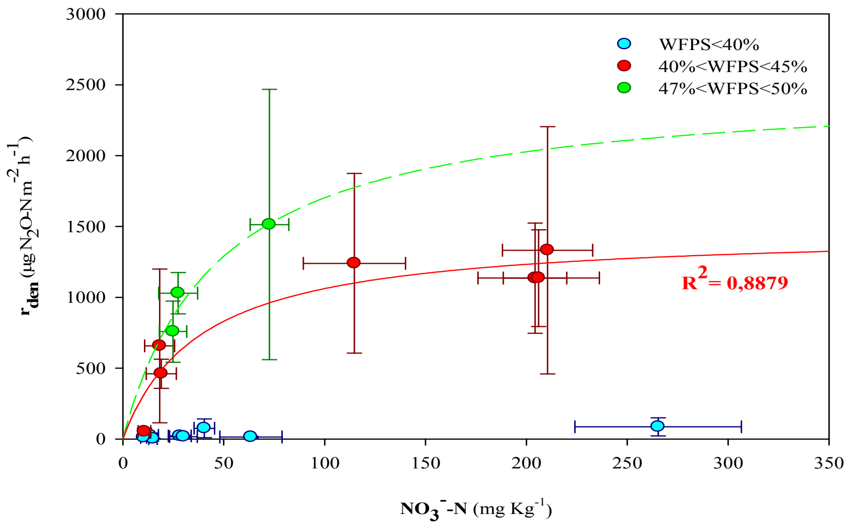


Figure 6-1: Actual denitrifiaction rate (r_{den}) v.s. soil nitrate concentration at increasing range of soil WFPS (mean values from each sampling date). R^2 is the coefficient of determination for the Nonlinear Regression (One site saturation equation, f= Bmax* (x)/(Km + (x), where Bmax= maximum rate= 1469,5970 µg N_2O-N $m^{-2}\ h^{-1}$ and Km= half-saturation constant= 38,4795 mg NO_3^--N Kg^{-1}).

As discussed in more detail in Chapter 1, Section 1.2.1, the Michaelis-Menten form is the type of relation usually reported to describe denitrification dependence on soil nitrate and to predict denitrification rate in most N-cycling models. Anyway the half-saturation constant K_M and the maximum denitrification rate can show a huge ranges of values depending on soil texture, climatic factors and soil management practices.

In this study, at values of WFPS in the range between 40% and 45%, 37,5 mg NO_3^--N Kg^{-1} was the soil concentration giving a denitrification rate of 50% of the maximum value, about 1470 µg N_2O-N m^{-2} h^{-1}. Nevertheless, as illustrated by the dotted green line in Figure 6-1 the plateau appeared to increase at higher range of soil WFPS's.

It's interesting to notice the WFPS threshold value, that is the WFPS below which the O_2 content inside the soil core is enough high to inhibit denitrifying enzymes (Smith and Tiedje, 1979), is quite lower than the values reported in literature (Rolston et., 1984; Arcara et al., 1999; Henault and Germon, 2000; Vallejo et al, 2001; Vallejo et al., 2004). For istance, as far as concern irrigated croplands under Mediterranean conditions, Vallejo et al. (2004) found a threshold volumetric moisture content (θ_d) of 0,285 cm^3 cm^{-3}, corresponding to a soil WFPS values of 65%, in the top-layer of a sandy-loam soil, with bulk density value of 1,47 g cm^{-3} and total organic matter content of 1,4%.

Anyway soil denitrifying micro-organisms are able to produce N_2O over a wide range of oxygen pressure, moreover the limiting value of WFPS for bacterial denitrification can show marked variations depending on the soil texture (Barton et al., 1999) and not always the empirical WFPS term is able to normalize the water regimes of intact soil cores for soil type differences (Schjønning et al., 2003).

At the experimental site the clay soil is characterized by a bulk density on average close to 1,00 g cm^{-3}, with values increasing up to about 1,15 g cm^{-3} by the end of winter period, probably as a result of compaction through winter rains.

Even if usually, because of their fine structure, clay soils have low percentages of macropores and very high percentages of micropores, in Mediterranean regions the dynamics of continuos macropores can be strongly affected by cracks formations (Fig.6-2) during spring and summer period (Vogel et al, 2005). The shrinking of the soil at decreasing water content produces in fact a very variable network of macropores which allow a quicker and wider water infiltration during rainfall or irrigation events, at the same time loosing water much faster through drainage and evaporation.

Anyway since it can be assumed denitrification mostly occurs inside the micropores of the soil aggregates (Nomik, 1956; Arah and Smith, 1989; Seech and Beuchamp, 1988), it might be argued anaerobic denitrifying microsites could be still very active at relatively high intra-aggregated WFPS, also when the total WFPS seems to be low because of the air filled macropores.

Figure 6-2: Crack formation at the surface of the fine textured soil in the experimental field during summer period.

Therefore the WFPS threshold value near 40% found in this study may be probably characteristic of the fine textured soil analysed under Mediterranean conditions.

Denitrification rate showed to be regulated by the soil aeration state also. Since oxygen gradient along the soil profile is strongly affected by the soil water content, with air porosity decreasing at increasing value of WFPS, the dependence of denitrifying activity on oxygen supply can be analysed by the use of soil WFPS as well.

As shown in Figure 6-3, at soil nitrate concentration below 15 mg NO_3^--N Kg^{-1}, denitrification rate showed very slight values despite of increasing soil WFPS.

Differently, at not limiting soil nitrates, denitrification rate exhibited significant correlations with soil WFPS (Table 6-2), exponentially rising at increasing values of soil WFPS, moreover with a higher steepness of the curve at increasing ranges of soil nitrates taken into account (Fig.6-3).

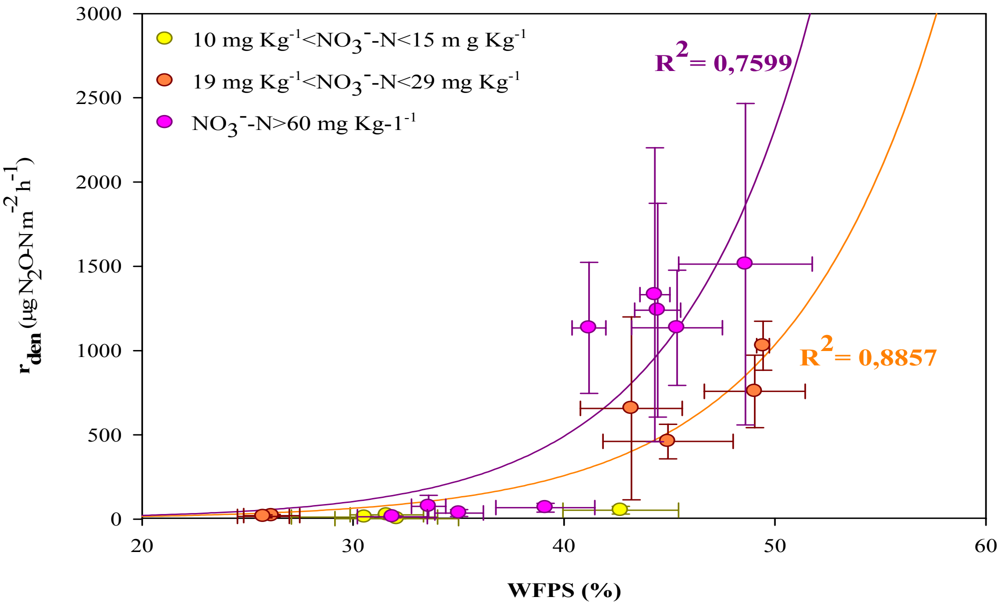


Figure 6-3: Actual denitrifiaction rate (*r_{den}*) v.s. soil WFPS at increasing range of soil nitrate concentration (mean values from each sampling date). R^2 is the coefficient of determination for the Nonlinear Regressions (Exponential growth, 1 parameter, equation, f= exp(a*x), where a= 0,1388 at 19 mg Kg^{-1}<NO_3^--N<29 mg Kg^{-1} and a= 0,1549 at NO_3^--N>40 mg Kg^{-1}).

Steep non linear functions, like exponential, power and sigmoidal functions, are usually employed in models to describe denitrification dependence on soil aeration status (Grundmann and Rolston, 1987; Parton et al., 1996; Heinen, 2006 b), since coefficients of oxygen diffusion inside the soil are non-linearly related to soil air filled pore space (Bakken et al., 1987).

Considering the regressions curves for denitrification rate variations as function of soil nitrate and WFPS, actual denitrification rate can be calculated as:

$$r_{den\ predicted} = \mathbf{k\ f(NO_3^-)\ g(WFPS)} \qquad \mathbf{(1)}$$

where

$f(NO_3^-)$= Michaelis-Menten function describing r_{den} dependence on soil nitrate concentration at 40%<WFPS<45% (Fig.6-1)

g(WFPS)= exponential function describing r_{den} dependence on soil WFPS at NO_3^--N>60mg Kg^{-1}, that is at nitrate concentration close to the plateau and so only slightly influencing r_{den} (Fig.6-2)

K= Correction factor= direction coefficient of the linear regression r'= K $r_{den\ measured}$, with r'= $f(NO_3^-)$ g(WFPS) (Fig.6-4).

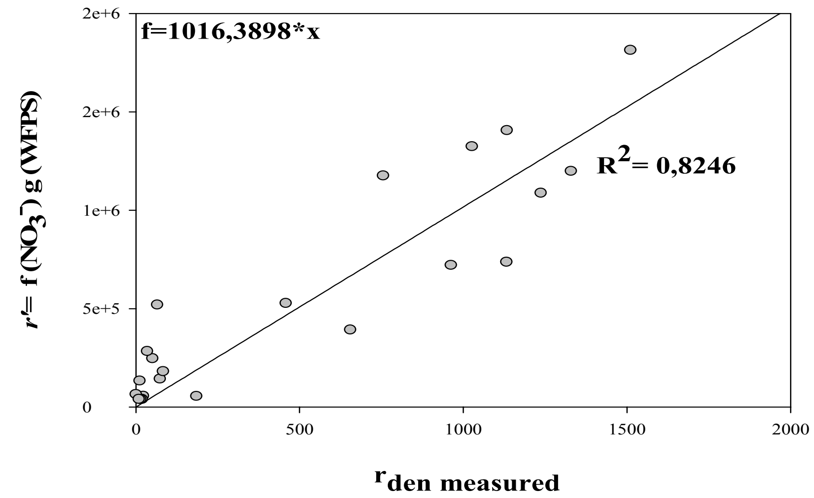


Figure 6-4: Calculation of the correction factor K as the direction coefficient of the linear regression r'= K $r_{den\ measured}$. R^2 is the coefficient of determination for the linear Regression.

As shown in Figure 6-5, the denitrification process appeared to be predictable with satisfying approximation by considering soil nitrate concentration and WFPS.

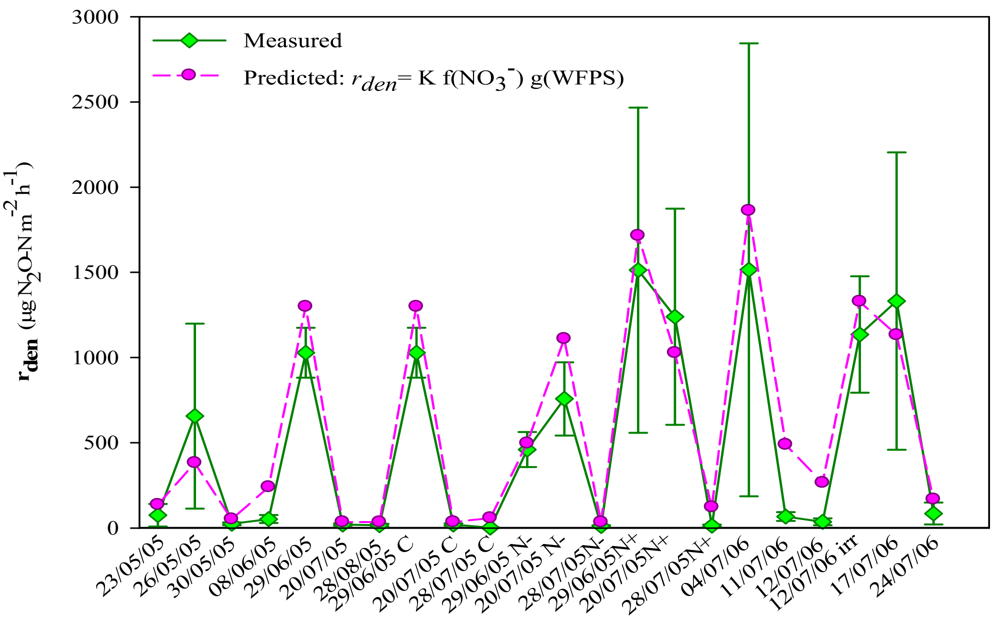


Figure 6-5: Comparison between predicted and measured actual denitrification rates for all sampling dates in the course of the *Zea mays* growths in 2005 and 2006.

Also Vallejo (Vallejo et al., 2004) found that the denitrification process, measured on intact soil cores, could be effectively simulated by considering soil temperature, nitrate avilability and water content.

As already pointed out before, in this study there were too few sampling dates differing only for soil temperature at similar not limiting values of soil nitrate and WFPS, so that it was not possible to determine significant relations between denitrifying activity and both seasonal and day-to-day variations of soil temperature.

Anyway for completeness sake, in Figure 6-6, the regression curve for denitrification rate dependence on temperature of soil is illustrated as well, based on the Vant'Hoff law:

$$\mathbf{f = Q_{10}\text{^}((T\text{-}T_r)/10)}$$

where:

f= the rate of the biological process analysed

Q_{10}= the increase factor in **f** at a 10 °C (or 10 K) increase in T

T= temperature of a given soil layer (°C or K)

T_r= reference temperature where f_t=1

Many N-cycling models use the exponential Van'Hoff or Arrhenius equations to relate denitrifying activity to soil temperature and normally values from 2 to 3 are used for Q_{10}, even if it can greatly vary depending on the temperature range considered for its calculation (Heinen, 2006 b).

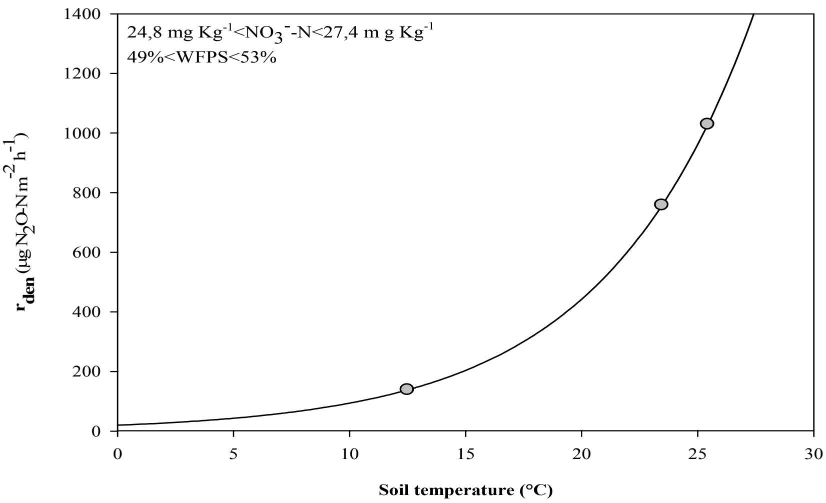


Figure 6-6: Actual denitrifiaction rate (r_{den}) v.s. soil temperature at increasing range of soil WFPS (mean values from each sampling date). R^2 is the coefficient of determination for the Nonlinear Regression (Vant'Hoff law, f= Q_{10}^((T-Tr)/10)).

In this study the value of Q_{10} derived by the Vant'Hoff equation, was 4,72 for T_r= 20, anyway this topic will not be discussed further now, since the scanty data available to derive the regression curve.

6.3.2 N_2O fluxes v.s. soil NO_3^- concentration and WFPS

Similarly to actual denitrification rate, also N_2O fluxes from soil appeared to be insensitive to changes in soil nitrate concentration at values of WFPS below 40% (Fig.6-7), while at values above 40% they showed to increase with rising values of nitrate availability, with a steeper slope at rising range of soil WFPS considered (Table 6-3 and Fig.6-7).

Table 6-3: Significant correlations between N_2O fluxes and both soil nitrate and WFPS. Pearson product-moment Test: * P< 0,05, ** P< 0,01, *** P< 0,001.

	N_2O fluxes v.s NO_3^- (40%<WFPS< 45%)	N_2O fluxes v.s NO_3^- (47%<WFPS< 50%)	N_2O fluxes v.s WFPS (19 mg Kg^{-1}<NO_3^--N<29 mg Kg^{-1})	N_2O fluxes v.s WFPS (NO_3^--N>60 mg Kg^{-1})
r^2	0,7293***	0,7779*	0,6798*	0,6907***

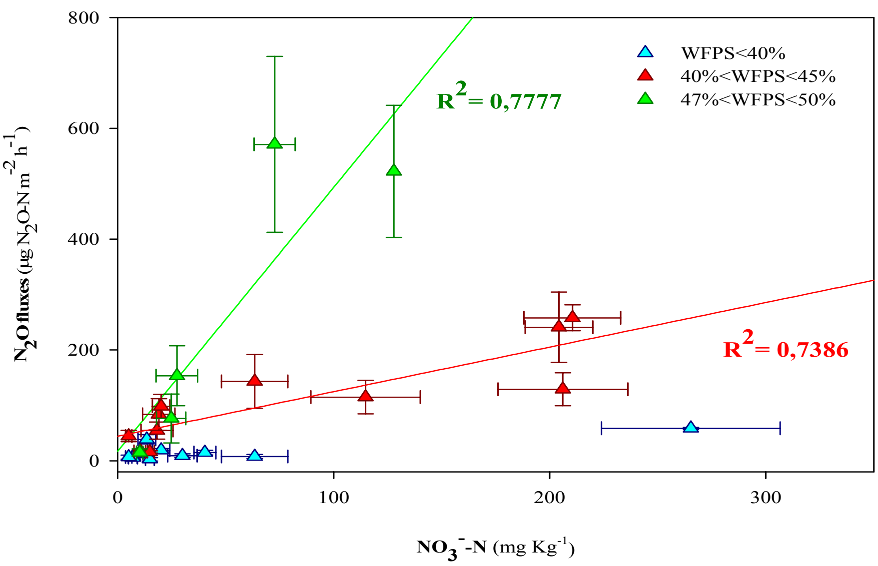


Figure 6-7: N_2O fluxes from soil v.s. soil nitrate concentration at increasing range of soil WFPS (mean values from each sampling date). R^2 is the coefficient of determination for Linear Regression (Linear equation, f=y_0+a*x, where y0= 44,2558 and a= 0,8044 at 40%<WFPS<45% and y0= 17,1141 and a= 4,7624 at 47%<WFPS<50%).

This finding is in agreement with the inhibitory and retarding effect of rising soil NO_3^- concentration on N_2O reduction to N_2 via bacterial denitrification, determining a marked increase of the N_2O/N_2 ratio (Blackmer and Bremner, 1978; Cho and Mills, 1979; Christensen, 1985; Kroeze et al., 1989), as explained in more detail in Chaper 1, section 1.3.3.

Moreover, once again similarly to denitrifying activity the amount of N_2O evolved from soil showed to be strongly affected by the soil water content, exhibiting very slight values at low soil nitrate concentration, while rising exponentially with increasing soil WFPS at not limiting soil nitrates and via a steeper curve at the highest range of soil WFPS considered (Table 6-3 and Fig.6-8).

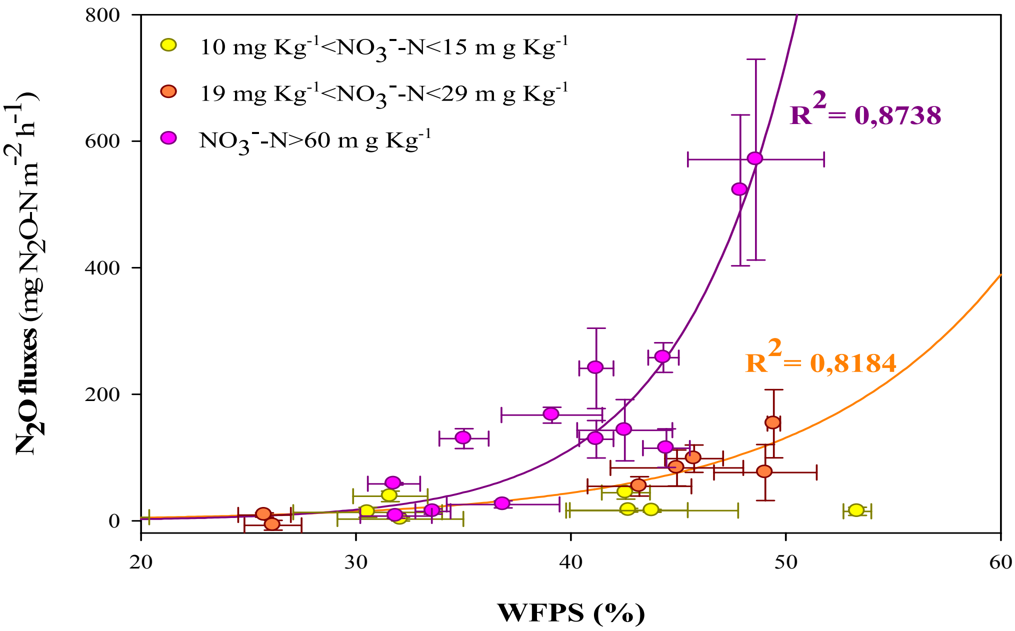


Figure 6-8: N_2O fluxes from soil v.s. soil WFPS at increasing range of soil nitrate concentration (mean values from each sampling date). R^2 is the coefficient of determination for the Nonlinear Regressions. (Exponential growth, 2 parameter, equation, f= a*exp(b*x), where a= 0,5723 and b= 0,1087 at 19mg Kg^{-1}<NO_3^--N<29mg Kg^{-1} and a= 0,0675 and b= 0,1856 at NO_3^--N>40mg Kg^{-1}).

Afterwards, the N_2O emissions from soil measured in the field, were compared with the predicted values, obtained by applying the regression curves described above to the values of soil nitrates and WFPS detected at the site, that is:

$$\mathbf{N_2O\ fluxes}_{predicted} = \mathbf{k\ f(NO_3^-)\ g(WFPS)} \qquad \mathbf{(2)}$$

where:

f(NO_3^-)= linear function describing N_2O fluxes dependence on soil nitrate concentration at 40%<WFPS<45% (Fig.6-7)

g(WFPS)= exponential function describing N_2O fluxes dependence on soil WFPS at NO_3^--N>60mg Kg^{-1} (Fig.6-8)

K= Correction factor= direction coefficient of the linear regression ***f'***= K **N_2O fluxes** $_{measured}$, with ***r'***= f(NO_3^-) g(WFPS) (Fig.6-9).

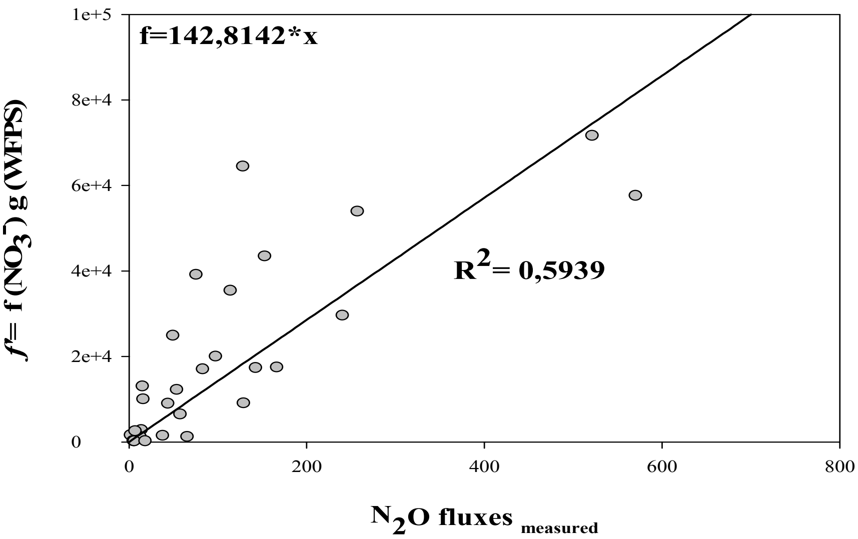


Figure 6-9: Calculation of the correction factor K as the direction coefficient of the linear regression *f'*= K N_2O fluxes $_{measured}$. R^2 is the coefficient of determination for the linear Regression.

As shown in Figure 6-10 the predictions gave acceptable results, anyway they appeared not to be as fitting as the predictions of actual denitrification rate (Fig.6-5), since the dependences on soil parameters were not as strong as for actual denitrification rate.

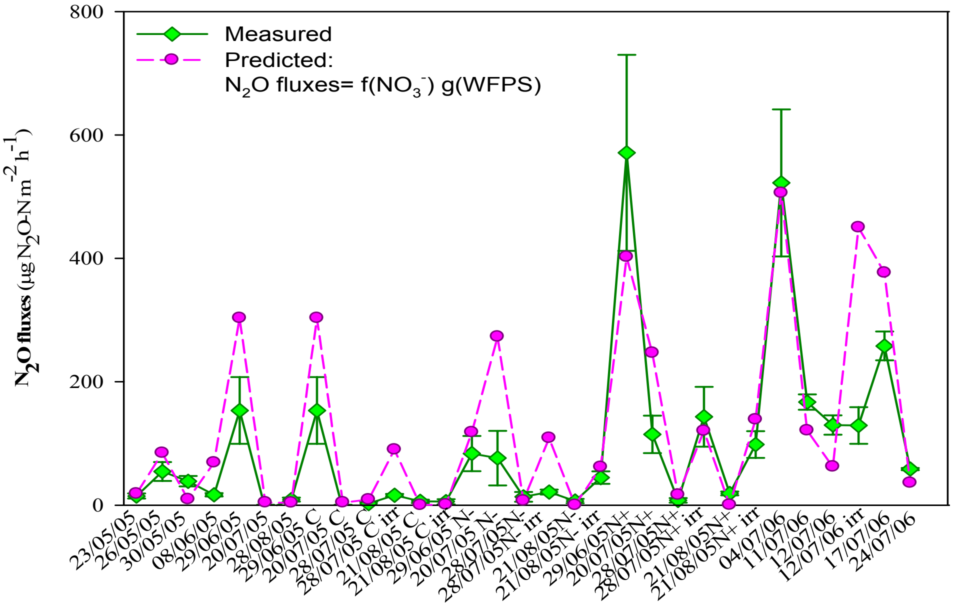


Figure 6-10: Comparison between predicted and measured N_2O fluxes from soil for all sampling dates in the course of the *Zea mays* growths in 2005 and 2006.

6.3.3 Actual denitrification rate as predictor parameter for N_2O emissions from soil

A positive significant correlation was found on the whole between the amount of N_2O evolved from soil and actual denitrification rate (Pearson product-moment test, $P<0,05$), supporting the idea of using actual denitrification rate for N_2O fluxes prediction.

Anyway, as shown in Figure 6-11, relatively high emissions were detected at very slight values of denitrifying activity as well, and as a matter of fact, N_2O emissions showed no significant relation with actual denitrification rate at soil WFPS below 40%.

It might be assumed these moderate N_2O peaks were a result of nitrifying activity, in agreement with the positive relation found between N_2O fluxes and soil NH_4^+ concentration at soil

WFPS<40% in the course of the maize crop in 2006 (see section 3.3.6 in Chapter 3).

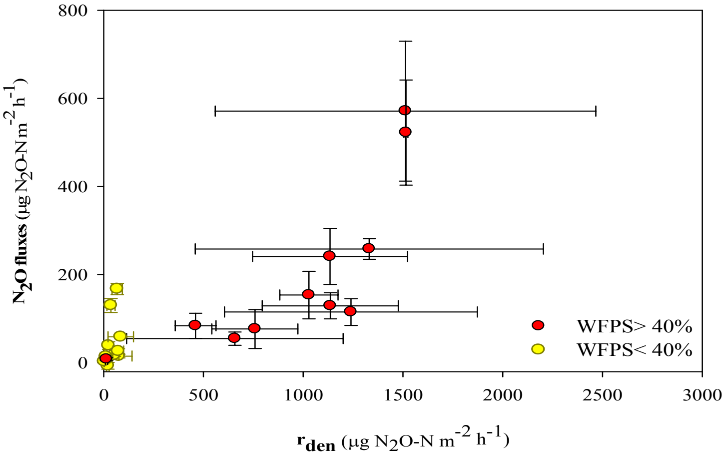


Figure 6-11: N_2O fluxes from soil v.s. actual denitrification rate (mean values from each sampling date) at different range of soil WFPS.

Otherwise, at soil WFPS<40% N_2O fluxes exhibited an exponential dependence on actual denitrification rate (Fig.6-12).

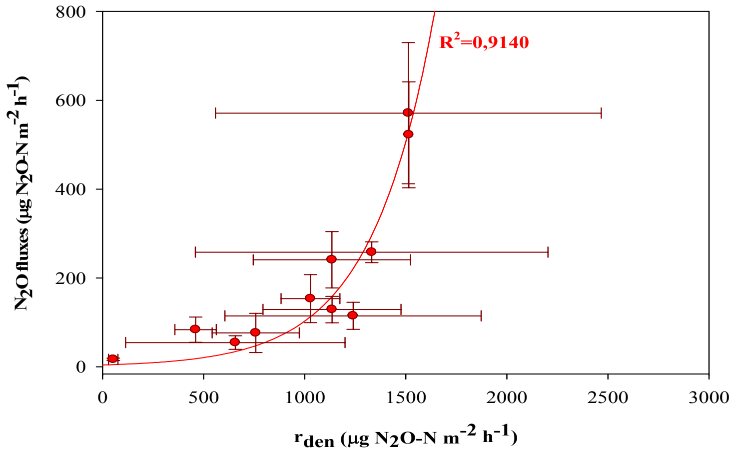


Figure 6-12: N_2O fluxes from soil v.s. actual denitrification rate (mean values from each sampling date) at WFPS> 40%. R^2 is the coefficient of determination for the Nonlinear Regression (Exponential growth, 2 parameter, equation, f= a*exp(b*x), where a= 4,2457 and b= 0,0032).

Predicting functions for N_2O fluxes from soil were derived considering both only the relationship with actual denitrification rate (3) and their dependence on the combined effect of actual denitrification rate, soil nitrate concentration and WFPS (4):

$$\mathbf{N_2O\ fluxes}_{\mathbf{predicted}} \mathbf{= k\ h(}\boldsymbol{r_{den\ measured}}\mathbf{)} \qquad \mathbf{(3)}$$

$$\mathbf{N_2O\ fluxes}_{\boldsymbol{predicted}} \mathbf{= k'\ f(NO_3^-)\ g(WFPS)\ h(}\boldsymbol{r_{den\ measured}}\mathbf{)} \qquad \mathbf{(4)}$$

where:

$f(NO_3^-)$= linear function describing N_2O fluxes dependence on soil nitrate concentration at 40%<WFPS<45% (Fig.6-7)

g(WFPS)= exponential function describing N_2O fluxes dependence on soil WFPS at NO_3^--N>60mg Kg^{-1} (Fig.6-8)

$h(r_{den})$= exponential function describing N_2O fluxes dependence on measured values of actual denitrification rate (Fig.6-12)

k= Correction factor= direction coefficient of the linear regression ***y'*** v.s. **N_2O fluxes** $_{measured}$, with ***y'***= h($r_{den\ measured}$) (Fig.6-13 A).

k'= Correction factor= direction coefficient of the linear regression ***y'*** v.s. **N_2O fluxes** $_{measured}$, with ***y'***= f(NO_3^-) g(WFPS) h($r_{den\ measured}$) (Fig.6-13 B)

Moreover, besides the real values measured of actual denitrification rate, functions were derived on the basis of the predictable values of actual denitrification rate from the direct measurements of soil NO_3^- and WFPS through equation (1), that is:

$$\mathbf{N_2O\ fluxes}_{\boldsymbol{predicted}} \mathbf{= k''\ h(}\boldsymbol{r_{den\ predicted}}\mathbf{)} \qquad \mathbf{(5)}$$

$$\mathbf{N_2O\ fluxes}_{\boldsymbol{predicted}} \mathbf{= k'''\ f(NO_3^-)\ g(WFPS)\ h(}\boldsymbol{r_{den\ predicted}}\mathbf{)} \qquad \mathbf{(6)}$$

where:

h($r_{den\ predicted}$)= exponential function describing N_2O fluxes from the predicted values of actual denitrification rate through equation (1)

k''= Correction factor= direction coefficient of the linear regression ***y'*** v.s. **N_2O fluxes** $_{measured}$, with ***y'***= h($r_{den\ predicetd}$) (Fig.6-13 C).

K'''= Correction factor= direction coefficient of the linear regression ***y'*** v.s. **N_2O fluxes** $_{measured}$, with ***y'***= f(NO_3^-) g(WFPS) h($r_{den\ predicted}$) (Fig.6-13 D).

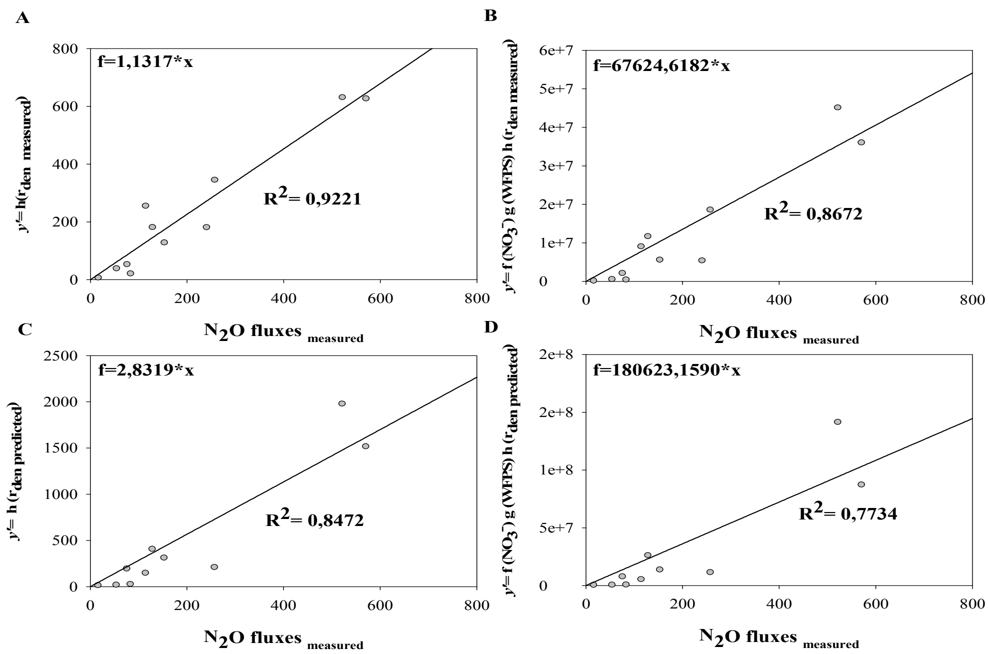


Figure 6-13: Calculation of the correction factors for the different N_2O predicting functions, as the direction coefficient of each linear regression *y'* v.s. N_2O fluxes $_{measured}$. R^2 is the coefficient of determination for the linear Regressions.

It's noteworthy all predicting functions (3), (4), (5) and (6), considering N_2O fluxes dependence on actual denitrification rate, appeared to be more fitting than equation (2), based only on direct measurement on soil NO_3^- and WFPS (Fig.6-14).

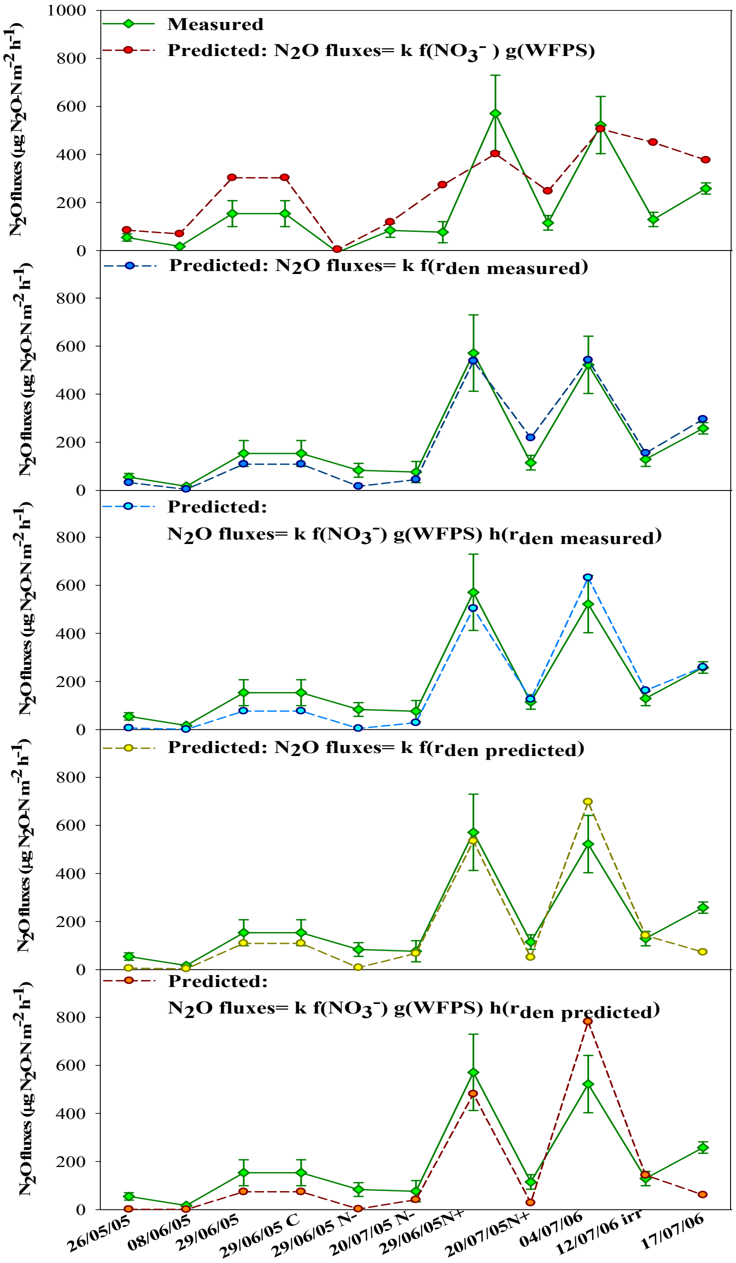


Figure 6-14: Comparison between measured N_2O fluxes from soil and predicted values via equations (2), (3), (4), (5) and (6), for all sampling dates at WFPS>40% in the course of the *Zea mays* growths in 2005 and 2006.

In fact as shown in Figure 6-15, illustrating the residuals of the different predicting equations from the real values of N_2O fluxes measured in the field, the function *N_2O fluxes $_{predicted}$= k f(NO_3^-) g(WFPS)* exhibited the highest residuals in most of the sampling dates, even if only predicted values of N_2O fluxes from soil via equation (3) had residuals significantly lower.

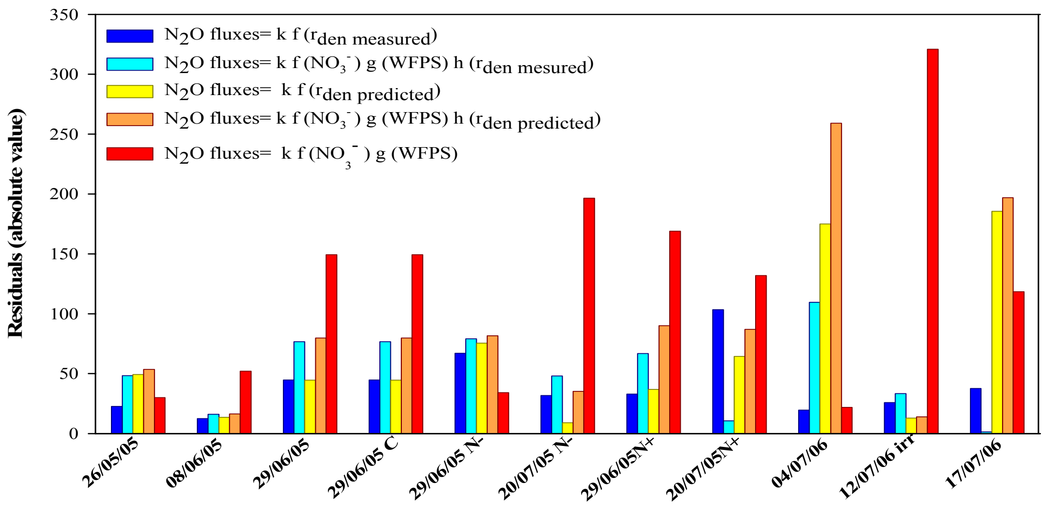


Figure 6-15: Comparison of residuals between measured and predicted N_2O fluxes from soil for the different predicting equations (2), (3), (4), (5) and (6). Residuals for N_2O fluxes= k f($r_{den\ measured}$) were lower than for N_2O fluxes= k f(NO_3^-) g(WFPS) (One Way Analysis of Variance, Multiple Comparisons versus Control Group Dunn's Method, P<0,05).

Therefore, this finding besides pointing out the relevance of the parameter "actual denitrification rate" in N_2O predicting models, suggests that after an initial characterization of denitrifying activity in a given soil (and it's relationship with the amount of N_2O evolved from soil in the field), it might be possible to estimate emissions via models considering their dependence on the parameter "actual denitrification rate", in its turn not directly measured, but derived from soil key drivers such as NO_3^- concentration, WFPS and temperature.

A demonstration is given in Figure 6-16, regarding two sampling dates during the manipulation experiment when denitrification rates were not analysed while N_2O fluxes measurements were performed in-situ in the field (see Chapter 5).

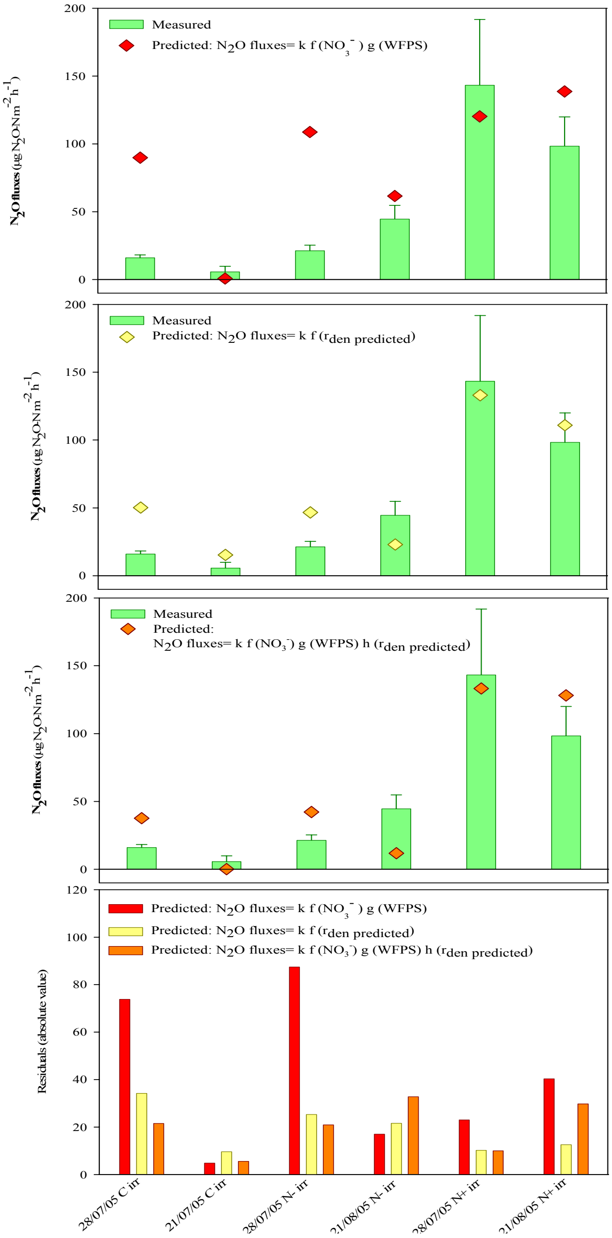


Figure 6-16: Comparison between measured N_2O fluxes from soil and predicted values via equations (2), (5) and (6), for two sampling dates at WFPS>40% during the manipulation experiment in the course of the *Zea mays* growths in 2005. Comparison of residuals between measured and predicted N_2O fluxes from soil for the different predicting equations (2), (5) and (6) are shown as well.

Once again predicting functions (5) and (6) accounting for N_2O fluxes dependence on actual denitrification rate (in its turn not directly measured but derived from soil NO_3^- and WFPS), appeared to be more fitting than equation (2), based only on N_2O fluxes relations with soil NO_3^- and WFPS.

This appears to be quite interesting considering actual denitrification measurements can be labour expensive, depending for instance on the number of sample replicates, soil texture and climate conditions (think how tiring can be collecting intact soil cores from a clay soil in summer period under Mediterranean conditions, moreover in the middle of a transpiring maize crop).

Of course the idea of using actual denitrification rate as a predictor parameter for indirect emission estimation need to be supported by further investigations, since up to the present only few studies have been conducted performing both measurements of actual denitrification and N_2O fluxes from soil, moreover with denitrification assessment methods not completely comparable.

As far as concern this study there are at least three topics needing a more detailed investigation.

The first regards the relatively high peaks of N_2O fluxes detected at WFPS<40%, suggesting the predictive power of actual denitrification rate in the clay soil analysed may drop under accentuated dry conditions and lead to underestimation of total emissions from soil.

The second deals with the very huge errors often characterizing actual denitrification estimates (because of the high spatial variability) and how they might affect through error propagation parameter estimation, both in empirical and process models.

Finally it would be proper to verify the existence of a significant correlation between actual denitrification rate and N_2O fluxes measured at the field scale (Eddy Covariance technique by means of TDL).

6.4 CONCLUSIONS

Correlation and regression analyses on the whole set of data relating to the fine textured soil in the course of the maize cropping cycles in 2005 and 2006 (both monitoring activities and the manipulation experiment) pointed out that actual denitrification rate can be effectively predicted by considering its dependence on soil characteristics such as nitrate concentration and WFPS.

At values of WFPS above 40%, denitrification showed to increase with rising values of nitrate availability according to a Michaelis-Menten kinetic, with an half-saturation constant of 37,5 mg NO_3^--N Kg^{-1} and a maximum value of actual denitrification rate of 1470 μg N_2O-N m^{-2} h^{-1}, at WFPS's in the range between 40% and 45%. Similarly, at not limiting soil nitrates (NO_3^--N Kg^{-1}> 15 mg Kg^{-1}), denitrification rate exhibited a significant correlations with soil water content, exponentially rising at increasing values of soil WFPS.

The WFPS threshold value of 40% detected in this study is lower than the values usually reported in literature (close to 60%) and it might be argued it is probably characteristic of the fine textured soil analysed, subject to cracks formation under Mediterranean conditions.

Actual denitrification rate appeared in its turn a good predictor parameter for estimating N_2O emissions indirectly, without flux measurement.

N_2O fluxes showed indeed a marked exponential relationship with denitrification rate at WFPS> 40%, stronger than their dependences on soil NO_3^- and water content and, as a matter of fact, simple predicting functions for N_2O fluxes from soil derived also considering their dependence on actual denitrification rate showed to be more fitting than predicting equations based only on direct measurement of soil nitrates and WFPS.

These findings suggest that actual denitrification rate may be a good predictor parameter to develop reliable empirical models and/or a useful tool to parameterise and calibrate existing process models in other to achieve more appropriate estimations of N_2O at a local scale.

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