Different Cover Crops Affect Nitrogen Fluxes in Mediterranean Vineyard

Leonardo Sulas,* Luca Mercenaro, Giuseppe Campesi, and Giovanni Nieddu

ABSTRACT

Compared to traditional soil tillage, the establishment of cover crops in Mediterranean vineyards has several advantages such as better soil fertility; however, vigor and N concentration of grapevine [*Vitis vinifera* (L.)] organs may be affected. A 3-yr experiment was performed in Sardinia (Italy) to: (i) quantify the N fixation ability by burr medic [*Medicago polymorpha* (L.)] grown in a vineyard and its potential benefit for grapevines; (ii) compare the effects of different management systems (grass or legume cover crop vs. soil tillage) on grapevines. Total N fixed by burr medic was 125 kg ha^{-1} yr^{-1} and it was twice as high as the N contents of grapevine annual organs. Soil tillage promoted higher cluster and cane dry matter (+38% and +31%), compared to cover crop treatments. Legume cover crop induced higher N concentration in leaves and canes. The N content detected in grapevine annual plant growth with the legume cover crop reached 61 kg ha^{-1} yr^{-1}. According to an indirect ^{15}N dilution approach, the higher ^{15}N enrichments detected in organs of grapevines, did not indicate that legume N was utilized by grapevine. However, lower ^{15}N enrichment was occasionaly detected, indicating 6 and 13% of N derived from legume in clusters and canes, respectively. Compared to grapevines with grass cover, the N contribution from legume cover, estimated by the N-difference method, induced 24, 24, and 31% of N increase in leaves, clusters, and canes, respectively. The increases corresponded to 10% of legume fixed N.

Core Ideas

- Burr medic cover crop supplied 125 kg ha^{-1} year^{-1} of fixed N to the vineyard.
- Twice the N needs of grapevine annual organs were satisfied by legume cover crop.
- Legume cover crop promoted + 25% of total N compared to grass cover crop.

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Promoting environmental sustainability in viticulture requires increasing knowledge of all the issues related to vineyard ecosystems. The soil management plays a key role in the response of the grapevine to the environment and in the expression of its productive and qualitative potential (Mercenaro et al., 2014). The awareness of the limitations related to the soil processing techniques has promoted the transition from monoculture to subsidiary management of the vineyard with cover crops. Since the early 1900s, cover crops have been used in Californian vineyards to reduce erosion, add or reduce N and grapevine vigor, when leguminous plants or gramineae are used, increase soil organic matter, improve soil structure, and water penetration. Cover crops proved to be a useful tool to limit the excessive vine vigor, resulting in changes in the quality of the grapes and wines (Nieddu et al., 2000a). The use of cover crop provides additional benefits, such as lessening the use of herbicides (Powles et al., 1996) and the associated risks related to contamination of ground water (Mitchell et al., 2007) and reduction of biodiversity in the agro-ecosystem (Danne et al., 2010; Sanguankeo and León, 2011).

Celette and Gary (2013) in a study performed in a vineyard near Montpellier (France) stated that the adoption of a permanent grass cover crop in a water-limited environment generates both water and N stress to grapevine, compared to bare soil. These authors found that the early growth limitation observed in grapevines with cover crop was due to an early N stress, suggesting that the soil N pool was depleted because of an earlier competition between grapevine and cover crop. After grapevine flowering, water appeared to be the most limiting factor for both grapevine growth and N uptake. The selection of the most suitable species and cultivars allowed a long intercropping and co-existence and ensured at the same time a good standard coverage of the soil (Volaire and Lelièvre, 2010; Mercenaro et al., 2014).

In Mediterranean climate, vineyard management combines no-till with the seeding of leguminous cover crops resulting in benefits including decrease in soil erosion, increase of C sequestration, and reduction in the use of synthetic fertilizers, without affecting vine yield or quality (Pardini et al, 2002; Baumgartner et al., 2008). Cover crops can also prevent the leaching of soil nitrate to ground water by taking and storing excess soil N (Justes et al., 2012). In

L. Sulas and G. Campesi, Consiglio Nazionale delle Ricerche, Istituto per il Sistema Produzione Animale in Ambiente Mediterraneo, Traversa La Crucca 3, località Baldinca, 07100 Sassari, Italy; L. Mercenaro and G. Nieddu, Università di Sassari, Dippartimento di Agraria, Viale Italia 39, 07100 Sassari, Italy. Received 22 May 2017. Accepted 25 Aug. 2017. *Corresponding author (l.sulas@cspm.ss.cnr.it).

Abbreviations: DM, dry matter; %Ndfa, proportion of nitrogen derived from the atmosphere; %Ndfl, proportion of grapevine organ nitrogen derived from legume; %Ndff, proportion of grapevine organ nitrogen derived from the fertilizer N.

addition to the several well-known advantages arising from the cover crop adoption in vineyards (Piano, 1999), the use of annual self reseeding legumes could reduce the need of nitrogenous chemical fertilizers because of their ability to biologically fix the atmospheric N (Unkovich and Pate, 2000). The amount of symbiotically fixed N in legumes can vary from 0 to more than 450 kg N ha^{-1} yr^{-1} (Peoples and Baldock, 2001; Herridge et al., 2008) and the amount of fixed N depends on several factors, such as legume species, soil management and environmental conditions (pH, soil N content, and humidity), and inoculation with selected strains of rhizobia. Therefore, the estimation of legume N fixation may be highly variable within the same species and its quantification at local level is desirable.

Patrick et al. (2004), using a mix of different legumes species, showed that leguminous cover crops have the capability to supply N to grapevines during the peaks of N demand in early and late spring, during bud burst and stem growth and during fruit set. Previous experiments performed in Mediterranean vineyards indicated that natural vegetation cover negatively affected grape yield and vine quality, whereas the introduction of the annual legume burr medic (*Medicago polymorpha* L.) as cover crop could result in grapevine vegetative and productive performances similar to traditional management (Nieddu et al., 2000b). Several studies highlighted positive aspects of legume crops in vineyard; however, additional information is still required when the cover cropping strategy is applied in both inter and intra rows (i.e., covering the entire vineyard floor). To improve knowledge regarding vineyard nutrition and proper soil management protocols, a multidisciplinary research was performed in a Mediterranean area. We hypothesized that different soil management could affect N availability in vineyard. Therefore, our specific objectives were to: (i) quantify the N fixation ability of burr medic grown in an entire vineyard floor and its potential benefit for grapevines and agroecosystem; (ii) compare the effects of different management (grass or legume cover crop vs. traditional tillage) on grapevines.

MATERIALS AND METHODS

Locations, Experimental Design, and Crop Management

The experiment was performed over three agronomical seasons between 2013 and 2015 in a private vineyard, located in North West Sardinia (Italy, 40°33′28″ N; 8°19′20″ E, elevation 40 m asl). The climate is Mediterranean with mild winters and hot dry summers, precipitations concentrated between October and May with an average annual rainfall of 580 mm and an average annual temperature of 16.2°C. The soil, classified as Lithic and Typic Xerorthents (Soil Survey Staff, 2014) is alluvial calcareous; the deep layer is 60 to 70 cm, characterized by sand 51%, clay 25%, and silt 24%; pH 7.4 and adequate contents of nitrogen (2 g kg^{-1}), P (29 mg kg^{-1}), organic matter (16 g kg^{-1}), and organic C (9 g kg^{-1}). The hydrological parameters, obtained by Richards equation, are 42.0 for field capacity and 20.0 for the wilting point, both expressed as volume percentage.

Grapevines cultivar Carignano (Nieddu et al., 2006) 16-yr old were spaced 2.7 by 1 m, trained with a spur-pruned cordon and grafted onto 779 Paulsen rootstock. The present study was conducted in a randomized complete block design with four replications. Each plot was 12 m long and 5.4 m wide (width of two inter-rows) and consisted of a central experimental row of 10 grapevines and two adjacent inter-rows on either side of the study row. Plots were separated by a single border row. The following floor management systems were compared: (i) legume cover crop (LC) of the annual self-reseeding burr medic cultivar Anglona; (ii) grass cover crop (GC) consisting of a summer semi-dormant perennial grass (cocksfoot), *Dactylis glomerata* L. 'Currie'; (iii) soil tillage (ST), as the traditional reference treatment.

Cover crops were established in the entire floor vineyard (intra and inter-rows) and sowing was done in Autumn 2012 by hand, using a seeding rate of 30 kg ha^{-1}. Weeds along the rows were manually removed. To quantify the legume N fixation according to the ^{15}N isotope dilution method (Warembourg, 1993), GC was used as non-fixing reference plant. A rate of 4 kg N ha^{-1} of ammonium sulfate enriched ^{15}N ($[NH_4]_2SO_4$, with an isotopic composition of 10 atom %^{15}N) was uniformly applied on the sampling areas (3 m^2 each) of both LC and GC, at crop emergence. The ^{15}N-enriched fertilizer was diluted in water and uniformly hand-sprayed at a rate of 1 L m^{-2} to allow a homogeneous distribution in the soil profile.

Dry matter (DM) production was determined at maturity by harvesting the biomass at 5 cm above the ground level over the ^{15}N enriched area within each experimental plot. Complete cover crop plants were also obtained by excavating a 40-cm wide and 40-cm deep hole. The root samples were wet sieved to separate the physically recoverable roots from the soil. Fresh shoots and roots were weighted and DM was determined by drying samples at 60°C, until constant weights were reached. Dry subsamples of shoots and roots were ground finely enough to pass through a 1 mm mesh and then submitted by dry combustion to elemental analyzer isotope ratio mass spectrometry at the laboratory Iso-Analytical Limited (Cheshire, UK) to determine both N concentration (%N) and the atom% ^{15}N. In June of each year, cover crop biomass residues were mulched.

Calculations

The proportion of N derived from the atmosphere (%Ndfa) in shoots and roots was calculated according to the ^{15}N isotopic dilution method using the following equation:

$$\%\text{Ndfa} = \left(1 - \frac{\text{atom}\%^{15}\text{N excess}_{\text{burr medic}}}{\text{atom}\%^{15}\text{N excess}_{\text{cocksfoot}}}\right) \times 100$$

where atom% ^{15}N excess = (atom% ^{15}N sample - atom% ^{15}N N_2 air) and atom% ^{15}N of air N_2 = 0.3663. The %Ndfa of each biomass portion was obtained using the ^{15}N excess of the legume shoots and roots and aboveground and belowground portions of the reference non-fixing crop. The amount of N fixed by the burr medic in N yield (kg ha^{-1}) was than computed as follows:

$$\text{Nfix (kg ha}^{-1}\text{)} = \text{burr medic N (kg ha}^{-1}\text{)} \times \left(\frac{\%\text{Ndfa}}{100}\right)$$

To evaluate on grapevines the potential N inputs coming from LC, compared to GC and ST, soil N was labeled using ammonium sulfate enriched ^{15}N ($[NH_4]_2SO_4$, with an isotopic composition of 10 atom %^{15}N). A rate of 40 kg N ha^{-1}, split in two applications of 20 kg N ha^{-1} each, was applied at grapevine leaf emergence and fruit set, respectively. The ^{15}N-enriched fertilizer was diluted in water and uniformly hand sprayed on the soil surface on both sides of the grapevine row.

From three grapevines per plot, subsamples of fully expanded leaves were collected at three phenological stages: pea size stage, veraison, and harvest. Fruit and cane subsamples were also collected

during harvest and dormancy, respectively and DM contents were determined. In addition, the N concentration (%N) and the atom% ^{15}N were determined at the laboratory Iso-Analytical Limited (Cheshire, UK). Using an indirect ^{15}N dilution approach, the N proportion of grapevine organs derived from legume (%Ndfl) was calculated by:

$$\%\text{Ndfl} = \left(1 - \frac{\text{Vine atom \%}^{15}\text{N excess}_{\text{with legume}}}{\text{Vine atom\%}^{15}\text{N excess}_{\text{without legume}}}\right) \times 100$$

where atom% ^{15}N excess with legume represents the ^{15}N enrichment of grapevine organs grown under burr medic cover crop. The amount of grapevine organ N derived by LC was calculated as follows:

$$\text{Vine N from legume} = \text{Vine N (kg ha}^{-1}) \times \left(\frac{\%\text{Ndfl}}{100}\right)$$

The proportion of the grapevine organ N derived from the fertilizer-N (%Ndff) applied to the plots was calculated by:

$$\%\text{Ndff} = \left(\frac{\text{atom \%}^{15}\text{N excess}_{\text{vine}}}{\text{atom\%}^{15}\text{N excess}_{\text{fertilizer}}}\right) \times 100$$

where atom% ^{15}N excess of ammonium sulfate fertilizer is 10.

Finally, the amount of grapevine organ N derived from fertilizer was calculated by

$$\text{Vine N from fertilizer} = \text{Vine N (kg ha}^{-1}) \times \left(\frac{\%\text{Ndff}}{100}\right),$$

and ^{15}N recovery is the percentage recovery of the 40 kg N ha^{-1} fertilizer N applied.

Statistical Analysis

Data regarding cover crops, specifically DM yields, N concentration and content of shoots and roots, and ^{15}N data were analyzed for each single year by one-way ANOVA using SPSS (SPSS Statistical Package 16; SPSS, Chicago, IL), with cover crop as fixed factor and block as random effect. Data regarding grapevine organs, specifically DM yields, N concentration and contents, and ^{15}N data were analyzed using the same statistical package, with soil management as fixed factor, whereas year and block were designed as random effect. Percentage values, of both cover crops and grapevine parameters were normalized by the arcsin transformation before analysis. When tratment differences were detected, means were compared by LSD test.

RESULTS

From September 2012 to July 2015, annual rainfall decreased from 679 mm (year 2012–2013) to 437 mm (2014–2015) with a reduction of 22 and 36% to the climate long-term average (Fig. 1). Temperatures were slightly higher than long-term values.

Cover Crop Shoot Dry Matter Yield, Nitrogen Concentration, and Content

The aboveground biomass of LC ranged from 3.7 to 4.9 t ha^{-1} compared to stable but lower biomass in GC (Table 1). The root contribution to the total plant biomass was approximately 10 and 66% for legume and grass cover plants, respectively. In contrast with its stable aboveground biomass yields, the GC

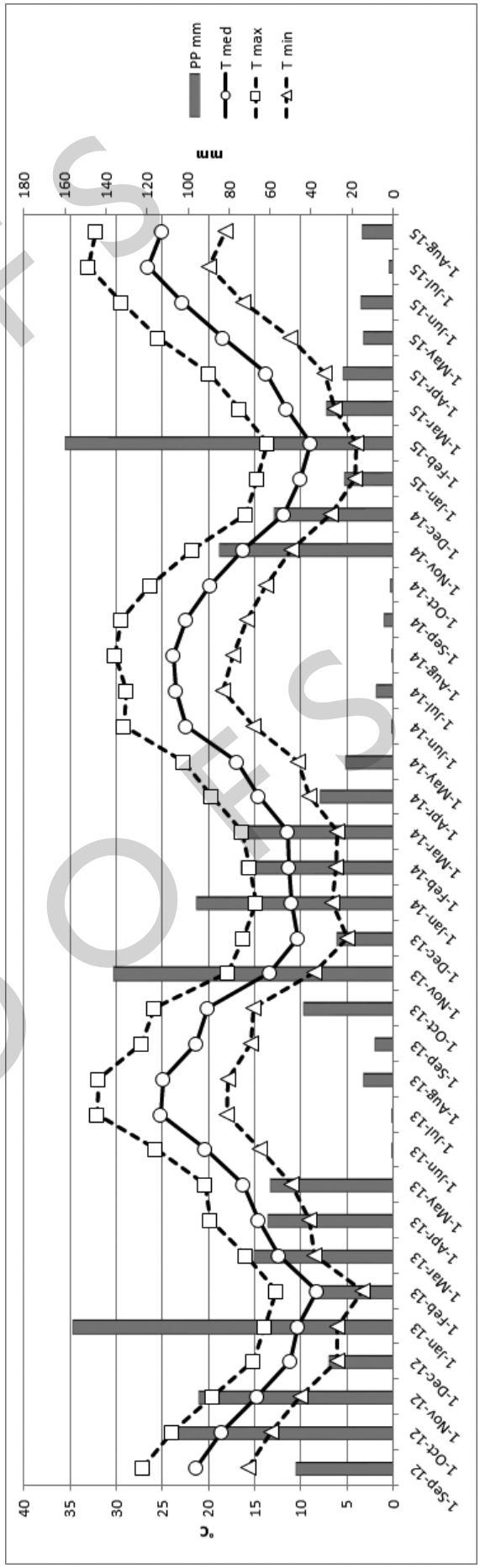

Fig. 1. Thermopluviometric average trend from September 2012 to August 2015.

treatment showed increasing values of root biomass from the first to the third year, when it was fourfold higher than aboveground biomass. The N concentration of biomass significantly varied between cover crop species and their plant components (Table 1). Both aboveground and belowground LC fraction reached peak values of 3.43 and 2.93% N, respectively. Those values were about twice as high as in GC shoots and roots. Overall, the N contents in the aboveground biomass of the two cover crops remained quite stable during the 3 yr but substantially higher in LC than in GC (Table 2). On average, GC had about 15 kg of N ha^{-1} yr^{-1} and LC had 130 to 150 kg N ha^{-1} yr^{-1}. The contribution given by the legume roots ranged from 10 to 15 kg ha^{-1} yr^{-1} of N and it was quite stable (Table 2). Conversely, the N content in GC showed an increasing trend, reaching a peak value of 50 kg ha^{-1} in the last year.

Burr Medic Atom% ^{15}N Excess, %Ndfa, and Fixed Nitrogen

The isotopic excess values were always higher in GC compared to the LC, whose ^{15}N content was "diluted" from atmospheric N (Table 2). Absolute legume shoot isotopic excess ranged from 0.0033 to 0.0222, whereas it varied from 0.0130 and 0.0557 in legume roots. The GC treatment showed higher values, ranging from 0.0440 to 0.2228 in aerial biomass. The 3-yr average of %Ndfa in the aerial biomass of burr medic was 84.9% and the corresponding amount of fixed N was 117.4 kg ha^{-1}. The %Ndfa and fixed N values of roots were 82.7% and 7.9 kg ha^{-1}, respectively, representing 7% of total plant fixed N. Therefore, relevant amounts of N could be added to the soil at the end of each season.

Nitrogen Concentrations, Yields, and Requirements in Annual Organs of Grapevine

Soil tillage promoted higher DM in clusters (+38%) and canes (+31%), compared to LC and GC treatments. Mean leaf DM was 2.78 g each, without significant differences due to treatment (Table 3). Treatments also influenced leaf and cane N percentage. Compared to ST, LC increased N concentration of canes and leaves, whereas GC decreased their N concentration. Leaf and cane total N content was significantly lower in the GC than in the LC or ST treatments. The cluster N content ranged from 11.77 to 9.48 kg ha^{-1} and it was affected by treatment. On average, the cumulative N content detected in leaves, clusters, and canes annually produced by the grapevine was 60.88 kg ha^{-1}. In the LC grapevines, it represented about half of the total fixed N that was quantified for burr medic.

Table 1. Shoot and root dry matter yield (t ha^{-1}) and N concentration (% N) of cover crop biomass measured from 2012 to 2015.

Year	Cover crop	Shoot	Root	Shoot	Root
		t ha^{-1}		% N	
2012–2013	Burr medic	3.9a†	0.5b	3.43a	2.37a
	Cocksfoot	1.2b	0.7b	1.31b	0.79b
2013–2014	Burr medic	3.7a	0.4b	2.66a	2.31a
	Cocksfoot	1.2b	2.9a	1.07b	0.83b
2014–2015	Burr medic	4.9a	0.5b	3.13a	2.93a
	Cocksfoot	1.1b	4.9a	1.29b	1.37b

† Values with different letters differ significantly ($P < 0.05$).

Table 2. Shoot and root N contents (kg ha^{-1}) and isotopic excess (% ^{15}N) of cover crop species from 2012 to 2015.

Year	Cover crop	Shoot	Root	Shoot	Root
		N content			
		kg ha^{-1}		Atom % ^{15}N	
2012–2013	Burr medic	132.7a†	10.2a	0.0033a	0.0130a
	Cocksfoot	15.8b	5.7a	0.0440b	0.2230b
2013–2014	Burr medic	131.1a	9.8a	0.0222a	0.0550a
	Cocksfoot	13.5b	24.5b	0.2228b	0.2086b
2014–2015	Burr medic	156.2a	15.1a	0.0137a	0.0557a
	Cocksfoot	14.2b	49.7b	0.0563b	0.1403b

† Values with different letters differ significantly ($P < 0.05$).

Table 3. Dry matter (DM), N percentage and content of annual vine organs subjected to different managements: soil tillage (ST), legume cover crop (LC), and grass cover crop (GC).

Treatments	DM, g vine $organ^{-1}$			N, % of DM			N ha^{-1}			
	Leaf	Cluster	Cane	Leaf	Cluster	Cane	Leaf	Cluster	Cane	Vine
ST	2.69	97.36a†	54.93a	1.9317ab	0.4475	0.6425b	41.66a	18.55a	13.36a	73.57
LC	2.87	69.42b	44.48b	2.0342a	0.4392	0.6908a	37.58 a	11.77b	11.36a	60.71
GC	2.83	71.58b	40.37b	1.8067b	0.3889	0.6017c	30.20 b	9.48c	8.69b	48.37
Significance	ns‡	**	*	*	ns	*	*	*	*	ns

* $P < 0.05$.

** $P < 0.01$.

† Means within a column followed by different letters are differ significantly.

‡ ns, not significant.

Leaf, Cluster, and Cane Atom% ^{15}N Excess, %Ndff, and ^{15}N Recovery

Leaf, cluster, and cane atom% ^{15}N was significantly affected by soil management. Regardless of the analyzed vine organs, higher values of atom% ^{15}N excess were always detected in LC and ST treatment (range 0.1411–0.2777), wheras the lowest in GC (Table 4). As expected, N derived from fertilizer followed the trend previoulsy described for atom% ^{15}N. The ^{15}N recovery (percentage recovery of the 40 kg N ha^{-1} fertilizer N applied) was significantly affected by treatment. In particular, comparison between treatments indicate no difference between ST and LC. However, ST and LC values were higher than GC in each analyzed grapevine parts. The highest Ndff percentage was almost 3% in LC clusters, whereas it ranged from 0.0650 to 0.3125 in GC grapevine organs, where the ^{15}N recovery was negligible. Overall, the significantly higher ^{15}N enrichment detected in annual organs of LC grapevines, compared to grapevines grown under ST and GC treatments, did not indicate that some legume N was utilized by grapevine organs. However, only occasionally across the 3 yr, unreplicated sporadic lower ^{15}N enrichments were detected in clusters and leaves, leading to percentages of N derived from legume of 6 and 13, respectively. In addition, the N contribution from LC to grapevines, if estimated by N-difference method from data reported in Table 3, indicated 24, 24, and 31% of N increase in leaves, clusters, and canes, respectively, when comparing LC vs. GC grapevines. On average, that increase corresponded to additional 12.4 kg ha^{-1} yr^{-1} of N incorporated in the annual organs produced by the LC grapevines, and to about 10% of total N fixed by burr medic. On the contrary, ST induced a significant 57% N increase in clusters, compared to GC.

DISCUSSION

The aboveground dry matter production of burr medic cover crop, as measured in late spring during each year of the study, averaged 4.2 t ha^{-1} and it was in line with previous results, obtained in Sardinia, concerning burr medic dry matter yields for both forage and cover crop utilizations (Nieddu et al., 2000a; Sulas and Sitzia, 2004). Yields were also similar to those obtained in other regions under similar climate conditions (Avendaño et al., 2005; Ovalle et al., 2006) or in other annual *Medicago* species (Fourie et al., 2001). Results also indicated remarkable differences between the two cover crops under comparison, in terms of both absolute biomass yields and N contents and its partitioning in aboveground and belowground organs. Much of the N incorporated in the legume biomass originated from the biological fixation of atmospheric N. The amount of fixed N confirmed the valuable N fixing ability of burr medic, as found in previous experiments for the same legume grown as forage species (Sulas and Sitzia, 2004). Even including the contribution from physically recoverable roots, our estimation of fixed N by burr medic may be considered conservative, because N rhizodeposition was not taken into account in this experiment. Moreover, the remarkable amount of N contained in the burr medic residues, once mineralized, can increase soil nitrate and ammonium contents, which represents an important N contribution for grapevines. To clarify the N fluxes in vineyard, the N supply from the LC that can be potentially available for grapevine was quantified. The fixed N from burr medic was able to potentially satisfy twice the N needs required for the nutrition of the leaves, canes, and clusters that were annually produced by the grapevine plants.

Many factors affect the N availability (soil properties, nutrient interactions, microbial populations, and soil pH), and among them soil management plays an important role. The timing and amount of N application or N naturally present can affect grapevine phenology, vigor, and yield. Fuentes et al. (2008) indicated that soil areas of maximum nutrient uptake and root water are located near the grapevine trunk. Guilpart et al. (2014) demonstrated that water and N stress around flowering induces a decrease of grapevine bud fertility. Sweet and Schreiner (2010) suggested that competition between grass cover crop and grapevines might be related more to N than to water, because grapevines could compensate for competition by directing root growth to deeper soil layers to obtain water, but in these layers of the soil profile N is less available. Pérez-Álvarez et al. (2013) showed that a reduction of soil NO_3- during bloom reduces yield and grapevine vigor. Cover crops had different effects on N availability compared to conventional tillage; however, not all cover crop compete with grapevine in the same manner. The legume cover crop progressively increased the soil N content over time by biologically fixing the atmospheric N (Pérez-Álvarez et al., 2015). As the grass and the self-regenerating legume soil cover competed with grapevines for soil N, the N concentration of grapevine leaves could be negatively affected. Similar results were also reported by King and Berry (2005) and Curtis et al. (2012).

In our experiment, LC effects on grapevines were evident in the higher N concentrations and amounts found in leaves and canes. The effects confirmed the results obtained in a parallel experiment conducted by Muscas et al. (2017). Compared to LC, they found that grapevines subjected to GC had lower leaf SPAD values during the seasons and were characterized by lower yield and pruning weight. Furthermore, the comparison of LC vs. ST in three growing seasons indicated a reduction of grapevine vigor and yield, 2 yr after the cover crop establishment.

Remarkable amount of N from the legume biomass left on floor in each season was monitored and the higher N concentration and amounts recorded in leaf, cluster, and cane grapevines were determined by the LC treatment. However, the higher ^{15}N-enrichments

Table 4. Isotopic excess (atom % ^{15}N), percentage of N derived from fertilizer (% Ndff), and percentage of ^{15}N recovery of annual vine organs subjected to different managements: soil tillage (ST), legume cover crop (LC), and grass cover crop (GC).

Treatments	Isotopic excess			Ndff			^{15}N recovery		
	Leaf	Cluster	Cane	Leaf	Cluster	Cane	Leaf	Cluster	Cane
ST	0.1411b†	0.1758b	0.1845b	1.1425b	1.7600b	1.8458b	0.1675a	0.0077a	0.0060a
LC	0.1994a	0.2777a	0.2427a	1.9967a	2.7783a	2.4267a	0.1858a	0.0079a	0.0067a
GC	0.0064c	0.0069c	0.0313c	0.0650c	0.0683c	0.3125c	0.0002b	0.0000b	0.0006b
Significance	**	**	**	**	**	**	*	**	**

* $P < 0.05$.

** $P < 0.01$.

† Means within a column followed by different letters are differ significantly.

detected in annual grapevine organs growing with LC, compared to ST and GC, indicated instead that no or little legume N was apparently utilized by grapevine organs. Our results could indicate the difficulty to trace the N transfer from legume biomass residues to grapevine organs, and/or a little N recovery from grapevine, using an indirect ^{15}N dilution approach. Several papers also documented similar findings. Brunetto et al. (2014) demonstrated that the rate of N derived from the litter (rye litter labeled with ^{15}N) was less than 2% for all the cultivar Niagara Rosada grape organs. Therefore, more than 98% of the N contained in the grapevine tested organs was derived from different N sources, rather than rye litter that apparently contributed little to grape nutrition in the short term. Previous reports pointed out that Chardonnay leaves derived less than 1% of their N from the litter, when tested 8 wk after deposition of the two cover plants (perennial ryegrass and white clover) labeled residues on the soil surface (Brunetto et al., 2011). Patrick et al. (2004) reported that in Chardonnay grapevines grown with a mixture of ^{15}N-labeled legume cover crop species, which provided 81 kg of N ha^{-1} to the soil, only 0.28% of the litter-derived N was recovered in the grapevine leaves at 20 wk after deposition of the residues on soil surface. Ovalle et al. (2010), within an experiment aimed at estimating the relative contribution of legume N to the nutrition of grapevines and with a similar methodology than in the current experiment, found that the amount of legume N estimated to be recovered by grapevines represented less than 10% of the amounts of N annually returned to the soil in above-ground legume biomass. Moreover, the possible accumulation of N in the roots and trunks, which was not evaluated in the present study, could lead to additional explanations.

In a soil type similar to our experimental vineyard, Sulas et al. (2012) demonstrated that N losses due to leaching could be not neglegible, especially from the end of summer to autumn. Therefore, low N rates can be explained by leaching as well as other losses such as (i) volatilization of NH_3–N during the decomposition of legume biomass, (ii) N_2O-N denitrification in soil micropores, and (iii) strong physical adsorption of soil mineral N (NH_4–N) (Brunetto et al., 2011). In addition, low percentage of litter-derived N may also be attributed to low mineralization of soil organic matter, which may have a complex part of the applied ^{15}N. The ability of soil microorganisms in competing with plants for N uptake is another factor that may limit vine N uptake. It is also possible that the N available from the legume litter was greater in the soil and was lost before the nutrient uptake by grapevines reached its peak at budbreak of the plants, due to an only partial synchronism between N mineralization and N uptake. As well, the labeled N may not reach the entire root system of grapevines, which is known to explore also deep soil layers (King and Berry 2005).

The availability of other sources of (unlabeled) mineral N in soil is an important factor and it is desirable that an experiment designed to investigate the transfer of biologically fixed N be conducted on an N-responsive site. Finally, a measured isotope dilution can only be regarded as an apparent indicator of the transfer of biologically fixed N, since the basic assumption (same ratio of labeled N to soil-derived unlabeled N is taken up by all species in all plots) may not hold true (Papastylianou and Danso 1991). Indeed, higher recovery of labeled fertilizer by the non-legume compared to the legume treatment can be due to N-sparing effect of the legume favoring grapevine organs and/or differences in cover crop plant densities.

CONCLUSIONS

Although several studies reported positive influence of cover crops in interrow vineyard, additional information, when covering the entire vineyard floor using grass or legume cover crops, comes from present study. In particular research allowed to quantify the N fixed in the aboveground and belowground biomass of burr medic cover crop established in the entire floor of a Mediterranean vineyard.

About 125 kg ha^{-1} yr^{-1} of atmospheric N was supplied to the vineyard from burr medic cover crop, which is roughly twice the amount of N produced annually in grapevine leaves, canes, and clusters.

As expected, cover crops affected some agronomic parameters of grapevines. Specifically, LC induced higher N concentration and content in leaves, clusters, and canes (+25% of total N), compared to GC. Grapevine annual organ N contents under ST, did not significantly differ from LC, probably due to the absence of competititon from a vegetative cover (GC), or weeds presence.

Specific results, obtained with an indirect ^{15}N dilution approach, demonstrated an evident difficulty to trace the N transfer from legume cover crop biomass mulched in floor to grapevine organs with apparently no or little N recovery from grapevine.

Further study are required for investigating the role of grapevine perennial organs such as trunk and root, for N sink and remobilization, as well as methodology improvement for succesful tracing of N fluxes in open field.

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