# 19th NITROGEN WORKSHOP

## Efficient use of different sources of nitrogen in agriculture – from theory to practice

## Skara, Sweden 27 June – 29 June 2016

Abstracts

SLU

# NITROGEN DYNAMICS AND N-CYCLING BACTERIAL COMMUNITIES IN A MEDITERRANEAN OLIVE ORCHARD UNDER A SUSTAINABLE FARMING SYSTEM

Silvia Pascazio[1], Carmine Crecchio[1], Marina Scagliola[1], Alba N. Mininni[1], Bartolomeo Dichio[2], Cristos Xiloyannis[2], Adriano Sofo[3]
*[1]Department of Soil, Plant and Food Sciences (DiSSPA), Università degli Studi di Bari "Aldo Moro", Via Amendola, 165, 70126 Bari, Italy, [2]Department of European and Mediterranean Cultures: Architecture, Environment and Cultural Heritage (DiCEM), Università degli Studi della Basilicata, Via San Rocco, 3, 75100 Matera, Italy, [3]3 School of Agricultural, Forestry, Food and Environmental Sciences (SAFE), Università degli Studi della Basilicata, Viale dell'Ateneo Lucano, 10, 85100 Potenza, Italy*

## Objectives

In the recent years, soils has been recognized to play a double role in the entire agro-ecosystem: it is important for a good production as well as for a healthy environment [1, 2]. The conventional, non-sustainable, agronomic practices should evolve in a more sustainable management addressed to ameliorate the ecological networks and nutrient cycling in which soil microorganisms are involved [3, 4, 5].

The main objective of this study was to analyze N dynamics in an olive orchard managed with sustainable agricultural practices. For improving the system and better characterizing the components responsible for the transformation of the different forms of soil N and their availability for plants, a biological and biochemical soil N indicator was used, and the expression of genes of N-cycling bacterial communities were investigated.

## Method

The trial was in a 2-ha mature olive grove located in Southern Italy managed using sustainable and organic agricultural practices for 15 years. Plants were drip irrigated with urban wastewater (total N = 18.3 mg $L^{-1}$; with a mean presence of *Escherichia coli*, Enterococci and *Clostridium* spores of 3500, 7000 and 2300 CFU 100 $mL^{-1}$, respectively) and lightly pruned every year The soil was permanently covered by spontaneous self-seeding weeds mowed twice a year. Cover crop residues and prunings were shredded and left on the ground as mulch. Two sampling areas were identified: along the row (under the emitters) and along the inter-row (rain-fed). Three soil samplings (composite bulk samples collected from the 0-10 cm soil layer) were performed in a year.
The degree of soil quality was expressed by the ratio Nc/Nk, where Nk is Kjeldahl total soil N, while Nc is a linear function of microbial biomass carbon and N mineralization capacity, combined with three enzyme activities, calculated by the following equation [5]:

Total N = $(0.38\times10^{-3})$ microbial biomass C + $(1.4\times10^{-3})$ mineralized N + $(13.6\times10^{-3})$ phosphomonoesterase + $(8.9\times10^{-3})$ $\beta$-glucosidase + $(1.6\times10^{-3})$ urease

The expression levels nitrogenase (*nifH*), ammonia monooxygenase (*amoA*), nitrite reductase (*nirK* and *nirS*), and nitrous oxide reductase (nosZ) were determined by real-time quantitative PCR, using specific primers and microbial reporter genes (from *Nitrosomonas europaea, Azospirillum irakense, Azospirillum brasiliense, Pseudomonas stutzeri* and *Pseudomonas fluorescens*). Total soil RNA extraction was carried out by RNA PowerSoilTM Total RNA Isolation Kit Sample (Mobio) followed by a DNase treatment. Retrotracripts were purified by using the Wizard SV Gel and PCR Clean-Up System (Promega).

Microbial biomass C was determined by the fumigation-extraction method [6]. Mineralized N was evaluated as the difference of inorganic N at the beginning and at the end of an incubation period [5]. Inorganic N was determined by the method of Bremner [7]. Urease, phosphomonoesterase, and β-glucosidase activities were measured following the methods described in [7], [8] and [9], respectively.
Together with N analyses, counts of specific N-cycling bacterial groups, soil organic matter, soil moisture and soil physical structure, were monitored.

## Results

In a soil, a large number of physical, chemical and biochemical properties are involved. However, due to the impossibility of considering all of them, it is of key importance to make a selection. In this study, the ratio Nc/Nk exhibited all the attributes of a good soil fertility indicator, as it was sensitive to changes that occur in the soil, capable of reflecting the improvement of soil quality and not too sensitive to environmental and fluctuations. Nc/Nk showed significant differences in the different areas of each orchard (row and inter-row), being generally higher along the row (in the areas wetted by drippers and with high organic matter content) and so indicating a better soil quality. This ratio, together to the microbial biomass carbon, N mineralization capacity and enzyme activities, gave a precise idea on nitrogen soil dynamics (fixation, mineralization, immobilization, organication, nitrification and denitrification) in the different parts of the olive orchard studied. In the areas along the row, a higher bacterial functional activity and diversity was also found, compared to the inter-row areas.

The sequences of DNA with high phylogenetic information content, such as the rRNA genes, has been used for the description of the N microbial networks, increasing our knowledge on the bacterial diversity in soils [10]. Studies based on DNA mainly provide information on the community structure, while the RNA studies, and in particular the analysis of mRNA expression, provide information on the activities of specific populations. Considering the different conditions in the different parts of the orchard (along the row, under the emitters, with high soil moisture; and along the inter-row, where cover crop residues and prunings were shredded) and the seasonal effects (mainly due to rainfall distribution, soil moisture and soil temperature), mRNAs from N-cycling communities were considered for this study. The number of ammonifying bacteria, proteolytic bacteria and nitrogen-fixing *Azotobacter* in the wetted areas under the drippers were significantly higher than along inter-rows, whereas denitrifying *Pseusomonas* were not significantly different between the two parts of the orchard. The higher bacterial counts along the row were accompanied by higher expressions of *nifH, amoA, nirK* and *nirS*.

## Conclusions

This study confirms the need for Mediterranean orchards to encourage farmers to practice soil management based on organic matter inputs associated with zero tillage, in order to improve soil fertility. The increase of knowledge on biochemical processes of the soil microorganism involved in soil N dynamics influencing N availability for plants, can lead to optimize management strategies for a modern and multifunctional concept of agriculture, based on product quality, environmental protection, resource saving and promotion of human health.

## References

[1] Sofo, A., Palese, A.M., Casacchia, T., Dichio, B., Xiloyannis, C. 2012. In: Ahmad, P., Prasad, M.N.V. (eds) Abiotic Stress Responses in Plants. Springer, New York, USA. p. 105-129
[2] Ding, G.C., Piceno, Y.M., Heuer, H., Weinert, N., Dohrmann, A.B., Carrillo, A., Andersen, G.L., Castellanos, T., Tebbe, C.C., Smalla, K. 2013. PLoS ONE 8: e59497
[3] Graf, D.R.H., Jones, C.M., Hallin, S. 2014 PLoS ONE 9: e114118
[4] Gil-Sotres, F., Trasar-Cepeda, C., Leirós, M.C., Seoane, S. 2005. Soil Biology & Biochemistry 37: 877-887
[5] Trasar-Cepeda, C., Leirós, M.C., Gil-Sotres, F., Seoane, S. 1998. Biology and Fertility of Soils 26: 100-106
[6] Vance, E.D., Brookes, P.C., Jenkinson, D.S. 1987. Soil Biology & Biochemistry 19: 703-707
[7] Bremner, J.M., 1995. In: Black, C.A. et al. (eds) Methods of Soil Analysis. ASA, CSSA, SSSA, Madison, WI, USA. p. 1179-1237
[8] Tabatabai, M.A., Bremner, J.M., 1972. Soil Biology & Biochemistry 4: 479-487
[9] Eivazi, F., Tabatabai, M.A., 1977. Soil Biology & Biochemistry 9: 167-172
[10] Eivazi, F., Tabatabai, M.A. 1988. Soil Biology & Biochemistry 20: 601-606