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Quantification of N_2-fixation from *Medicago polymorpha* L. grown as cover crop in vineyard

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Abstract. Cover crops in vineyards help the reduction of chemical inputs and soil erosion risk as well as the improvement of soil fertility. In a two-year experiment carried out in Sardinia (Italy), the nitrogen (N) fixed by burr medic (*Medicago polymorpha* L.) grown as cover crop in a vineyard was quantified. The potential of N fixation in burr medic was estimated by the ^{15}N isotopic dilution method. In late spring, dry matter legume forage mass ranged from 3.7 to 4.0 t ha^{-1}, in 2012-13 and 2013-14, respectively. On average, the aerial fixed N was 120 kg ha^{-1} year^{-1} and represented about twice of the total N in grapevine organs (leaves, fruits and canes). If burr medic below-ground N is also taken into account, legume contribution to the total N of grapevine could be even higher. Results indicated that the N requirements from the annual organs of the grapevine could be potentially covered by atmospheric N via legume cover crop, compared to soil tillage treatment in vineyards.

Keywords. Burr medic – ^{15}N – Fixed N – Vine organs – Vineyard.

Quantification de la fixation de N_2 de* Medicago polymorpha *L. cultivé comme plante de couverture dans les vignobles

Résumé. *Les cultures de couverture dans les vignobles permettent la réduction des intrants chimiques et des risques d'érosion des sols et l'amélioration de la fertilité des sols. Dans une expérience de deux ans menée en Sardaigne (Italie), l'azote fixé par la luzerne polymorphe (*Medicago polymorpha *L.) cultivée comme plante de couverture dans un vignoble a été quantifié. Le potentiel de fixation de l'azote par la luzerne polymorphe a été estimé par la méthode de dilution isotopique ^{15}N. À la fin du printemps, la masse de MS des légumineuses fourragères se situait de 3,7 à 4,0 t ha-1, respectivement pour la première et la deuxième année. En moyenne, l'estimation de l'azote fixé aérien était de 120 kg ha^{-1} an^{-1}, ce qui représente deux fois l'azote total dans les organes de la vigne (feuilles, branches et grappes). Si l'azote en dessous du sol pour la luzerne polymorphe est également pris en compte, la contribution des légumineuses à l'azote total de la vigne pourrait être encore plus élevée. Les résultats indiquent que les besoins en azote des organes annuels de la vigne pourraient être potentiellement couverts par l'azote atmosphérique à travers la couverture de légumineuses, par rapport à un traitement des sols avec un travail traditionnel.*

Mots-clés. *Luzerne polymorphe – ^{15}N – N fixé – Organes de la vigne – Vignes.*

I – Introduction

Within the multifunctional agriculture, the use of annual self-reseeding legumes as cover crop is an important tool in order to support sustainable agroecosystems. The promotion of the environmental sustainability in viticulture requires increasing in the knowledge of all the issues related to vineyard ecosystems (Mercenaro *et al.*, 2014). In addition to the several well-known advantages arising from the cover crops in vineyards (Piano, 1999), the use of annual self reseeding legumes could allow to avoid the need of nitrogenous chemical fertilizers for their ability to fix biologically the atmospheric N (Unkovich and Pate, 2000). Legume plants are the only cover crop species able to produce net N inputs for the agroecosystem. Patrick *et al.* (2004), using a mix of different legumes species, showed that leguminous cover crops have the potentiality to supply N to grapevines during the times of peak nitrogen demand in early spring and fruit set. However, the amount of fixed N depends on several factors such as legume species, management and environmental conditions (pH, soil N content and humidity), inoculation with selected strains of rhizobia, etc. Therefore, the estimation of legume N fixation is highly variable within the same species. For this reason, the quantification of the N fixation carried out at local level is preferable. The objective of this work is to quantify the N fixation ability of a legume in an intercropped Mediterranean vineyard and the potential benefit that vines could receive from the N produced by legume cover crops.

II – Materials and methods

The experiment was carried out over two agronomical seasons in 2012-13 and 2013-14, in a private vineyard located in North Sardinia (Italy). Carignano vines, trained by VSP, were grafted onto 779 Paulsen and spaced 2.7 x 1 m. The climate is Mediterranean with an average annual rainfall of 540 mm and an average temperature of 16.2 °C. The soil in the site is calcareous, with sand-clay-silt texture and pH 7.4. Within three adjacent rows, each plot consisted of a central row along 10 vines, under a randomized block design with four replications. In order to quantify the legume N fixation, the following inter-row treatments were compared: (*i*) a legume cover crop by burr medic (*Medicago polymorpha* cv Anglona); (*ii*) a grass cover crop by a summer semi-dormant perennial grass, cocksfoot (*Dactylis glomerata* cv Currie), used as non-fixing reference plant (NFS).

A rate of 4 kg N ha^{-1} of enriched ^{15}N fertilizer (10 atom %^{15}N enriched ammonium sulfate) was applied on the sampling areas of both NFS and burr medic. At maturity, dry matter (DM) production was determined by cutting the aerial biomass at 5 cm above ground level over the ^{15}N-enriched area within each experimental plot, and drying the material at 65°C in a forced-air oven until it reached a constant weight. Dry samples ground finely enough to pass through a 1 mm mesh were subjected to elemental analyser isotope ratio mass spectrometry (Cheshire, United Kingdom) to determine both N and the atom%^{15}N content. The proportion of burr medic N derived from the atmosphere (%Ndfa) was calculated by the ^{15}N dilution method (Warembourg, 1993). The amount of N fixed was calculated by multiplying burr medic N yield (kg ha^{-1}) per %Ndfa/100.

Another inter-row treatment, (*iii*) based on the traditional soil tillage was used as a baseline for comparing the potential N benefits from legume cover crop to vines. Therefore, on the vines, either intercropped with burr medic either managed under the soil tillage, subsamples of leaves, fruits (at maturity) and canes (in winter) were collected and their DM and N contents were determined.

Data were analysed by ANOVA and separation of mean values by the least significance difference (LSD) test at 5% of probability.

III – Results and discussion

Aerial biomass in burr medic ranged from 3.7 to 4.0 t ha^{-1} over the two years, whereas it was, on average, 1.2 t ha^{-1} in cocksfoot, due to its slow establishment (Table 1). Nitrogen concentration was remarkably higher in the legume biomass as well as the N yield that was affected by both DM yield and N concentration. On the contrary, compared to NFS, the isotopic excess values were lower in the legume with ^{15}N amounts diluted by atmospheric N. The Ndfa values of burr medic ranged from 90 to 92%.

The amount of fixed N in aerial phytomass of burr medic was, on average, 120 kg ha^{-1} $year^{-1}$. This was close to the values previously recorded for the same annual medic cultivar in a near site (Sulas and Sitzia, 2004) and for other Mediterranean forage legumes (Sulas *et al.*, 2009). In spite of the additional N supplied by burr medic via biological N fixation was remarkable, no significant effects on yield and berry quality were recorded in the vines during the study period. Our findings are in agreement with Bair *et al.* (2008), who carried out an experiment aimed at providing N to organically produced Concord grape through legume used as cover crops. These authors found that, although legume treatments resulted in increase of the N availability from grape bloom until the onset of ripening (veraison), no differences were detected between legume treatments and control for yield and sugar content. When comparing vine intercropped with burr medic vs. vine managed by using the traditional soil tillage treatment, the N concentration of the annual organs of vine (i.e., leaves, fruits and canes) where similar or slightly higher in the legume cover crop treatment (Fig. 1), suggesting a possible positive effect in N levels of vine annual organs.

Table 1. Dry matter yield (DMY), N concentration and yield, isotopic excess of species used as cover crops. Proportion of N derived from the atmosphere (%Ndfa) and amount of fixed N in the aerial phytomass of burr medic

Species	Year	DM t ha^{-1}	N g kg^{-1}	N yield kg ha^{-1}	Atom % ^{15}N %	Ndfa %	Fixed N kg ha^{-1}
Burr medic	2012-2013	4.0a	34.3a	132.7a	0.0033a	92.1	123.0
	2013-2014	3.7a	26.6a	131.1a	0.0222a	90.0	117.0
Cocksfoot	2012-2013	1.2b	13.1b	15.8b	0.0440b	-	-
	2013-2014	1.2b	10.7b	13.5b	0.2228b	-	-

Means followed by the same letter within each column are not significant different at P≤0.05.

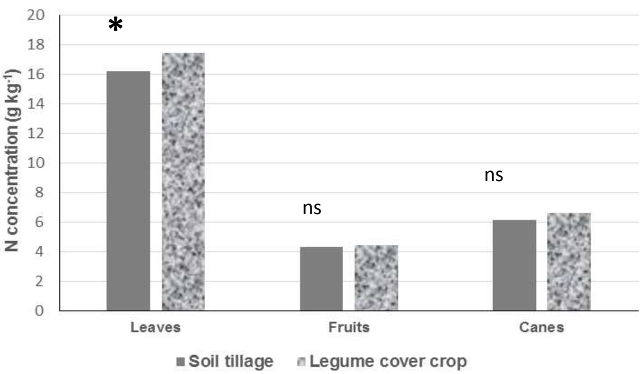

Fig. 1. Nitrogen concentration in the annual organs of vines (* = P<0.05).

On the opposite, the absolute values of N yields from the annual organs of vines revealed low values in fruits and canes of vines intercropped with burr medic (Table 2), and wider variations of N yields compared to those of N concentrations, as shown in figure 1. However, it is important to point out that the fixed N in the aerial biomass of burr medic was twice as high as the total N production of vine annual organs. This means that the amount of the fixed N by the legume cover crops is, presumably, able to cover the N requirements of leaves, clusters and canes annually produced by vines and, consequently, represents an important N contribution to the system represented by the soil – vine – cover crop.

Table 2. Nitrogen yield in annual organs and total per vine on hectare basis. Fixed N in the aerial biomass of the legume and ratio Fixed/Total nitrogen

Treatment	Year	Leaves	Grapes	Canes	Total	Fixed N	Fixed/Total
		------------------------ N kg ha^{-1} ------------------------------					%
Soil tillage	2012-2013	30.6	19.9	15.7	66.2	0	-
Burr medic	2012-2013	28.9	14.9	14.9	58.7	123.0	2.0
Soil tillage	2013-2014	26.5	18.1	11.0	55.6	0	-
Burr medic	2013-2014	32.8	10.5	10.6	53.9	117.1	2.2

However, our estimation of burr medic N fixation can be considered conservative because legume below ground N and N rhizodeposition were not taken into account in this experiment. On the other hand, the N content of vine trunk and roots, which are capable of storing large amounts of N for later use, needs to be measured for better understanding of the N fluxes in vine plants. Moreover, two year of experiment are not enough to determine differences in yield and must quality of the vines subjected to different soil management. For future, it is also important to evaluate the size of the legume contributions to the nitrogen in each organ of vine plants.

IV – Conclusions

The present research has highlighted the important role of annual self reseeding legumes used as cover crops in a Mediterranean vineyard, in fixing the atmospheric N biologically. Aerial biomass of the legume fixed about 120 kg ha^{-1} year^{-1} of N, representing an important N net input to the system, soil – vine – cover crop. This can potentially meet about twice the N needs for the nutrition of leaves, branches and clusters annually produced by the vine plant. Therefore, burr medic proved to be a valuable component in the soil – vine – cover crop system.

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