Plant Soil (2010) 334:247–259
DOI 10.1007/s11104-010-0379-1

REGULAR ARTICLE

Estimating the contribution of nitrogen from legume cover crops to the nitrogen nutrition of grapevines using a ^{15}N dilution technique

Carlos Ovalle · Alejandro del Pozo · Mark B. Peoples · Arturo Lavín

Received: 22 October 2009 / Accepted: 1 April 2010 / Published online: 20 April 2010

Abstract Cover cropping is increasingly being used as a form of sustainable soil management in grapevine (*Vitis vinifera* L.) production. The objective of the current study was to determine the effect of a legume cover crop on a set of growth and production variables of a vineyard, and to quantify the relative importance of legume nitrogen (N) as a source of N for the vine plants and to compare this to the current recommended practice of annually applying 40 kg fertilizer-N ha^{-1}. The study was carried out in a 5-yr-old vineyard cv. Cabernet Sauvignon at the Cauquenes-INIA Experimental Center in one of the wine production areas of Chile. The treatments were: control without cover crop (C); a legume mixture of early maturing cultivars of subterranean clover (*Trifolium subterraneum* L.) and burr medic (*Medicago polymorpha* L.) (EMC); or a legume mixture of late maturing cultivar of subterranean clover and balansa clover (*T. michelianum* Savi) (LMC). Average inputs of legume N generated by the EMC and LMC treatments represented 112 and 161 kg above-ground legume N $ha^{-1}yr^{-1}$, respectively. Grape dry matter production was increased significantly ($P \leq 0.05$) by 48–61% and the amount of N accumulated in grape bunches was enhanced by 74–105% after 2 years of legume cover crop. However, no significant ($P > 0.05$) effects of cover cropping were observed on vine leaf, canes, roots or trunk biomass. To estimate the relative contribution of legume and fertilizer N to the N nutrition of grapevines ^{15}N-enriched fertilizer (10 atom% ^{15}N excess) was applied to the soil in micro-plots within all treatments as two split applications of 20 kg N ha^{-1}. The ^{15}N composition of vines grown with or without cover crops were subsequently compared. Any measured reduction in the ^{15}N enrichment of the tissues of cover cropped vines relative to the nil cover crop control was assumed to have resulted from the uptake of legume N. Recovery of ^{15}N in vine plant parts was used to calculate the efficiency of use of fertilizer N. Surprisingly the ^{15}N data

Responsible Editor: Elizabeth M. Baggs.

C. Ovalle (✉)
Centro Regional de Investigación Quilamapu,
Instituto de Investigaciones Agropecuarias,
Casilla 426,
Chillán, Chile
e-mail: covalle@inia.cl

A. del Pozo
Facultad de Ciencias Agrarias, Universidad de Talca,
Casilla 747,
Talca, Chile

M. B. Peoples
CSIRO Plant Industry,
P.O. Box 1600, Canberra, Australian Capital Territory
2601, Australia

A. Lavín
Centro Experimental Cauquenes,
Instituto de Investigaciones Agropecuarias,
Casilla 165,
Cauquenes, Chile

indicated that the fruit had the lowest reliance upon legume N for growth (only 7–13% cf 14–56% of the N in other organs), even though grape bunches were the only plant part to positively respond to the cover cropping treatments. This suggested that either the temporal patterns of N mineralization in soil and N uptake by vines over the growing season was such that much of the mineral N utilized during fruit development was not directly derived from legume residues, or that N remobilized from other plant parts within the vines was the major source of N for grape production rather than N newly assimilated from soil. The amount of legume N estimated to be recovered by cover cropped vine plants (12–15 kg N ha^{-1}), was similar to the calculated contribution from 40 kg of fertilizer-N applied to vines (11-12 kg ha^{-1}). This amount of N represented <10% of the amounts of N annually returned to the soil in above-ground legume material in the case of the cover cropping treatments, and was equivalent to a recovery of 27–30% of the N applied in fertilizer.

Keywords Sustainable soil management · N transfer · *Medicago polymorpha* · *Trifolium michelianum* · *Trifolium subterraneum* · *Vitis vinifera*

Introduction

Cover cropping with nitrogen (N) fixing forage legumes is a technology increasingly being used in grapevine (*Vitis vinifera* L.) production systems in various wine producing regions of the world, such as the USA, Australia, Italy, France and South Africa (Miller et al. 1989; Masson and Bertoni 1996; Nieddu et al. 2000; Masson and Gintzburger 2000; Fourie et al. 2006). Cover cropping in vineyards can potentially provide a range of benefits. These include a reduced risk of erosion, enhanced biological activity in soil, increased soil organic matter content and nutrient availability, and improvements in soil porosity and aggregate stability, and increased soil water holding capacity (Frye and Blevins 1989). There have also been reports of cover crops reducing weed incidence and improving the control of some nematodes species (Aballay and Insunza 2002). Cover cropping has only recently been introduced into vineyards in Chile, and their effects have not been widely studied. In particular, little is known about how important N derived from legume cover crops may be as a means of either replacing or supplementing annual applications of N fertilizer to vines. Predicting the impact of an increased N supply on grapevines is difficult due to a poor understanding of the quantitative response of vines to N, and the temporal patterns of both the partitioning of N within the annual above-ground vegetative plant parts and fruits, and the storage and remobilization of N from perennial structures during the growing season (Peacock et al. 1989; Wermelinger and Koblet 1990; Treeby and Wheatley 2006).

Forage legumes species differ in their capacity to symbiotically fix atmospheric N_2, and in the N content of their stems, leaves and roots (e.g. Ledgard and Steele 1992; Peoples and Baldock 2001; Campillo et al. 2003; Peoples et al. 2004), and consequently different legume cover crops may also vary in the amounts of N that could potentially be returned to the soil in a vineyard. There is a wide range of productive fodder legumes that could be suitable as cover crops in Chile. For example, in low rainfall areas (<400 mm annual rainfall), hualputra (*Medicago polymorpha* L.) has a wide variability in phenology, biomass production and N_2 fixation (del Pozo et al. 2000, 2002; Ovalle et al. 2006). In areas of high rainfall, late maturing cultivars of subterranean clover (*Trifolium subterraneum* L.), balansa clover (*Trifolium michelianum* Savi), yellow serradela (*Ornithopus compressus* L.), and pink serradela (*O. sativus* Brot.) could be utilized with this purposes (Avendaño et al. 2005; Ovalle et al. 2005).

Numerous studies have demonstrated improvements in crop N status and production benefits from using annual (e.g Dou et al. 1994; Guldan et al. 1997a, b; Rochester and Peoples 2005) or perennial legumes (e.g Ovalle et al. 2007a, b; Sanchez et al. 2007) as cover crops, mulch or green manure. Rhizodeposition of N via exudates and secretions from living legume roots is one potential pathway that vines could benefit from cover crops (Wichern et al. 2008), and the direct transfer of N via mycorrhizal hyphae connections between the roots of legumes and their neighboring vines might be another (Haystead et al. 1988). However, while some researchers have found evidence of a transfer of N from legumes to a companion species during growth (Høgh-Jensen and Schjoerring 2000; Xiao et al. 2004), others have failed to detect direct below-ground transfer (Ledgard et al.

1985; Giller et al. 1991; Trannin et al. 2000). The main source of plant-available forms of legume N is generally believed to result from the decomposition and mineralization of organic legume residues at the end of the growing season following the death of the legume (Danso et al., 1993; Trannin et al. 2000; Fillery 2001). This would particularly be the case for forage legumes in the absence of grazing animals (Peoples et al. 2004).

The rate by which legume organic residues are mineralized to plant-available forms of soil mineral N (nitrate and ammonium) depends on the chemical composition of the legumes, residue management and soil properties (Ladd et al. 1985; Fillery 2001; Peoples et al. 2004). In annual cropping systems <20–25% of the N in forage legume residues or green manures is commonly recovered in the short-term, although legume N can sometimes represent up to 30–33% of the N requirements of a following crop (Fillery 2001; Crews and Peoples 2005; Peoples et al. 2009). However, much less information is available in systems with perennial cropping systems. In an organic raspberry (*Rubus idaeus* L.) production system where white clover (*T. repens*) was used as a cover crop, recoveries of legume N by the raspberry plants were estimated to have ranged from 2.5–7.0% in fruits to 18–22% in canes and leaves using an indirect ^{15}N dilution method (Ovalle et al. 2007a, b). In a coffee (*Coffea arabica* L.) plantation, ^{15}N studies also suggested that 30% of the N_2 fixed by a legume cover crop may be transferred to the coffee plants (Snoeck et al. 2000).

Ovalle et al. (2007c) has previously demonstrated that differences in biomass production can be achieved by mixtures of early maturing cultivars (EMC) of subterranean clover and burr medic or mixtures of late maturing cultivars (LMC) of subterranean clover and balansa clover. These authors also reported that annual legume cover crops could significantly increase the concentrations of plant-available soil N in vineyards in the sub-humid Mediterranean zone of Chile, but no data were reported concerning their impact on vine productivity. The objective of the present study was to evaluate the relative effect of EMC and LMC legume cover crops on a number of growth and production variables of the vineyard, to estimate the size of the contributions of the legumes to the N nutrition of grapevines, and to compare this to current practices of applying 40 kg fertilizer-N ha^{-1} to vineyards each year, usually in the form of ammonium sulfate.

Materials and methods

Experimental site and treatments

The experiment was carried out in the Experimental Center of Cauquenes-INIA (35°58' S, 72°17′ W; 140 m above sea level), Maule Region, in the eastern part of coastal mountain range of Chile. Long-term average minimum temperatures of the coldest month (July) is 4.8°C and maximum temperature in warmest month (January) is 29°C. Data of rainfall was obtained from an automatic meteorological station located in the same Experimental Center: total rainfall was 806, 745 and 406 mm, in spring (September-December) was 88, 110 and 47.6 mm, and in summer (January-April) was 28, 58 and 80.1 mm, in 2005, 2006 and 2007, respectively.

The study was undertaken between May 2005 to June 2007, in a 5-yr old vineyard of cv. Cabernet Sauvignon, planted at 2.5 m row width × 0.5 m spacing between vines on a shallow, hilly granitic soil (fine, kaolinitic, mesic Typic Palexeralfs). At the beginning of the study soil pH (water) in the top 10 cm was 6.7, organic matter (OM) was 1.5%, and the levels of availability for N (2*M* KCl), P (0.5*M* $NaHCO_3$) and K (1*M* NH_4OAc) were 1.93 mg kg^{-1}, 5.47 mg kg^{-1}, and 0.53 $cmol_{(+)}$ kg^{-1}, respectively.

The experimental treatments were: control without cover crop and the ground kept bare (C), legume cover crops consisting of either a mixture of early maturing cultivars (EMC) of subterranean clover (*Trifolium subterraneum* L.) and burr medic (*Medicago polymorpha* L.), or late maturing cultivars (LMC) of subterranean clover and balansa clover (*Trifolium michelianum* Savi). Four replicate plots (25 m x 4 m) for each legume treatment were sown on 31 May 2005 in a randomised block design. Table 1 presents details of cultivars, seed rate, pasture establishment and management. The vineyard received supplementary drip irrigation (30 L $plant^{-1}week^{-1}$) from September to March in each year. All treatments were irrigated with the same water source. Although no analyses of the irrigation water were undertaken during the experiment, the water came from rivers flowing from the mountains that are not subject to nitrate

Table 1 Treatments, legume species and cultivars, and seeding rate used for cover crops

Treatment	Species	Cultivar	Legume seeding rate (kg ha^{-1})
Control without cover crop (C)			0
Legumes mixture of early maturing cultivars (EMC)			
	Sub clover	Seaton Park	12
	Burr Medic	Santiago	8
Legumes mixture of late maturing cultivars (LMC)			
	Sub clover	Antas	12
	Balansa clover	Paradana	5

contamination and irrigation water was believed not to represent a significant external input of N for the vines.

Labeling the soil N pool with ^{15}N

To allow the uptake of legume N by vines to be calculated the soil N within "micro-plot" areas (3 m x 2 m on the row) was labeled using ^{15}N enriched $(NH_4)_2SO_4$ (10% atom excess ^{15}N). The ^{15}N-enriched fertilizer was added at a rate of 40 kg N ha^{-1} as two applications: 50% at leaf emergence (September 2006), and 50% during fruit set (December 2006). Labeled fertilizer was applied to four vines inside each micro-plot as a solution diluted 1% (weight / volume) in water to the soil surface in the area of soil moistened by drip irrigation (2 m x 0.5 m) on either side of the row of planted vines. Micro-plots were surrounded by a raised soil barrier to prevent run-off and lateral movement of ^{15}N, and each micro-plot area was delineated with wooden stakes.

Legume biomass harvest and tissue analyses

The above-ground biomass production of the legume cover crops was determined using five quadrants of 1 m×0.5 m per plot, distributed at both sides of the vineyard rows. In the first growing season cover crops were sampled on 31 October 2005, and in the second season above-ground biomass was determined on 15 August, 21 September and 3 November 2006. Samples were dried in forced ventilation oven at 65°C for 48 h and the dry weight recorded. After sampling, the remainder of the cover cropped area was mown to a height of around 5 cm and the cut foliage was left as a mulch. The N concentration of the legume dry matter was determined using Kjeldahl digestion.

Vine sampling, analyses and calculations

Vine leaves were sampled in December 2006 and February 2007 (at the time of maximum canopy development), dried in an oven with forced ventilation at 65°C for 48 h. Number of bunches and grape production was evaluated for five plants per replicate at the time of fruit harvest in April 2006 and 2007. A destructive sampling of the whole plant (included roots) was undertaken in five plants per replicate at the end of the growing season in June, 2007 for determination of total biomass and N content. The $^{15}N/^{14}N$ isotopic ratio was determined for various plant parts sampled from the two central vines from each micro-plot area. Total N in vine tissues were determined using Kjeldahl digestion, while analyses for ^{15}N composition were undertaken using an optical emission spectrometer ^{15}N analyzer NOI-6PC (FAN, Germany), as described by Axmann (1990).

The recovery of legume N by grapevines was estimated using an indirect ^{15}N dilution approach that has been applied in similar studies in other cropping systems (e.g. Ofosu-Budu et al. 1995; Trannin et al. 2000; Hood 2001). With this technique the ^{15}N concentration in the tissue of vines growing within the ^{15}N-labeled micro-plots with legumes were compared to vines growing in micro-plots without cover crop, and the percentage of the vine N derived from the legume (Ndfl%) was calculated using Eq. 1:

$$\text{Ndfl\%} = 100 \times \left[1 - \left(\textit{Vine atom\%}^{15}\textit{N excess with legume} / \textit{Vine atom\%}^{15}\textit{N excess without legume}\right)\right] \quad (1)$$

Where atom %^{15}N excess represents a measure of the enrichment of vine samples in ^{15}N relative to the background concentration of ^{15}N occurring naturally in atmospheric N_2 (0.3663 atom %^{15}N):

$$\textit{Vine atom\%}^{15}N\ \textit{excess} = \left(\textit{Vine sample atom\%}^{15}N\right) - 0.3663 \quad (2)$$

The calculation using Eq. 1 essentially assumes that all of the N generated by the legume came from symbiotically fixed atmospheric N_2 which by definition has an atom %^{15}N excess value of zero. In the case of the current experiment with grapevines where the demand for N by the various annual above-ground plant parts may be partially met from the remobilization of N reserves from perennial structures in addition to soil mineral N (Treeby and Wheatley 2006), it was assumed when comparing ^{15}N-enrichments of individual plant organs of vines that the extent of utilization of older, unlabeled N reserves for growth was identical in all treatments.

The amount of vine N originating from legume cover crops was calculated from Ndfl% and measures of N accumulated by each of the individual plant parts (Vine N) using Eq. 3:

$$\text{Vine N from legume} = \text{Vine N} \times (\text{Ndfl\%})/100 \quad (3)$$

The ^{15}N enrichment data could also be utilized to estimate the proportion of the vine N derived from the 40 kg fertilizer-N ha^{-1} applied to the micro-plots (Ndff) in both the absence and presence of cover crops using Eq. 4:

$$\text{Ndff\%} = 100 \times \left[\left(\textit{Vine atom\%}^{15}N\ \textit{excess}\right)/\left(\textit{atom\%}^{15}N\ \textit{excess of applied fertilizer}\right)\right] \quad (4)$$

Since the ^{15}N-enriched fertilizer was applied as 10 atom%^{15}N excess ammonium sulfate Eq. 4 could be simply re-expressed as:

$$\text{Ndff\%} = 10 \times \left[\left(\textit{Vine atom\%}^{15}N\ \textit{excess}\right)\right] \quad (5)$$

The amount of vine N originating from fertilizer was calculated from Ndff% and measures of amounts of N accumulated in different plant parts of the vines using Eq. 6:

$$\text{Vine N from fertilizer} = \text{Vine N} \times (\text{Ndff\%})/100 \quad (6)$$

Statistical analyses

The experimental design was complete random blocks with 3 treatments and 4 replications. The statistical analysis was carried out using the statistical program SAS system. T Student's test and ANOVAs were performed, on pasture and vine biomass, tissue N concentration and ^{15}N data.

Results

Legume cover crops

Leguminous material represented 95–98% of the total above-ground dry matter (DM) that grew in the cover crop treatments in 2005-06 and 99-100% in 2006-07. In both growing seasons, DM production by LMC was significantly higher ($P \leq 0.05$) than the EMC (Table 2). The N concentration of the legume biomass was 2.90 and 3.15%, in the EMC and LMC, respectively, but this difference between the two legume mixtures was not significant ($P \geq 0.05$). Above-ground inputs of N by the legume cover crops represented 68 and 128 kg N ha^{-1} in the EMC in 2005-2006 and the 2006-2007 growing seasons, respectively (average 112 kg shoot N ha^{-1}yr^{-1}), and 157 and 194 kg N ha^{-1} in the LMC treatment (Table 2, average 161 kg shoot N ha $^{-1}$yr^{-1}).

Biomass production, N content in vine plants

Grape production for vines growing in association with EMC was lower than those in the control in the first growing season (2005-2006), but was 48–61% higher than the control for both the EMC and LMC treatments in the second growing season ($P<0.05$, Table 3). However, despite this improvement in fruit dry matter in response legume cover cropping, determinations of cane, trunk and root biomass were similar ($P<0.05$) regardless of whether vines were grown with cover crops or not (Fig. 1). As a net result total vine biomass under cover cropping was only around 15% greater than the control (Fig. 1). The

Table 2 Above-ground dry matter (DM) and N content of legume cover crops in a vineyard established in a granitic soil, in two growing seasons

Cover crop	2005-2006		2006-2007	
	DM (kg ha^{-1})	N (kg Nha^{-1})	DM (kg ha^{-1})	N (kg Nha^{-1})
EMC	2330±573 b*	68±16 b	5391±915 b	157±26 b
LMC	4070±765 a	128±23 a	6148±555 a	194±18 a

*Means (±SE) followed by different letters in the same column differ significantly according to t Student's test ($P \leq 0.05$)

annual components of vines (leaves, cane and fruit) represented 41–44% of the total recovered biomass.

The N concentrations measured in 2007 were higher than the control in grape bunches under both cover crops ($P \leq 0.05$), and in the canes and trunk of the LMC treatment (Table 4). The total amount of N accumulated by vine plants by the end of the second growing season, was also significantly ($P \leq 0.05$) higher in treatments with cover crops (8.0 and 9.1 g $plant^{-1}$ in EMC and LMC mixtures, respectively) than in control (6.9 g $plant^{-1}$; Table 4).

The annual components of the vines represented 46–51% of the total plant N (Table 4). The largest amounts of N accumulated in all treatments were found to be in the leaves and trunk (each containing between 2.1–2.8 gN $plant^{-1}$), with slightly lower amounts being recovered in the roots (1.3–2.0 gN $plant^{-1}$; Table 4).

Recovery of legume N by vine plants

The overall enrichment of ^{15}N measured in the roots and leaves were lower than that detected in the canes and fruit in all treatments (Table 5). This presumably reflected the presence of unlabelled N accumulated in root structures during previous growing seasons, and suggested that leaves had a higher dependence upon remobilization of older sources of N from perennial tissues for growth than uptake of newly assimilated soil N relative to other plant parts (Treeby and Wheatley 2006).

The significantly lower ^{15}N-enrichments detected in vines growing with cover crops than the control provided evidence that some legume N was utilized for growth by all plant parts in the cover cropped treatments (Table 5). Estimates of the proportion of the vine N derived from the legumes (Ndfl%) showed that vine plants growing with the late maturing mixture of annual legumes (LMC) generally had a higher reliance upon legume N for growth than vines with the EMC cover crop (Table 5), which was consistent with the larger inputs of legume N in the LMC treatment (Table 2). Analyses indicated that Ndfl ranged from 7–13% of the N in grape bunches, to 39–56% of the N in roots in the EMC and LMC treatments, respectively. Legume N provided between 14 and 20% of the N in vine canes and leaves (Table 5).

The total contribution of legume cover crops to the N nutrition of vine plants was calculated from the ^{15}N data of individual plant parts to be 1.46–1.92 gN $plant^{-1}$ or about 12–15 kg N ha^{-1} with no significant differences between the two cover crop treatments (Table 6). This represented between 18–21% of the N

Table 3 Number of bunches per plant and grape biomass production for grapevines growing with or without cover crops in two growing seasons

Treatment	2005-2006		2006-2007	
	Number of bunches	Grape production (kg ha^{-1})	Number of bunches	Grape production (kg Nha^{-1})
Control	11±2.4 a*	7,368±378 a	9±1.5 b	5,952±289 b
EMC	8.3±1.3 a	4,728±274 b	10±2.5 b	8,824±474 a
LMC	10.2±2.1 a	6,736±293 a	14±2.4 a	9,560±348 a

*Means (±SE) followed by different letters in the same column differ significantly according to t Student's test ($P \leq 0.05$)

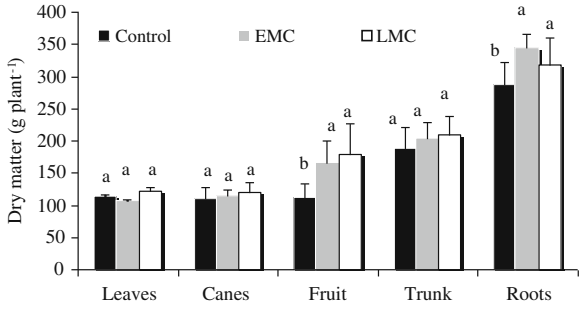

Fig. 1 Dry matter production (g plant^{-1}) of the components of vine plants growing with or without cover crops in 2006-2007. Leaves were sampled in February (at maximum canopy development), fruits on April (at harvest) and canes, trunk and roots in June 2007. Means followed by different letters in a column are significantly different according to Duncan test ($P \leq 0.05$)

requirement of the grapevine (compare the total plant data in Table 4 with Table 6), and a recovery of <10% of the N annually returned to the soil in above-ground legume residues. The N contribution of legume cover crops can also be estimated by N-difference for comparative purposes from the total plant N data presented in Table 4 (see Hood 2001). Using this procedure the N provided by legumes was calculated by subtracting the N accumulated by the control vines in the absence of legumes (6.92 gN plant^{-1}) from the N content of vines grown with the EMC (8.03 gN plant^{-1}) and LMC treatment (9.09 gN plant^{-1}; Table 4). The resulting additional N in the cover cropped vines attributable to legumes (1.11 and 2.17 gN plant^{-1}) was equivalent to 14% and 24% of the N requirements of EMC and LMC grown vines, respectively.

Recovery of fertilizer N by vine plants

Since the same amount of ^{15}N-enriched fertilizer was applied to micro-plots in all treatments as is currently applied to vineyards, the ^{15}N data presented in Table 5 could also be used to determine the proportion of the vine N derived from fertilizer (Ndff%) that might be achieved following standard fertilizer recommendations. Estimates of Ndff% indicated that between 8% (LMC roots) and 27% (Control fruits) of the N present in different plant parts came from fertilizer. Total contributions fertilizer N were calculated to be between 1.34 and 1.50 gN plant^{-1}, equivalent to 11-12 kg N ha^{-1} in both the absence and presence of legume cover crops (Table 6). These values represented 27–30% recovery of the 40 kg N ha^{-1} fertilizer N applied during the growing season.

Discussion

Productivity and N inputs by legume cover crops

The amount of biomass accumulated by the legume cover crops in the second year of the project was comparable to, and in some cases, greater than, DM yields previously reported for the same species under permanent pasture conditions in the same agro-

Table 4 Determinations of N concentration and N content in above-ground and root biomass for grapevines grown with or without legume cover crops in 2006-2007

Treatment	Leaves[a]	Canes	Fruit	Trunk	Roots	Total plant
N concentration (%)						
Control	1.85±0.22 a*	0.44±0.03 b	0.55±0.03 b	1.12±0.11 b	0.42±0.06 a	Nd
EMC	1.70±0.16 a	0.45±0.03 ab	0.64±0.06 a	1.13±0.20 b	0.58±0.13 a	Nd
LMC	2.02±0.28 a	0.47±0.02 a	0.70±0.07 a	1.36±0.10 a	0.51±0.13 a	Nd
N content (g plant^{-1})						
Control	2.41±0.31 a	0.49±0.04 a	0.61±0.22 b	2.12±0.21 b	1.29±0.01 b	6.92±0.68 b
EMC	2.15±0.35 a	0.52±0.04 a	1.06±0.16 a	2.30±0.23 b	2.00±0.02 a	8.03±0.74 a
LMC	2.81±0.39 a	0.56±0.42 a	1.25±0.12 a	2.85±0.27 a	1.62±0.02ab	9.09±1.22 a

[a] Leaves were sampled in February (at maximum canopy development), fruits on April (at harvest) and canes, trunk and roots in June 2007

* Means (±SE) followed by different letters in a column are significantly different according to Duncan test ($P \leq 0.05$). nd: non determined

Table 5 Measures of ^{15}N enrichment (atom% ^{15}N excess) in various plant parts of grapevines and estimates of the proportion of the N in those plant parts derived from the legume cover crop (Ndfl)

Plant part[1] and treatment	18-12-2006		08-02-2007		Mean	
	^{15}N enrichment	Ndfl%[a]	^{15}N enrichment	Ndfl%[a]	^{15}N enrichment	Ndfl%[a]
Leaves						
Control	0.81±0.07 a	–	1.45±0.34 a		1.13±0.21 a	–
EMC	0.72±0.04 b	13±1.2 a	1.26±0.25 b	15±1.3 b	0.99±0.14 ab	14±1.5 b
LMC	0.72±0.03 b	13±1.2 a	1.13±0.31 b	28±3.1 a	0.93±0.17 b	20±3.1 a
Canes						
Control	2.08±0.20 a	–	2.59±0.37 a	–	2.34±0.29 a	–
EMC	1.74±0.31 b	19±3.2 b	2.17±0.25 b	19±2.7 a	1.96±.0.28 b	19±4.0 a
LMC	1.84±0.31 b	13±3.9 b	2.19±0.22 b	18±2.3 a	2.02±0.25 b	15±3.7 a
Fruits						
Control	–	–	2.67±0.85 a		–	–
EMC	–	–	2.51±0.16 a	7±0.62 b	–	
LMC	–	–	2.38±0.91 b	12±0.94 a	–	–
Roots						
Control	–	–	1.32±0.28 a		–	–
EMC	–	–	0.95±0.17 b	39±4.3 b	–	–
LMC	–	–	0.85±0.19 b	56±5.3 a	–	–

*Means (SE) followed by different letters in the same column differ significantly according to Duncan's test ($P \leq 0.05$)

[a] Calculated using Eq. 1 as described in the Materials and Methods

ecological area (Avendaño et al. 2005; Ovalle et al. 2005) and represented above-ground inputs of 157–194 kg legume N ha $^{-1}$ for the EMC and LMC treatments, respectively. Since up to 40–50% of the total N in a forage legume may be below-ground associated with rhizodeposition of N and the N contained in the nodulated roots (Peoples and Baldock 2001; Unkovich et al. 2010), the total annual input of legume N by the cover crops in 2006-2007 could have represented up to 300–400 kg N ha^{-1}.

Table 6 Estimates of the amounts of N recovered by grapevines from legume cover crops or fertilizer

Treatment	Leaves	Canes	Fruit	Trunk[a]	Roots	Plant	Total
N derived from legume cover crops[b]							
	(g N plant^{-1})						(kg Nha^{-1})
EMC	0.29 b*	0.10 a	0.07 a	0.22 b	0.78 a	1.46 a	11.7 a
LMC	0.57 a	0.09 a	0.16 a	0.21 b	0.90 a	1.92 a	15.4 a
N derived from fertilizer[c]							
	(g N plant^{-1})						(kg Nha^{-1})
Control	0.35 a	0.13 a	0.16 a	0.55 a	0.17 b	1.36 a	10.9 a
EMC	0.27 a	0.11 a	0.27 b	0.55 a	0.19 b	1.34 a	10.7 a
LMC	0.32 a	0.12 a	0.30 b	0.62 a	0.14 b	1.50 a	12.0 a

*Means followed by different letters in the same column differ significantly according to t Student's test ($P \leq 0.05$)

[a] N recovered by the trunk was estimated using data of ^{15}N enrichment of canes

[b] Calculated from data presented in Table 5 using Eq. 3 as described in the Materials and Methods

[c] Calculated from data presented in Tables 4 and 5 using Eqs. 5 and 6 as described in the Materials and Methods

Three possible sources of N could have contributed these large amounts of N generated by the legume cover crops: (a) soil mineral N, (b) N-fertilizer, and (c) biologically fix atmospheric N_2. While specific measures of the reliance of the legumes upon N_2 fixation for growth relative to soil or fertilizer N were not undertaken, similar studies in the granitic soils of the region have previously indicated that >90% of the N contained in subterranean clover and burr medic can be derived from N_2 fixation (Ovalle et al. 2006). Data collected in other forage legume systems also suggest that rates of N fertilizer up to 50 kg N ha^{-1} can have relatively little effect N_2 fixation (Ledgard and Steele 1992), so it would appear unlikely that the two split applications of 20 kg fertilizer-N ha^{-1} supplied to the vineyard would have been sufficient to significantly suppress N_2 fixation by cover crops assimilating several hundreds of kg of N. Therefore, it seems reasonable to suggest that the majority of the N accumulated by the legumes in the present study also came from N_2 fixation.

Impact of cover crops on the N-economy of vines

The increased grape production and N concentration of vines growing in association with legume cover crops described here has been also reported elsewhere in the world. For example, a long-term (10 year) experiment undertaken in South Africa with Chardonnay/99 Richter vines growing in association with single leguminous species (either *Vicia faba*, *Medicago* spp., or *T. subterraneum*), found greater grape production, and higher N concentrations in leaf petioles and in grape juice than those in vines grown without cover crop (Fourie et al. 2006). The results are also not dissimilar to that reported for organic raspberry after 3 years growing in association with a white clover cover crop, where fruit yield tended to be higher, although not statistically, from the control without cover crop (Ovalle et al. 2007a). However, there can be delays in the vine's response to the increased inputs of N so the long-term production benefits of cover-cropping could larger than reported here. For example, an Australian study indicated that at least two growing seasons were required before grape yields were improved by increased applications of fertilizer N (Treeby and Wheatley 2006).

Between 27–35% of the vine N was partitioned in leaves. This was similar to the findings of a grapevine investigation undertaken in Europe (Wermelinger and Koblet 1990), but contrasts with a North American study conducted in a 23 year old Pinot noir vineyard which found relatively larger amounts of N in the roots and trunk than leaves (Schreiner and Scagel 2006), indicating that older and bigger vines may have the capacity to store more N in root and trunk reserves than measured in the present trial in a 5 year old vineyard.

The rate of N uptake by vines increases from bud break and reaches a maximum close to bloom and fruit set during late spring—early summer (Wermelinger and Koblet 1990; Schreiner and Scagel 2006). The soil temperature and water conditions most favorable for rapid mineralization of the N in organic matter are also likely to occur in spring (Mengel 1996; Fillery 2001; Peoples et al. 2009). As a consequence, the supply of plant-available N from the legume residues might be expected to coincide with the time of peak N demand by the grapevine (Ovalle et al. 2007c; Fourie et al. 2007), and should theoretically be conducive with high efficiencies of N uptake by vines and result in a lowered risk of N losses from the system (Crews and Peoples 2005). Certainly the concentrations of soil mineral N (on the top 20 cm), measured on four occasions between August and November 2006, were found to be significantly higher under the LMC (14–194 mg N kg^{-1}) than in EMC treatment (22–63 mg N kg^{-1}), with soil mineral N under both cover crop treatments being greater than the control (2–10 mg N kg^{-1}; Ovalle et al. 2007c). The maximum concentrations soil mineral N where observed in September i.e. during budbreak and early canopy development of grapevine.

The indirect ^{15}N dilution method applied here to determine the recovery of legume N by grapevine has also been used in other cover crops studies (Snoeck et al. 2000; Ovalle et al. 2007b) including grapevine (Patrick et al. 2004). However, it should be noted that the estimates of the relative importance of legume N to the N nutrition of the various vine tissues presented in Tables 5 and 6 calculated using Eq. 1 will inevitably be conservative since it was assumed that all the legume N taken up the vine had been derived from atmospheric N_2. Based on past experience with legumes grown on similar soil types one might expect that most, but certainly not all, of the cover crop N to have come from N_2 fixation (Ovalle et al. 2006). So while it seems likely that the

^{15}N-enrichment of the legume N would have been low, it was unlikely to have been zero. Consequently, the data in Tables 5 and 6 could undervalue the true contribution of legume N to vines. There may also be inherent methodological problems with the use of the indirect ^{15}N dilution technique, and with the application of ^{15}N-enriched fertilizer to label the soil N pool, which can lead to the uptake of legume N being underestimated (Høgh-Jensen and Schjoerring 2000; Hood 2001; Peoples et al. 2009). Nonetheless, it was encouraging in the present study that similar estimates of the contributions of legume N to vine nutrition were obtained using both the ^{15}N dilution approach (18–21% of the vine's N requirements) and an alternative, non-isotopic, N-difference method (14–24%).

The percentage of the vine leaf N calculated to have been derived from the cover crops (14–20%, Table 5) was considerably higher than reported by Patrick et al. (2004) who estimated only 0.28% of N in vine leaves to have come from legume N using similar ^{15}N methodologies. However, much less legume N was applied (81 kg ha^{-1}) in the study described by Patrick et al. (2004) and the rainfall during their experiment was only 45 mm. The drier soil would undoubtedly have restricted decomposition and mineralization of the organic legume residues to a greater extent than under the soil conditions experienced in the current experiment.

Importantly, the data in Table 5 indicated that despite the fruit being the main organ to increase biomass (Fig. 1) and N accumulation (Table 4) in response to legume cover cropping, it was the plant tissue that the ^{15}N data suggested had the lowest reliance upon of legume N for growth (7–13% compared to 14–20% for leaves, 15–19% for canes and 39–56% for roots; Table 5). It is interesting to speculate on possible explanations for this result. Since the leaves and canes largely assimilate much of their N prior to flowering and the developing grape bunches only become a sink for N after flowering (Wermelinger and Koblet 1990; Treeby and Wheatley 2006), one possible explanation for the observation may be that the relative contribution of legume N to the soil mineral N pool changed over the course of the growing season and more of the soil mineral N recovered by the leaves and canes was derived directly from legume residues than was subsequently then mineralized and taken up during fruit development.

An alternative explanation may be that the fruit had a heavier reliance on N remobilized from other structures from within the vine than upon the uptake of N assimilated from the soil. This is consistent with the data presented by Treeby and Wheatley (2006) who concluded that remobilized N provided about two-thirds of the N measured in grape berries at harvest. However, this is not a totally satisfactory explanation for although the ^{15}N-enrichments of the fruit did resemble those detected in the canes (Table 5), they were not identical, and they differed markedly from the other plant parts. Perhaps the truth lies in a combination of these two possibilities.

Comparison of recoveries of legume and fertilizer N by vines

The total amount of legume N taken up by vines in the cover cropped treatments (12–15 kg N ha^{-1}) was remarkably similar to the amount of fertilizer N used by vines (11-12 kg N ha^{-1}: Table 6). The apparent recovery of legume N by vines was estimated to represent <10% of the above-ground N and possibly <5% of the total N accumulated by the cover crops each year. While these recoveries are low, such low short-term recoveries of legume N by other plants are not uncommon (Fillery 2001; Crews and Peoples 2005; Peoples et al. 2009). Comparative recoveries of the ^{15}N-enriched fertilizer by vines were calculated to be 27–30% of the total fertilizer N applied, which was not too dissimilar from values previously reported for other crops (Crews and Peoples 2005; Gardner and Drinkwater 2009; Peoples et al. 2009). However, the estimates of the proportion of vine N derived from fertilizer observed here for various plant parts (ranged from 8–13% in roots to 24–27% in fruit) were generally higher than detected by Peacock et al. (1989) when investigating the impact of the time of application of fertilizer N to vineyards on N uptake (Ndff <10% of vine tissue N).

Legume and fertilizer N not recovered by vines

More than 90% of the N inputs provided by the self-replenishing legume cover crops, and 70% of the fertilizer N was not taken up by the grapevines. Unfortunately, the experimental design was such that it was not possible to distinguish between the contributions of below-ground pools of N or the

above-ground mulch as sources of legume N for vines, or determine the fate of the legume N that was utilized by the vines. Nor was the soil profile sampled to determine now much residual ^{15}N remained at the end of the 2006-2007 growing season to assess the extent of N losses from the cover cropped or bare soil treatments. However, it is possible to speculate based information reported in the scientific literature about the N-dynamics of legume and fertilizer N in other agricultural systems.

There are data that suggest below-ground legume N may be a more important source of N for crops than above-ground residues (Peoples et al. 2009). Other research indicates that rhizodeposits of N from living roots and other labile forms of legume N are readily immobilized by soil micro-organisms (Murphy et al. 1998; Wichern et al. 2008). Legume sources of N are usually considered to be less susceptible to loss processes than fertilizer and large proportions of legume N inputs tend to remain as organic N or are incorporated in soil microbial biomass to be slowly released for plant use over subsequent years (Fillery 2001; Crews and Peoples 2005; Gardner and Drinkwater 2009; Peoples et al. 2009). Consequently, it would be anticipated that the continued use of legume cover cropping in vineyards would result in a build-up of soil organic carbon and N, and there would be a net reduction in green-house gas emissions due to the substitution of legume sources of N for fertilizer N (Peoples and Baldock 2001; Boddey et al. 2009; Peoples et al. 2009).

Conclusions

The use of cover crops in a vineyard growing in low fertility granitic soils of the Mediterranean region of Chile presented clear advantages over the traditional N fertilized, bare soil management system in terms of improved N nutrition for grapevines. This study demonstrated that mixtures of annual legumes could produce large amounts of dry matter of high N content, resulting in large inputs of N into the system that can contribute similar amounts of N to vines as the current fertilizer recommendations. Grape production was increased by this additional N supply by legume cover crops, but no differences were observed in the leaf, cane, trunk or root biomass. The transfer of legume N to the leaves, roots and woody components of vines were verified using a ^{15}N technique which indicated that around 20% of the total N accumulated by the grapevines were provided by the legume cover crops. However, the ^{15}N data also suggested that legume N was not directly responsible for the observed improvements in grape production in the second year of the experiment. It is believed that this finding was a consequence of N accumulation by the vegetative components and fruit occurring at different times during the growing season. It is proposed that either there were dynamic changes in the dominant sources of organic N mineralized in the soil over time, and/or that the fruit relied more heavily on N remobilized from elsewhere in the vine than on N assimilated from the soil.

Acknowledgements This study was supported by FONDECYT (project N° 1050116).

References

Aballay E, Insunza V (2002) Evaluación de plantas con propiedades nematicidas en el control de *Xiphinema index* en uva de mesa cv. Thompson Seedless en la zona central de Chile. Agric Téc (Chile) 62:357–365

Avendaño J, Ovalle C, del Pozo A, Fernández F, Porqueddu C (2005) Mezclas de trébol subterráneo con otras leguminosas anuales para suelos graníticos del secano mediterráneo subhúmedo de Chile. Agric Téc (Chile) 65:165–176

Axmann H (1990) Methods for ^{15}N determination. In: Hardarson G (ed) Use of nuclear techniques in studies of soil-plant relationships. Training Course Series N° 2. Internacional Atomic Energy Agency (IAEA), Vienna, pp 55–59

Boddey RM, Alves BJR, Soares LH deB, Jantalia CP, Urquiaga S (2009) Biological nitrogen fixation and mitigation of greenhouse gas emissions. In: Emerich DW, Krishnan HB (Eds) Agronomy Monograph 52 Nitrogen Fixation in Crop Production, Am. Soc. Agron., Crop Sci. Soc. Am., and Soil Sci. Soc Am. Madison, Wisconsin, USA. Chapter 14 pp 387–413

Campillo R, Urquiaga S, Pino I, Montenegro A (2003) Estimación de la fijación biológica de nitrógeno en leguminosas forrajeras mediante la metodología del ^{15}N. Agric Téc (Chile) 63:169–179

Crews TE, Peoples MB (2005) Can the synchrony of nitrogen supply and crop demand be improved in legume and fertilizer-based agroecosystems? A review. Nutr Cycl Agroeco 72:101–120

Danso S, Palmason F, Hardarson G (1993) Is nitrogen transferred between field crops. Soil Biol Biochem 25:1135–1137

Del Pozo A, Ovalle C, Aronson J, Avendaño J (2000) Developmental responses to temperature and photoperiod in ecotypes of *Medicago polymorpha* L. along an

environmental gradient in central Chile. Ann Bot (London) 85:809–814

Del Pozo A, Ovalle C, Aronson J, Avendaño J (2002) Ecotypic differentiation in *Medicago polymorpha* along an environmental gradient in central Chile. II. Winter growth as related to phenology and temperature. Plant Ecol 160:53–59

Dou Z, Fox RH, Toth JD (1994) Tillage effect on seasonal nitrogen availability in corn supplied with legume green manures. Plant Soil 162:203–210

Fillery IRP (2001) The fate of biologically fixed nitrogen in legume-based dryland farming systems: a review. Aust J Expl Agric 41:361–381

Frye WW, Blevins RL (1989) Economically sustainable crop production with legume cover crops and conservation tillage. J Soil Water Conserv 44:57–60

Fourie JC, Louw PJE, Agenbag GA (2006) Cover crop management in a Chardonnay/99 Richter vineyard in the Coastal wine grape region, South Africa. 2. Effect of different cover crops and cover crop management practices on grapevine performance. S Afr J Enol Vitic 27:42–50

Fourie JC, Agenbag GA, Louw PJE (2007) Cover crop management in a Chardonnay/99 Richter vineyard in the Coastal Region, South Africa. 3. Effect of different cover crops and cover crop management practices on organic matter and macro-nutrient content of a medium-textured soil. S Afr J Enol Vitic 28:61–68

Gardner JB, Drinkwater LE (2009) The fate of nitrogen in grain cropping systems: a meta-analysis of ^{15}N field experiments. Ecol Applic 19:2167–2184

Giller KE, Ormesher J, Awah FM (1991) Nitrogen transfer from *Phaseolus* beans to intercropped maize measured using ^{15}N-isotope dilution methods. Soil Biol Biochem 23:330–346

Guldan SJ, Martin CA, Lindemann WC, Cueto-Wong J, Steiner RL (1997a) Interseeding legumes into Chile: legumes benefits to a following crop of forage sorghum. HortTechnology 7:146–149

Guldan SJ, Martin CA, Lindemann WC, Cueto-Wong J, Steiner RL (1997b) Yield and green-manure benefits of interseeded legumes in a high-desert environment. Agron J 89:757–762

Haystead A, Malajczuk N, Grove TS (1988) Underground transfer of nitrogen between pasture plants infected with vesicular-arbuscular mycorrhizal fungi. New Phytol 108:417–423

Høgh-Jensen H, Schjoerring JK (2000) Below-ground nitrogen transfer between different grassland species: direct quantification by ^{15}N leaf feeding compared with indirect dilution of soil ^{15}N. Plant Soil 227:171–183

Hood R (2001) Evaluation of a new approach to the nitrogen-15 isotope dilution technique, to estimate crop N uptake from organic residues the field. 34:156–161

Ladd JN, Amato M, Aodes JM (1985) Decomposition of plant material in Australian soil. III. Residual organic and microbial biomass C and N from isotope-labeled legume material and soil organic matter, decomposing under field condition. Aust J Soil Res 23:603–611

Masson P, Bertoni G (1996) Essai d'enherbement d'un vignoble méridional á base de trèfle souterr ain: synthèse de six années d'expérimentation. In: XI-Kolloquium Begrünung im Weinbau, Internationaler Arbeitsjkreis im Weinban, Kaltern Sud Tyrol, Italie. pp 28–31

Masson P, Gintzburger G (2000) Les légumineuses fourragères dans les systèmes de production méditerrané ens: utilisations alternatives. Cah Opt Méd 45:395–406

Mengel K (1996) Turnover of organic nitrogen in soils and its availability to crops. Plant Soil 181:83–93

Miller PR, Graves WL, Williams WA (1989) Cover crops for California Agriculture. University of California, Division of Agriculture & Natural Resources, Berkeley, p 24

Murphy DV, Fillery IRP, Sparling GP (1998) Seasonal fluctuations in gross N mineralisation, ammonium consumption, and microbial biomass in a Western Australian soil under different land uses. Aust J Agric Res 49:523–535

Nieddu G, Graviano O, Lostia A, Porqueddu C (2000) Effects of *Medicago polymorpha* cover cropping in Sardinia vineyards. Cah Opt Méd 45:449–452

Ledgard SF, Steele KW (1992) Biological nitrogen fixation in mixed legume / grass pastures. Plant Soil 141:137–153

Ledgard SF, Freney JR, Simpson JR (1985) Assessing nitrogen transfer from legumes to associated grasses. Soil Biol Biochem 17:575–577

Ofosu-Budu KG, Noumura K, Fujita K (1995) N_2 fixation, N transfer and biomass production of soybean cv. Bragg or its supernodulating nts1007 and sorghum mixed-cropping at two rates of N fertilizer. Soil Biol Biochem 27:311–317

Ovalle C, del Pozo A, Avendaño J, Fernández F (2005) Adaptación, crecimiento y producción de nuevas leguminosas forrajeras anuales en la zona mediterránea de Chile. II. Comportamiento de las especies en suelos graníticos del secano interior subhúmedo. Agric Téc (Chile) 65:265–277

Ovalle C, Urquiaga S, del Pozo A, Zagal E, Arredondo S (2006) Nitrogen fixation in six forage legumes in Mediterranean central Chile. Acta Agric Scand 56:277–293

Ovalle C, Gonzalez MI, del Pozo A, Hirzel J (2007a) Cubiertas vegetales en producción orgánica de frambuesa: efectos sobre el contenido de nutrientes del suelo y en el crecimiento y producción de la planta de frambuesa. Agric Téc (Chile) 67:271–280

Ovalle C, Gonzalez MI, Hirzel J, Pino I, del Pozo A, Urquiaga S (2007b) Contribution and transfer of nitrogen from cover crops to raspberry plant using isotopic techniques with ^{15}N. Acta Hort 777:465–472

Ovalle C, del Pozo A, Lavín A, Hirzel J (2007c) Cubiertas vegetales en viñedos: comportamiento de mezclas de leguminosas forrajeras anuales y efectos sobre la fertilidad del suelo. Agric Téc (Chile) 67:384–392

Patrick AE, Smith R, Keck K, Berry AM (2004) Grapevine uptake of ^{15}N-labeled nitrogen derived from a winter-annual leguminous cover-crop mix. Am J Enol Vitic 55 (2):187–190

Peacock WL, Christensen LP, Broadbent FE (1989) Uptake, storage, and utilization of soil-applied nitrogen by Thompson seedless as affected by time of application. Am J Enol Vitic 40:16–20

Peoples MB, Baldock JA (2001) Nitrogen dynamics of pastures: nitrogen fixation inputs, the impact of legumes on soil

nitrogen fertility, and the contributions of fixed nitrogen to Australian farming systems. Aust J Exp Agric 41:327–346

Peoples MB, Angus JF, Swan AD, Dear BS, Hauggaard-Nielsen H, Jensen ES, Ryan MH, Virgona J (2004) Case studies of N-dynamics in legume-based pasture systems. In: Mosier AR, Syers K, Freney JR (eds) Agriculture and the nitrogen cycle: assessing the impacts of fertilizer use on food production and the environment. The Scientific Committee on Problems of the Environment (SCOPE). Island, Covelo, pp 103–114

Peoples MB, Hauggaard-Nielsen H, Jensen ES (2009) The potential environmental benefits and risks derived from legumes in rotations. In: Emerich DW, Krishnan HB (Eds) Agronomy Monograph 52 Nitrogen Fixation in Crop Production, Am. Soc. Agron., Crop Sci. Soc. Am., and Soil Sci. Soc Am. Madison, Wisconsin, USA. Chapter 13pp 349–385

Rochester I, Peoples M (2005) Growing vetches (*Vicia villosa* Roth) in irrigated cotton systems: inputs of fixed N, N fertiliser savings and cotton productivity. Plant Soil 271:251–264

Sánchez EE, Giayetto A, Cichón L, Fernández D, Aruani MC, Curetti M (2007) Cover crops influence soil properties and tree performance in an organic apple (*Malus domestica* Borkh) orchard in northern Patagonia. Plant Soil 292:193–203

Schreiner RP, Scagel CF (2006) Nutrient uptake and distribution in a mature 'Pinot noir' vineyard. HortScience 41:336–345

Snoeck D, Zapata F, Domennach A (2000) Isotopic evidence of the transfer of nitrogen fixed by legumes to coffee trees. Biotechnol Agron Soc Environ 4:95–100

Trannin WS, Urquiaga S, Guera G, Ibijbijen J, Cadisch G (2000) Interspecies competition and N transfer in a tropical grass-legume mixture. Biol Fertil Soils 6:441–448

Treeby MT, Wheatley DM (2006) Effect of nitrogen fertilizer on nitrogen partitioning and pool sizes in irrigated Sultana grapevines. Aust J Expl Agric 46:1207–1215

Unkovich MJ, Baldock J, Peoples MB (2010) Prospects and problems of simple linear models for estimating symbiotic N_2 fixation by crop and pasture legumes. Plant Soil (in press)

Wermelinger B, Koblet W (1990) Seasonal growth and nitrogen distribution in grapevine leaves, shoots and grapes. Vitis 29:15–26

Wichern F, Eberhardt E, Mayer J, Joergensen RG, Müller T (2008) Nitrogen rhizodeposition in agricultural crops: Methods, estimates and future prospects. Soil Biol Biochem 40:30–48

Xiao Y, Li L, Zhang F (2004) Effect of root contact on interspecific competition and N transfer between wheat and faba bean using direct and indirect ^{15}N techniques. Plant Soil 262:45–54