

Article

N₂ Use in Perennial Swards Intercropped with Young Poplars, Clone I-214 (*Populus* × *euramericana* (Dode) Guinier), in the Mediterranean Area under Rainfed Conditions

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Abstract: Intercropping perennial legumes with trees can reduce Nitrogen (N) losses, due to the high amount of N accumulated in stable forms in the soil and permanent soil cover during the whole year. Although N cycling improvement in mature agroforestry systems (AFS) was well documented, there is a lack of knowledge regarding systems in transition to AF. In this work, we studied the association of two perennial forage crops, namely ryegrass (*Lolium multiflorum* Lam.) and sulla (*Hedysarum coronarium* L.), with 1-year old poplars, to evaluate: (i) the agronomic performance of sulla and ryegrass with vs. without intercropped poplar trees; (ii) the N-fixing ability of sulla in association with trees; (iii) the N transfer effect from sulla and growth promoting effect on poplar; and finally (iv) the nitrate leaching reduction due to the presence of poplar trees associated to forage crops. The layout was arranged in a two-factor randomized complete blocks design (RCB) with three replicates. The first factor tested (crop species) implied two different swards, namely sulla and ryegrass. The second factor (cropping system) included two different systems: PAST i.e., a pastoral system without trees, and SIPAST, i.e., a silvo-pastoral system with one poplar tree row beside the sward. Sulla resulted more productive than ryegrass when associated with trees (+35%). No clear trend was observed about the tree influence on N-fixation in sulla, but the amount of N fixed resulted higher in sulla grown in the SIPAST near the trees (+35%). Poplar plants, even in the first year after planting, resulted effective yet in reducing the nitrate flux from the crops towards ditches. Further investigations are needed to study other swards in young AF and better understand the N dynamics; in particular, it could be worth to assess the nutrient flux in the soil solution.

Keywords: agroforestry; legume; N-fixation; ¹⁵N natural abundance; sulla; ryegrass



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1. Introduction

Agroforestry (AF) is defined as “the deliberate integration of woody vegetation (trees and/or shrubs) as an upper storey on land, with pasture (consumed by animals) or an agricultural crop in the lower storey” and is gaining pace as a land-use strategy to cope with climate change and provide environmental, economic, and social benefits [1,2]. Agroforestry systems (AFS) can improve agricultural landscapes by increasing biodiversity and vegetation complexity. At the same time, AFS are well proved to increase land productivity, reduce soil erosion and water losses, improve natural biodiversity conservation, soil fertility, carbon sequestration, and greenhouse gas (GHG) emission mitigation [1,3–8]. AFS reduce many environmental risks, such as wild-fire, and mitigate the extreme weather conditions by reducing fluctuations in air/soil temperatures and humidity and breaking strong winds above the soil surface [5,9–11]. However, the transition towards a wider adoption of AF by farmers could be challenging, considering the (i) high level of required knowledge and experience, (ii) high initial financial and labor investments that do not result immediately

in higher income for the farmer [5,12] and (iii) absence of policy frameworks to effectively support the conversion from specialized crops to AF [13].

Among the many benefits (i.e., ecosystems services) that AFS are able to provide, there is no clear evidence of a net increase in soil N availability, especially in Mediterranean areas, mostly due to rare studies conducted on N₂-fixing trees in these conditions. Contrastingly, legume trees and shrubs are more present in AF studies conducted elsewhere (see e.g., Ribeiro-Barros et al. [14]). Hence, the integration of N₂-fixing crops between trees can be a solution to increase the land productivity and reduce the reliance on external inputs by increasing N availability and then both tree and crop growth [15]. Intercropping perennial legumes with trees can also reduce N losses, due to the higher amount of N accumulated in stable forms in the soil derived from biological N₂-fixation (BNF) and N root compartmentation [16]. Furthermore, perennial legumes ensure a year-long permanent soil cover, thus reducing N losses due to soil erosion and nitrate leaching [17]. Moreover, the amount of N derived from BNF in perennial legumes can be even higher than the standard N-fertilization rates commonly adopted in conventional agriculture [18]. When the soil N is a limiting factor (e.g., the legume crop is intercropped with a non-legume crop, outcompeting for soil N), legumes can make heavier investments in energy and photosynthates required for BNF. Indeed, in previous studies leguminous crops and trees cultivated under low N availability were showed to allocate greater energy to nodulation and fixation processes, and a greater number of nodules and higher BNF rate were observed compared to when N was supplied as fertilizer [19,20]. On the other hand, the competition produced by non-legume trees for water [6], light and nutrients [20] eventually could limit growth and N₂-fixation in the herbaceous legume crop [15].

To date, several studies were carried out focusing mainly on the effects of the shade of trees on legume yields [21–27], and assessing the biophysical interactions at the tree-crop interface in tree-based legume cropping systems [28–31]. However, from an agroecological point of view, it is of paramount importance also to examine the effect of trees on BNF in the understory, and the potential N-transfer effect through which legumes can increase tree growth in AFS, thus reducing their reliance on mineral N fertilisers [32–35]. The N fixed by legumes can be released into the soil through the falling of plant portions (e.g., senescent leaves or branches), or even directly through root exudates, that reach the non-legume crop roots mediated or not by arbuscular mycorrhizal fungi (AMF) hyphal nets. N transfer was demonstrated to occur in several experiments of intercropping between cereals and legumes [36,37], but always with high variability depending on peculiar environmental and managerial conditions. Nevertheless, in many studies the conclusion was that increased growth of non-legumes intercropped with legumes might have been derived basically by the increased competitive ability of roots to capture N from the soil, rather than from a real N transfer from the legume to the non-legume [38,39].

Specifically, the assessment of the tree competitive mechanisms on growth and BNF processes of the legume swards is a crucial point for optimizing resource use and re-designing systems that can tolerate competition in AF conditions, especially in the Mediterranean basin [15,40,41]. In this context, investigating synergies and trade-offs between the two main components of the AFS represents a priority and allows to assess whether the diversification due to introducing perennial herbaceous species can improve the resilience of cropping systems to variability of weather conditions.

In most of the studies conducted on N cycling in AFS including forage legumes, productive and qualitative parameters as affected by the interactions between trees and crops are reported [5,26,42,43], but only in mature AF systems. However, even if undervalued and not deepened in scientific literature, the response of a system in transition to AF appears as extremely interesting to understand. Actually, information on transitional phases to AFS could be relevant to support efficient design of AFS, in order to maximize the value of farmers' investments and orient policy in the field. In particular, the potential competition exerted by young tree plants on forage crops at the level of the soil (i.e., water and nutrients) should be elucidated. This is because young trees have high water and

nutrient needs and in the first years after plantation the roots of young trees share the same soil layers with herbaceous crops, with high competition effects likely to affect crop growth.

In Italy, among the most used perennial forage crops, the Italian ryegrass (*Lolium multiflorum* Lam.) is one of the most appreciated and employed as forage and pasture crops due to its high productivity and high nutritional value as livestock feed [44]. Also, sulla (*Hedysarum coronarium* L.), a typical biennial legume widely grown in Central and Southern Italy, could be particularly beneficial for: (i) its capability to exploit clay and alkali soils under rainfed conditions and (ii) its high nutritive value as pasture and hay, mostly due to its high protein content [45]. Among trees, in Italy poplars (*Populus* spp.) represent an important wood source, characterized by high productions, usually destined to the plywood industry, with rotations of only 10 years [46]. Poplars, thanks to their quick aboveground and root growth, are effective for controlling soil erosion and can be grown also in presence of grazing livestock [47]. One of the most used poplars is the clone I-214, characterized by very fast growth and wood technological features [48].

This research aimed to assess the differences between a sulla-poplar and a ryegrass-poplar AF and, specifically, investigated:—(i) the agronomic performance of sulla and ryegrass as forage crops with vs. without intercropped poplar trees; (ii) the N₂-fixing ability of sulla in association with 1 year-old poplar trees; (iii) the N transfer effect from sulla and growth promoting effect on poplar; and finally (iv) the nitrate leaching reduction due to the presence of poplar trees associated to forage crops. We hypothesized that: (i) the presence of young, fast-growing poplar trees can reduce, compared to sole crops, the forage yield in sulla and ryegrass; (ii) the N₂-fixation level in sulla is increased in presence of poplar trees compared to pure stand; (iii) the percentage of N derived from N₂-fixation is higher in poplar plants growing in intercropping with sulla than with ryegrass, as part of the total amount of N₂ fixed by the legume is released into the soil and can be absorbed by poplar roots; (iv) nitric-N in the soil is less abundant in treatments with than without poplar trees, with higher NO₃[−] scavenging effect in sulla than ryegrass.

2. Materials and Methods

2.1. Site Descriptions and Experimental Design

The rainfed plot field experiment was established at the Centre for Agri-Environmental Research “Enrico Avanzi” of the University of Pisa (CiRAA), San Piero a Grado, Pisa, Italy (43°41′07.6″ N 10°20′32.2″ E, 1 m above sea level and 0% slope). The experiment was replicated in time and space on two similar fields; field 1: 2020–2021 and field 2: 2021–2022. The area object of the study is characterized by an average annual rainfall of 892 mm and 14.9 °C of annual mean temperature (long-term data, 1993–2021). On average, for the 0–0.3 m horizon, the soil texture [49] of the two fields was loam (12.64 g 100 g^{−1} clay, 44.00 g 100 g^{−1} silt, 43.36 g 100 g^{−1} sand) with a pH value of 8.31, 1.69 g 100 g^{−1} of organic matter (Walkley–Black method) [50], 1.04 g kg^{−1} of total nitrogen (Kjeldahl method) [51], 7.97 mg kg^{−1} of available P₂O₅ (Olsen method) [52].

The experiment complied with a two-factor randomized complete block design (RCBD) with three replicates (each plot sizing 56 m²). The first factor (CROP) included two different swards: (i) sulla (SUL) cv. Silvan, (ii) ryegrass (RYE) cv. Teanna. The second factor (SYSTEM) included two different cropping systems: PAST, i.e., a specialised pastoral system with forage crops grown without intercropped trees, and SIPAST, a silvo-pastoral system with poplar (clone I-214) tree rows planted, North-South oriented, between the ditch and the forage crop. Five poplar trees have been planted on the row every 2 m along one side of each plot (plot length: 8 m), 2 m away from drainage ditches, and 1 m apart from the first row of the forage crop.

2.2. Crop Management

In both years, the two fields were mouldboard ploughed at the end of August at 0.3 m depth and then, in September, 100 kg ha^{−1} of P₂O₅ were applied as triple superphosphate (0-46-0) in both years. Immediately before the sowing, rotary harrowing was carried out

for seedbed preparation. The sowing was carried out with a mechanical plot seeder on 11 November 2020 and 18 October 2021 for field 1 and 2 respectively, at the following seeding rate: (i) sulla, 38 kg ha⁻¹; (ii) ryegrass, 30 kg ha⁻¹. Each crop was sown with an inter-row distance of 0.15 m. One-year-old stems of poplars were manually planted on 23 February 2021, in the field 1 and 3 February 2022, in the field 2. The space in between tree rows and ditches, on one side, and forage crops, on the other side, were managed by occasional mowing to control weeds during the year.

2.3. Data Collection and Chemical Analysis

To evaluate soil characteristics, before the sowing, three soil cores per plot (0–30 cm and 30–60 of depth, pooled per each depth) were sampled with an auger. To assess the Nitrogen dynamics in the soil, soil total N (STN, g kg⁻¹) and nitrates (NO₃⁻, mg kg⁻¹) were measured by collecting soil samples at two depths, 0–30, 30–60 cm, with diversified sampling schemes among the SYSTEM levels: (i) three soil cores were sampled for SIPAST, one between the ditch and the central tree of the plot (DITCH), one between the central tree of the plot and the first row of the forage crop (TREE), and one 4 m into the forage crop (SWARD); (ii) 2 soil cores were collected for PAST, one between the ditch and the first row of the forage crop (DITCH), and one 4 m into the crop (SWARD) (Figure 1).



Figure 1. Soil sampling positions within, respectively, the system SIPAST (a) and PAST (b). System SIPAST: DITCH was the sampling point between the ditch and the central tree of the plot; TREE was the sampling point between the central tree of the plot and the first row of the forage crop; SWARD was the sampling point located 4 m into the forage crop. System PAST: DITCH was the sampling point between the ditch and the first row of the forage crop; SWARD was the sampling point located 4 m into the forage crop.

Mowing was performed during the year to simulate animal grazing. Sulla and ryegrass aboveground biomass (AGB) were mowed two times in both years: on 26 April and 3 June 2021, on field 1, and 4 March and 10 May 2022, on field 2. The first harvest in 2021 was delayed in the season due to slow crop growth after waterlogging caused by intense rainfall during winter. At each cutting date, the plant biomass was collected on two sampling areas of 0.25 m² each per two distances from the side border of the plot, i.e., 1-m and 4-m apart (total area 0.5 m² per position per plot), to test whether there were differences in plant growth related to their distance (factor AREA, Ar) from the ditches or from the tree rows, respectively for PAST and SIPAST systems. The crop biomass was separated from the weeds, fresh weighted and then oven-dried at 60 °C until constant weight. Besides the aboveground biomass mowed during the season, also root samples of sulla and ryegrass (considered as the non-N₂-fixing reference crop), were collected at the end of the vegetative season (September) on two sampling areas (pooled) with the same sampling scheme as for AGB (1-m; 4-m). For each distance (1-m and 4-m), undisturbed soil samples were extracted by a spade on two areas (pooled) of 0.018 m² each (0.2 m width × 0.3 m length × 0.3 m depth). The samples were gently washed with water and the taproots belonging to different plants were separated. Trunk height and diameter (at the foot and at 130 cm) measured

at the time of planting and at the end of the vegetative season in November on all poplar trees in each plot, to assess their growth increment (%). Moreover, in the typical pruning time for our pedoclimatic context, leaves and pruning branches were sampled (pooled per each plant), in May (spring), and November (autumn) on the two neighbor trees on each side of the central one in the plot.

To assess the level of N₂-fixation of the legume crop and the N-transfer to poplars, sulla (shoot and root) and poplar (leaves and branches) biomass samples were oven-dried at 60 °C until constant weight and then milled with lab grinders mounted with 1 mm-sieve. Around 5 mg of the resulting material was inserted in tin capsules and then analyzed with an elemental analyser (Flash 2000, Thermo Scientific, Bremen, Germany) coupled in continuous flow mode to a Thermo Delta V Advantage isotope ratio mass spectrometer (Thermo Scientific, Bremen, Germany), to measure N content (N %) and ¹⁵N atom excess compared to the atmosphere (δ¹⁵N ‰), according to the methodology described in Peoples et al. [53].

Meteorological data were collected using a public weather station (43°40'51.60'' N, 10°16'48.00'' E) managed by the Tuscany Regional government (www.sir.toscana.it (accessed on 1 June 2023)). The weather station was equipped with a pluviometer and a thermometer measuring, respectively total rainfall and air temperature every 15 min.

2.4. Calculations

Nitrogen yield (N_{yield}) (g N m⁻²) was calculated by multiplying AGB dry matter (DM) (g m⁻²) per its N% content. The proportion of N derived from the atmosphere (%N_{dfa}) in legume shoots and roots was estimated according to ¹⁵N natural abundance method [53] using the equation:

$$\%N_{dfa} = \left(\frac{\delta^{15}N_{reference} - \delta^{15}N_{legume}}{\delta^{15}N_{reference} - B} \right) \times 100$$

where δ¹⁵N_{reference} is the δ¹⁵N value of the reference plant, B is the δ¹⁵N of sulla deriving N exclusively from N₂-fixation, namely when grown on a N-free medium, and δ¹⁵N_{legume} is the δ¹⁵N value of the legume under study. Reference values were determined on samples of ryegrass, that is a non-nodulating species, grown in neighbor plots. The B value for sulla (−1.3 δ¹⁵N) was retrieved from Gentili et al. [54]. Nitrogen fixed (N_{fixed}) (g N m⁻²), was calculated as N_{yield} × %N_{dfa}/100.

To assess the percentage of N in poplar trees that derived from the transfer of biologically fixed N through the soil (%N_{dft}), the equation proposed by Snoeck et al. [32] was used:

$$\%N_{dft} = \left(\frac{\delta^{15}N_{tree-ref} - \delta^{15}N_{tree-leg}}{\delta^{15}N_{tree-ref} - B} \right) \times 100$$

where δ¹⁵N_{tree-ref} was measured on samples of poplars intercropped with ryegrass, while δ¹⁵N_{tree-leg} was obtained from poplars intercropped with sulla.

2.5. Statistical Analysis

Statistical analyses were performed using R software [55]. The effect of System (S), Crop (C) and Area (Ar) was tested on cumulative AGB and N_{yield} (i.e., the total AGB and N_{yield} produced over multiple mowings) and N concentration of the forage crops. The effect of S, C and Ar was evaluated using the lm() function for fitting linear models of the “stats” package, and then a three ways ANOVA was performed. A two-way ANOVA was carried out also to evaluate the effect of System and Area on sulla shoot and root N-fixing related parameters, i.e., δ¹⁵N, %N_{dfa}, cumulative N_{fixed} (i.e., the total N_{fixed} produced over multiple mowings).

For soil nitrogen parameters, i.e., STN and NO₃⁻, we tested the effect of System, Crop Area and their interactions. For the factor Area (Ar), in case of soil N parameters, DITCH

and SWARD positions were considered to compare the effect of the two cropping systems (PAST and SIPAST). An additional analysis was performed only for SIPAST plots, to assess the effect of Crop and Area including also the third sampling position between the central tree and the beginning of the plot (TREE).

Finally, one-way ANOVA was carried out to evaluate the effect of neighbor Crop on tree growth parameters.

The significance of the effect “year” was tested on all the parameters analyzed. For AGB, Nyield and Nfixed the effect of the year was not significant and thus the results are presented averaged over the two fields, while the results of each year were kept separated for all the remaining parameters. Tukey’s HSD post-hoc test was carried out for pairwise multiple comparisons using the “emmeans” R package [56] with the emmeans() function. Bartlett’s test was used to check the homogeneity of variance and the Shapiro–Wilk test to check the normality of residuals. Detection and removal of outliers were carried out with the Bonferroni outlier test using the “car” package [57] with the outlierTest() function. Data transformation was not necessary.

3. Results

3.1. Weather Data

In field 1 (2020–2021 growing season), the cumulative rainfall registered from the sowing to the end of the growing season (November 2020–June 2021) was 697 mm, a value close to long-term average (626 mm). Compared to the long-term average, far higher rainfall occurred in December 2020 and January 2021 (267 and 234 mm, respectively, vs. 90 and 93 mm of long-term average). In field 2 (2021–2022), the cumulative rainfall measured in the growing season (i.e., from October 2021 to May 2022) was lower compared to the long-term average (563 mm vs. 697, respectively). In particular, rainfall was notably lower in October 2021 (44 mm) than the long-term average (114 mm). The rainfall distribution was similar between the two years of the experimentation in autumn and spring, but was different in winter: (i) in September–November the precipitation observed were 241 and 243 mm in 2020 and 2021, respectively; (ii) in December 2020–February 2021 the total rainfall was 540 mm, while in the following year it was only 125 mm; (iii) in March, April, and May the cumulative rainfall registered were 99 mm in 2021, and 144 mm in 2022. The daily mean temperature along the crop vegetative season was 12.3 °C and 11.5 °C in 2020–2021 and 2021–2022, respectively. The long-term trend showed similar value when compared to the 2020–2021 value, and a greater value compared to the 2021–2022 value (Figure 2).

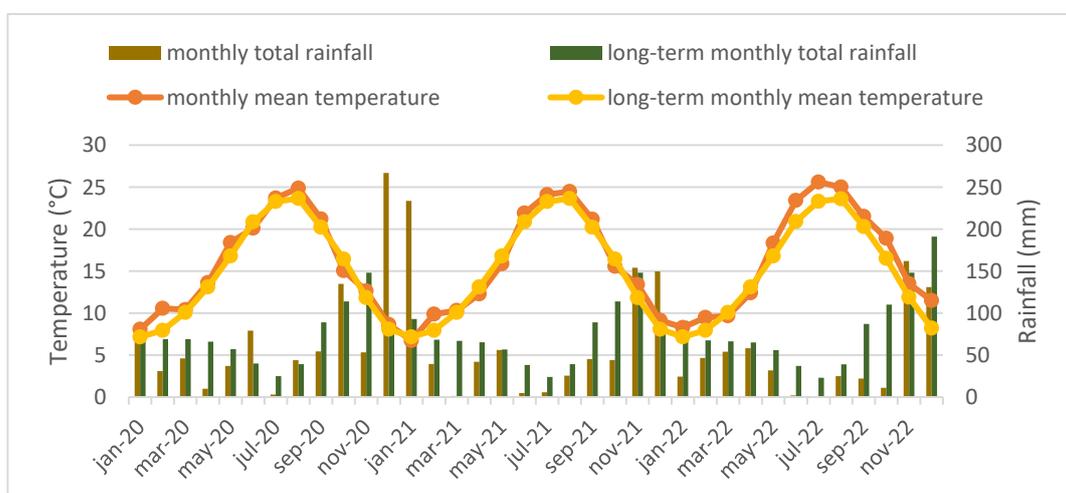


Figure 2. October 2019–May 2022 and long-term (1993–2021) monthly total rainfall (mm) and mean air temperature (°C), San Piero a Grado (Pisa, Italy).

3.2. Cumulative Forage Yield, Nitrogen Yield and Amount of Nitrogen Fixed

Results from the statistical analysis showed that AGB, Nyield and Nfix were not affected by S and Ar. Instead, AGB ($p \leq 0.05$) and Nyield ($p \leq 0.001$) were affected both by C (Table S1). AGB showed a significant interaction S \times C, whereas for Nfix we observed a significant C \times Ar interaction.

Averaged over the two experimental years/fields, AGB and Nyield resulted significantly higher for SUL than RYE (+19% and +65%, respectively for AGB and Nyield) (Table 1). Although not significantly, the average value of the area showed increased AGB value at 4-m position (645.44 vs. 551.71 g DM m⁻² for 4-m and 1-m respectively). Nyield and Nfixed did not show significant differences or any clear tendency regarding the averaged values of System and Area. Figure 3 shows the average AGB value in each System \times Crop treatment, averaged over the two years/fields. In the SIPAST system, sulla resulted significantly more productive than ryegrass (695.09 vs. 461.44 g DM m⁻²), while the two crops were not statistically different from each other within the PAST system, nor with the two crops grown in the SIPAST system. Figure 4 shows the 2-year average value of Nfixed for sulla grown in each area of sampling in each system treatment. Within each cropping system, no differences were found in the amount of fixed N between the two areas, but in SIPAST-1-m Nfixed was higher than PAST-1-m (17.89 vs. 11.70 g N m⁻²).

Table 1. Mean value averaged over the two experimental years (mean \pm standard error) of: (i) sulla and ryegrass aboveground dry biomass (AGB) and N yield (Nyield); and (ii) mass weight of N derived from N₂-fixation (Nfixed) in sulla, as affected by the experimental factors system (S), area of sampling (Ar) and crop (C) and their interaction. Treatments with the same letter are not significantly different at $p \leq 0.05$ (Tukey's HSD test). PAST means pastoral, SIPAST means silvopastoral, SUL means sulla and RYE means ryegrass.

Factors	Levels	AGB (g d.m. m ⁻²)	Nyield (g N m ⁻²)	Nfixed (g N m ⁻²)
System (S)	PAST	588.01 \pm 40.64	12.42 \pm 1.62	14.49 \pm 1.36
	SIPAST	583.35 \pm 43.02	13.92 \pm 1.76	17.16 \pm 1.08
Area (Ar)	1-m	551.71 \pm 42.77	12.73 \pm 1.64	14.80 \pm 1.36
	4-m	618.33 \pm 39.71	13.61 \pm 1.75	16.86 \pm 1.13
Crop (C)	RYE	523.42 \pm 41.83 b	6.84 \pm 1.00 b	
	SUL	645.44 \pm 37.78 a	19.5 \pm 1.14 a	
S \times C	SIPAST SUL	695.09 \pm 35.44 a	20.82 \pm 1.28	
	PAST SUL	595.79 \pm 65.30 ab	18.18 \pm 1.88	
	SIPAST RYE	461.44 \pm 64.47 b	7.03 \pm 1.65	
	PAST RYE	580.24 \pm 51.30 ab	6.65 \pm 1.21	
S \times Ar	SIPAST 1-m	578.48 \pm 66.60	14.40 \pm 2.63	17.89 \pm 1.47 a
	SIPAST 4-m	587.81 \pm 58.25	13.44 \pm 2.46	16.43 \pm 1.68 ab
	PAST 1-m	527.17 \pm 56.59	11.06 \pm 1.96	11.70 \pm 1.49 b
	PAST 4-m	648.86 \pm 55.05	13.78 \pm 2.62	17.28 \pm 1.67 a

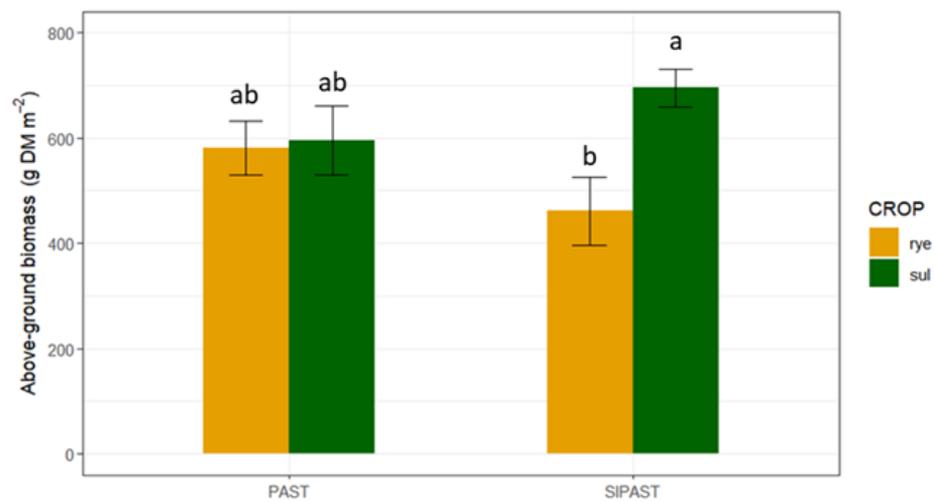


Figure 3. 2-yr average value of aboveground dry biomass (AGB) of the forage crops grown in the two cropping systems in 2020/21 and 2021/22. Vertical bars indicate standard error of the mean. Treatment combinations with the same letter are not significantly different at $p \leq 0.05$ (Tukey's HSD test). PAST means pastoral, SIPAST means silvopastoral, SUL means sulla and RYE means ryegrass.

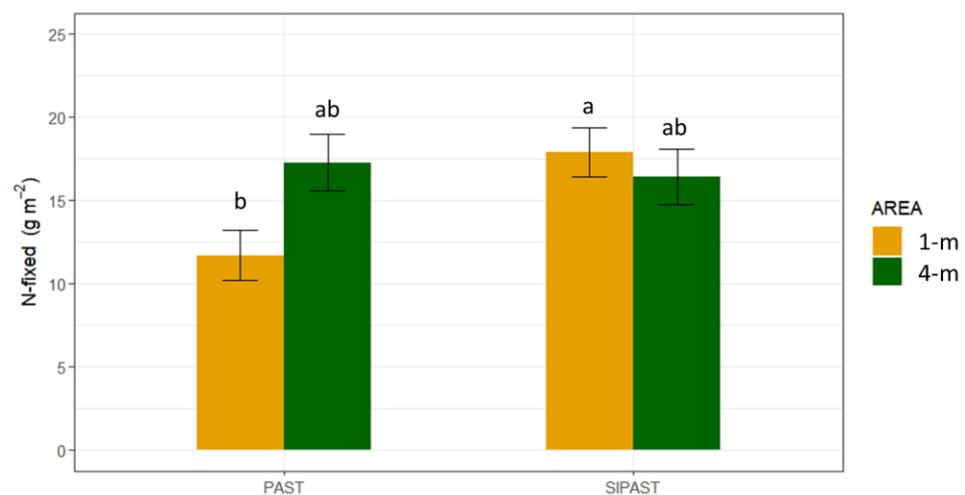


Figure 4. 2-yr average values of the mass amount of N derived from N₂-fixation (N-fixed) in sulla grown within the two cropping systems and sampled at the two distances from the lateral border of the crop. Vertical bars indicate standard error of the mean. Within each mowing, treatments with the same letter are not significantly different at $p \leq 0.05$ (Tukey's HSD test). PAST means pastoral, SIPAST means silvopastoral, 1-m means area 1 m from the first row of the crop, and 4-m means the central area of the crop, i.e., 4 m apart from the first row of the crop.

3.3. Sulla Shoot and Root N₂-Fixation

3.3.1. N%, $\delta^{15}\text{N}$ and %Ndfa in Field 1, Season 2020–2021

For the first year/field, the statistical analysis performed on shoots returned the following results: N% was affected only by C ($p \leq 0.001$), $\delta^{15}\text{N}$ was affected by S ($p \leq 0.05$) and Ar ($p \leq 0.01$), while %Ndfa was affected only by Ar ($p \leq 0.001$). For roots, it turned out that N% was affected by C ($p \leq 0.001$), while $\delta^{15}\text{N}$ and %Ndfa were affected both by S and Ar ($p \leq 0.05$). No significant interaction effect was recorded (Table S2).

Averaged over S and C, N% in shoots and roots resulted significantly higher in SUL (3.18 and 1.09%) than RYE (1.10 and 0.58%) (Table 2). Concerning the effect of the system on sulla shoots, averaged over the areas, $\delta^{15}\text{N}$ of PAST highlighted lower value than SIPAST (0.07 vs. 0.77‰), but %Ndfa did not show significant differences. Likewise, $\delta^{15}\text{N}$ of roots resulted significantly lower in the PAST plots (−96‰), while %Ndfa showed a significant

increase in the PAST system (+34%). On average, %Nd_{fa} in the shoots was higher in the first year (90.29%) than the second (53.34%). Regarding the mean effect of the sampling area on sula N shoots, averaged over the two systems, $\delta^{15}\text{N}$ showed a significantly lower value at 1-m position (-0.05 vs. 0.98‰ for 1-m and 4-m, respectively), which showed for %Nd_{fa} significantly higher value than 4-m (93.50 vs. 87.37%). Similarly, roots showed a significantly lower value for $\delta^{15}\text{N}$ at 1-m than 4-m position (2.32 vs. 5.49‰), and conversely an increased value at 1-m in comparison to 4-m sampling area (+41%).

Table 2. Shoot and root average value (mean \pm standard error) of N concentration (N%), ^{15}N atom excess respect to the atmosphere ($\delta^{15}\text{N}$, ‰) and percentage of N derived from N₂-fixation (%Nd_{fa}, %), as affected by the experimental factors system (S), area of sampling (Ar) and crop © in field 1 (2020/21) and field 2 (2021/22). Treatments with the same letter are not significantly different at $p \leq 0.05$ (Tukey's HSD test). PAST means pastoral, SIPAST means silvopastoral, SUL means sula, RYE means ryegrass, 1-m means area 1 m from the first row of the crop, and 4-m means the central area of the crop, i.e., 4 m apart from the first row of the crop.

Shoots					
Field	Factors	Levels	N% (% w/w)	$\delta^{15}\text{N}$ (‰)	%Nd _{fa} (%)
1	System	PAST	2.19 \pm 0.22	0.07 \pm 0.19 b	91.48 \pm 1.13
		SIPAST	2.08 \pm 0.22	0.77 \pm 0.27 a	89.10 \pm 1.40
	Area	1-m	2.16 \pm 0.21	-0.05 ± 0.16 b	93.05 \pm 0.76 a
		4-m	2.12 \pm 0.23	0.98 \pm 0.25 a	87.37 \pm 1.14 b
	Crop	RYE	1.10 \pm 0.05 b		
		SUL	3.18 \pm 0.06 a		
2	System	PAST	2.34 \pm 0.23	0.78 \pm 0.33	73.01 \pm 3.03
		SIPAST	2.48 \pm 0.24	0.33 \pm 0.20	76.93 \pm 2.40
	Area	1-m	2.48 \pm 0.23	0.84 \pm 0.21	71.75 \pm 2.74
		4-m	2.34 \pm 0.24	0.26 \pm 0.21	78.09 \pm 2.49
	Crop	RYE	1.56 \pm 0.18 b		
		SUL	3.26 \pm 0.13 a		
Roots					
Field	Factors	Levels	N% (% w/w)	$\delta^{15}\text{N}$ (‰)	%Nd _{fa} (%)
1	System	PAST	0.86 \pm 0.10	2.64 \pm 1.10 b	64.38 \pm 10.51 a
		SIPAST	0.81 \pm 0.09	5.17 \pm 1.23 a	42.30 \pm 11.65 b
	Area	1-m	0.84 \pm 0.07	2.32 \pm 1.07 b	67.08 \pm 10.29 a
		4-m	0.89 \pm 0.12	5.49 \pm 1.10 a	39.59 \pm 10.67 b
	Crop	RYE	0.58 \pm 0.05 b		
		SUL	1.09 \pm 0.25 a		
2	System	PAST	2.80 \pm 0.28	1.31 \pm 0.46	65.12 \pm 5.36
		SIPAST	3.21 \pm 0.28	0.83 \pm 0.28	71.25 \pm 3.10
	Area	1-m	3.01 \pm 0.27	1.46 \pm 0.36 a	62.69 \pm 3.78 b
		4-m	3.00 \pm 0.3	0.68 \pm 0.35 b	73.68 \pm 3.98 a
	Crop	RYE	2.23 \pm 0.23 b		
		SUL	3.78 \pm 0.10 a		

3.3.2. N%, $\delta^{15}\text{N}$ and %Nd_{fa} in Field 2, Season 2021–2022

Regarding the field 2, statistical analysis about shoot showed that N% was significantly affected only by C ($p \leq 0.001$), while $\delta^{15}\text{N}$ and %Nd_{fa} were not statistically affected by any factor. Roots showed the following significant effects: N% was affected only by C

($p \leq 0.001$), while $\delta^{15}\text{N}$ and %Ndfa were affected by Ar ($p \leq 0.05$), but no effect was showed by S. Similarly as for year 1, no significant interaction effect was observed (Table S2).

Averaged over the systems and the sampling areas, N% in shoots and roots resulted significantly higher in SUL (3.26 and 3.78%) than RYE (1.56 and 2.23%) (Table 2). About roots, considering the effects of system averaged over the sampling areas, the $\delta^{15}\text{N}$ in the PAST system showed a tendentially greater value than SIPAST (1.31 vs. 0.83‰) and %Ndfa highlighted a non-significant but lower value in the PAST plots than SIPAST (65.12 vs. 71.25%). Regarding the effect of the area, averaged over the systems, $\delta^{15}\text{N}$ showed a significantly lower value in area 4-m compared to 1-m (0.68 vs. 1.46‰). This resulted in a greater %Ndfa value in area 4-m with respect to area 1-m (73.68 vs. 62.69%).

3.4. Poplar Growth and N-Transfer

For field 1, the statistical analysis showed no significant effect of the intercropped forage crop species on the percentage of increment of height, diameter at 130 cm and diameter at foot in poplar plants grown in the SIPAST system. In the field 2, statistical analysis turned out only a significant effect of the crop on diameter at foot increment ($p \leq 0.01$) (Table S3).

In field 1 there was a greater diameter at 130 cm in poplar intercropped with SUL (+18%) compared to RYE plots, but this increment was not statistically relevant. To the contrary, in the field 2 it was highlighted how diameter at foot in RYE plots was significantly higher than SUL plots (12.5 vs. 6.28%), but these observations did not follow any clear tendency (Table 3).

Table 3. Average value (mean \pm standard error) percentage of increment of height, diameter at 130 cm and diameter at foot of poplars grown on the border of sulla (SUL) and ryegrass (RYE) in the silvopastoral system (SIPAST). Treatments with the same letter are not significantly different at $p \leq 0.05$ (Tukey's HSD test).

Field	Crop	Height Increment (%)	Diameter at 130 cm Increment (%)	Diameter at Foot Increment (%)
1	RYE	2.29 \pm 0.57	26.59 \pm 4.75	17.82 \pm 2.52
	SUL	2.41 \pm 2.78	32.35 \pm 3.50	16.54 \pm 2.53
2	RYE	12.22 \pm 1.92	6.79 \pm 1.14	12.50 \pm 1.77 a
	SUL	14.28 \pm 2.75	5.59 \pm 0.98	6.28 \pm 1.64 b

On both fields the $\delta^{15}\text{N}$ values of the poplar associated with RYE and SUL were evaluated to test a potential transfer of the N fixed by the sulla to the young poplar plants (%Ndft). The $\delta^{15}\text{N}$ of poplar in SUL plots was always averagely lower than in RYE plots, except for SUL in AUTUMN in the field 1, where the difference was not appreciable (5.37 vs. 5.08‰ for RYE and SUL respectively) (Table 4). About the %Ndft, it was possible to observe as in the poplar associated with SUL there was a variable percentage of N derived from the N-fixation. In the field 1 the average %Ndft was 19.27% in SPRING and 6.24% in AUTUMN, while in the field 2 we observed increased values, i.e., 55.80% in SPRING and 66.15% in AUTUMN.

Table 4. Average value (mean \pm standard error) of ^{15}N atom excess compared to the atmosphere ($\delta^{15}\text{N}$) and of percentage of N derived from transfer of N fixed by intercropped sulla plants (%Ndft) in pruning branches and leaves of poplar grown in the silvopastoral system (SIPAST) and sampled in May (SPRING) and September (AUTUMN) on the two experimental years/fields (Field 1 is 2020/21, Field 2 is 2021/22). SUL is sulla, RYE is ryegrass.

Experimental Field/Year	Crop	Date	$\delta^{15}\text{N}$	Ndft
Field 1	RYE	SPRING	5.01 \pm 0.24	19.27 \pm 4.50
	SUL	SPRING	3.79 \pm 0.32	
	RYE	AUTUMN	5.37 \pm 1.12	6.24 \pm 2.90
	SUL	AUTUMN	5.08 \pm 1.12	
Field 2	RYE	SPRING	2.70 \pm 0.45	55.80 \pm 13.89
	SUL	SPRING	0.21 \pm 0.51	
	RYE	AUTUMN	1.59 \pm 0.38	66.15 \pm 13.13
	SUL	AUTUMN	−0.53 \pm 0.38	

3.5. Soil Nitrogen Dynamics: STN and NO_3^-

3.5.1. Effect of the Two Sampling Areas DITCH and SWARD

Field 1, Season 2020–2021

For field 1, the statistical analysis, performed for two depths (0–30 and 30–60 cm) and two different dates of sampling (spring and autumn), showed that soil total nitrogen (STN) resulted not being affected by any factor, whatever the depth and the date. Differently, NO_3^- was affected by Ar in spring for both depths, 0–30 and 30–60 cm ($p \leq 0.05$). Moreover, a significant interaction between C and Ar was observed in 0–30 cm layer ($p \leq 0.05$). No significant effects were observed in autumn, instead (Table S4).

Concerning the mean effect of the area of sampling averaged over crop and system in spring, for the 0–30 cm horizon the NO_3^- in DITCH position (i.e., between ditch and crop) showed significant higher value than SWARD (6.33 vs. 4.08 mg $\text{NO}_3^- \text{ kg}^{-1}$), and likewise, at 30–60 cm depth, the DITCH position resulted significantly greater than SWARD (5.23 vs. 3.31 mg $\text{NO}_3^- \text{ kg}^{-1}$) (Table S5). Furthermore, in spring a significant interaction between crop and area of sampling was observed at 0–30 cm of depth, with RYE-DITCH showing higher NO_3^- content in comparison to RYE-SWARD (7.53 vs. 2.75 mg $\text{NO}_3^- \text{ kg}^{-1}$), while the SUL plots did not show differences related to the crop and sampling position (Table S5).

Field 2 Season 2020–2021

For field 2, the statistical analysis, performed for two depths (0–30 and 30–60 cm) and two dates of sampling (spring and autumn), turned out that STN was significantly affected by S, at each date in the 0–30 cm horizon ($p \leq 0.01$), and in autumn also at 30–60 cm depth ($p \leq 0.05$). STN resulted affected also by Ar at each date but only for the horizon 0–30 cm ($p \leq 0.01$ in spring and $p \leq 0.05$ in autumn). Likewise, NO_3^- was affected by Ar in each date and both depths, 0–30 ($p \leq 0.001$) and 30–60 cm ($p \leq 0.01$ in spring and $p \leq 0.05$ in autumn). Moreover, NO_3^- was affected also by crop in the horizon 0–30 cm at each date ($p \leq 0.01$ in spring and $p \leq 0.05$ in autumn), and at 30–60 cm in autumn ($p \leq 0.05$). An interaction C per Ar was highlighted for NO_3^- in spring at 0–30 cm ($p \leq 0.05$) (Table S4).

In field 2, considering the mean effect of cropping system averaged over crop and sampling area, in spring the STN in SIPAST at 0–30 cm depth was significantly higher than in PAST (1.13 vs. 1.03 g N kg^{-1}). Similarly, in autumn SIPAST revealed significantly greater STN content than PAST at 0–30 cm (1.12 vs. 1.02 g N kg^{-1}), but also at 30–60 cm of depth (1.02 vs. 0.92 g N kg^{-1}). About the effect of area of sampling, averaged over the system and crop, for both dates, but only for the 0–30 depth, STN resulted significantly higher in SWARD position than in DITCH (1.13 vs. 1.03, and 1.11 vs. 1.04 g N kg^{-1} for spring and autumn, respectively). Concerning the effect of the area, NO_3^- showed significant differences for both dates and depths of sampling: (i) in spring, it resulted significantly higher in SWARD than in DITCH at 0–30 cm (5.46 vs. 2.16 mg $\text{NO}_3^- \text{ kg}^{-1}$) and 30–60 cm

(5.11 vs. 2.53 mg NO₃⁻ kg⁻¹), and similarly (ii) in autumn SWARD showed increased value in comparison to DITCH at 0–30 cm (9.78 vs. 4.59 mg NO₃⁻ kg⁻¹) and 30–60 cm (3.38 vs. 2.02 mg NO₃⁻ kg⁻¹). Concerning the mean effect of crop value averaged over the system and area, NO₃⁻ showed in spring at 0–30 cm a significantly higher content in SUL plots than in RYE (5.09 vs. 2.53 mg kg⁻¹). Moreover, in autumn the same tendency at both depths was observed (8.60 vs. 5.88 and 3.39 vs. 2.02 mg NO₃⁻ kg⁻¹ for 0–30 and 30–60 cm horizons, respectively). In spring too, the interaction crop per area was studied, and the highest value was registered for SUL-SWARD (7.73 mg NO₃⁻ kg⁻¹) in comparison to all other combinations, that in turn did not differ to each other (Figure 5).

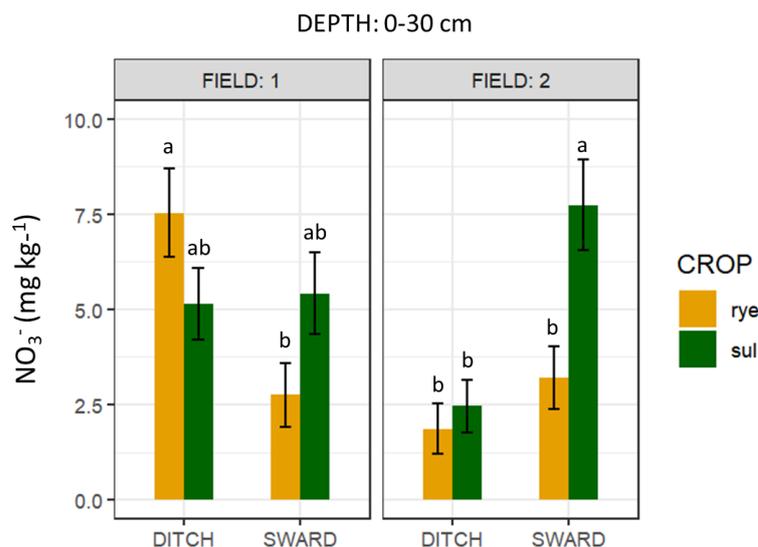


Figure 5. Average values of soil NO₃⁻ concentration at 0–30 cm of depth observed in spring as affected by forage crop species in the two different areas of sampling. Vertical bars indicate standard error of the mean. Within each mowing, treatments with the same letter are not significantly different at $p \leq 0.05$ (Tukey's HSD test). DITCH means sampling area between ditch and the first row of the crop, SWARD means sampling area 4 m into the crop, RYE means ryegrass and SUL means sulla.

3.5.2. Effect of the Three Areas of Sampling: DITCH, TREE and SWARD

Field 1 Season 2020–2021

The statistical analysis, performed for two depths (0–30 and 30–60 cm) and two dates of sampling (spring and autumn) only on SIPAST plot data, showed that in field 1 STN content resulted significantly affected by C at 0–30 cm of depth in each date ($p \leq 0.05$). NO₃⁻ was affected by Ar only in SPRING at depth of 0–30 ($p \leq 0.05$), and moreover, for this date and depth a significant C × Ar interaction was observed ($p \leq 0.05$) (Table S5).

Concerning the mean effect of the forage crop averaged over the areas of sampling, the STN content at 0–30 cm of depth in the RYE plots was significantly higher than SUL plots, both in spring (1.32 vs. 1.24 g N kg⁻¹) and autumn (1.31 vs. 1.25 g N kg⁻¹). About the effect of sampling area, averaged over the crops, NO₃⁻ showed significant differences only in spring at 0–30 cm depth, resulting in significantly higher nitrate content in DITCH position than TREE, i.e., the position between the central tree and the beginning of the crop (5.95 vs. 3.01 mg NO₃⁻ kg⁻¹), while the SWARD did not show any difference with respect to the other positions (4.33 mg NO₃⁻ kg⁻¹). In spring, the interaction C × Ar studied at 0–30 cm depth, revealed a higher value of nitrate content in SUL-SWARD and RYE-DITCH (6.22 and 6.58 mg NO₃⁻ kg⁻¹) in comparison to RYE-TREE, RYE-SWARD, and SUL-TREE (3.33, 2.44, and 2.68 mg NO₃⁻ kg⁻¹, respectively). Furthermore, nitrate content in SUL-DITCH (5.53 mg NO₃⁻ kg⁻¹) did not differ from SUL-SWARD, RYE-DITCH DITCH and RYE-TREE, but in turn was significantly higher than SUL-TREE and RYE-SWARD (Figure 5).

Field 2 Season 2021–2022

The statistical analysis, performed for two depths (0–30 and 30–60 cm) and two dates of sampling (spring and autumn), showed that STN resulted not being affected by any factor, while NO_3^- showed a significant mean effect of Ar at both depths in spring ($p \leq 0.01$) and autumn ($p \leq 0.05$). Besides this evidence, for NO_3^- the significant effect of C was highlighted, but only in spring, respectively at 0–30 ($p \leq 0.01$) and 30–60 cm depth ($p \leq 0.05$). Moreover, an interaction $C \times \text{Ar}$ was observed for NO_3^- in spring at 30–60 cm ($p \leq 0.05$) (Table S5).

Considering the average value of area of sampling averaged over the crop, in spring it was possible to observe that SWARD had a significantly higher nitrate content than DITCH position at 0–30 cm (5.80 vs. 1.79 $\text{mg NO}_3^- \text{ kg}^{-1}$), and moreover, SWARD highlighted a significant increase in nitrate in comparison to TREE and DITCH at 30–60 cm depth (6.25 vs. 3.12 and 2.12 mg kg^{-1}). Likewise, in autumn it was noticed at 0–30 cm depth that SWARD had significantly higher nitrate concentration than TREE and DITCH (10.68 vs. 4.46 and 5.13 $\text{mg NO}_3^- \text{ kg}^{-1}$), while at 30–60 cm, SWARD showed significantly higher values in comparison to TREE (3.58 vs. 1.39 $\text{mg NO}_3^- \text{ kg}^{-1}$). Conversely, DITCH (2.36 $\text{mg NO}_3^- \text{ kg}^{-1}$) was not different from the other two positions. Concerning the mean effect of the crop, averaged over the area of sampling, SUL plots resulted significantly higher than RYE plots only in spring, both at 0–30 (5.24 vs. 2.40 $\text{mg NO}_3^- \text{ kg}^{-1}$) and 30–60 cm depth (4.75 vs. 2.90 $\text{mg NO}_3^- \text{ kg}^{-1}$). In spring, it was also observed a significant $C \times \text{Ar}$ interaction, and a significant increase in nitrate content for SUL-SWARD (8.66 $\text{mg NO}_3^- \text{ kg}^{-1}$) in comparison to all other SUL combinations (averagely, 2.80 mg kg^{-1}), and RYE-TREE and RYE-DITCH (3.04 and 1.83 mg kg^{-1}). RYE-SWARD (3.83 mg kg^{-1}) did not show differences for all the interaction effects (Figure 6).

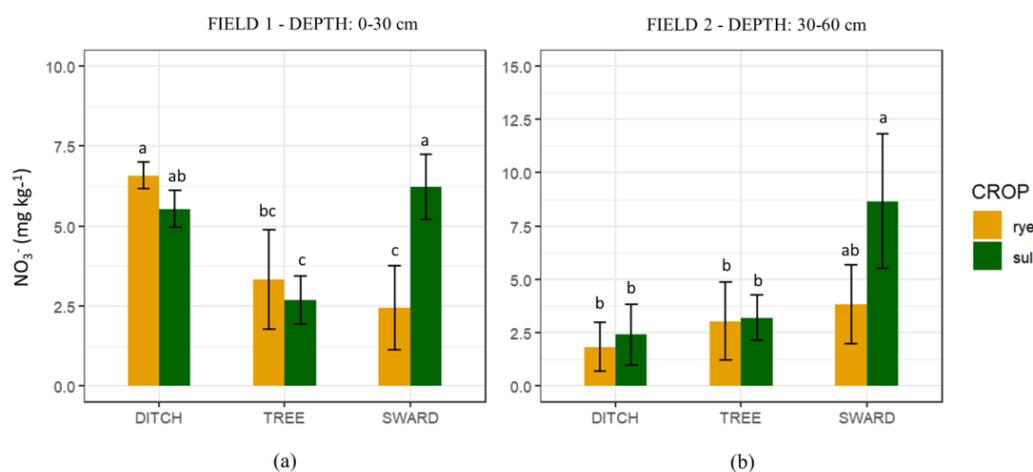


Figure 6. Average values of soil NO_3^- concentration as affected by the forage crop species for the silvopastoral system (SIPAST) in different areas of sampling in spring, respectively in field 1 (a) at 0–30 cm depth and in field 2 (b) at 30–60 cm depth. Vertical bars indicate standard error of the mean. Treatments with the same letter are not significantly different at $p \leq 0.05$ (Tukey's HSD test). DITCH means the sampling area between ditch and the first row of the crop, TREE means the sampling area between the trunk of the central tree within the row and the first row of the crop, SWARD means the central area of the crop, i.e., 4 m apart from the first row of the crop, RYE means ryegrass and SUL means sulla.

4. Discussion

To better understand the suitability of agroforestry systems in the Mediterranean area, it is of paramount importance to study this kind of associations in the early phases after the trees introduction. This is necessary to support efficient design of AFS and to guide farmers throughout the transition. In our trial, we introduced poplar trees in intercropping with different forage species with the purpose of investigating competition and facilitation

for N due to the intercropping during the first year of tree plantation, considering also the eventual reduction of potential nitrate leaching risk.

The trial consisted of two field plot experiments, that implied the same randomized block design, which was replicated in 2020 (Field 1) and 2021 (Field 2) on two similar fields. The herbage mass was collected during the first vegetative season in each field. In both years the meteorological conditions were adequate to allow the herbage to grow normally during the vegetative season also under rainfed conditions. However, differences in weather conditions caused some change in terms of crop management and herbage mowing times. In field 1, sowing was performed late, at the end of November, due to high soil moisture, and then after the sowing high precipitation were registered above the long-term average (540 mm among December and February). These different conditions determined different harvest stages, but despite this, two aboveground biomass harvests were performed per each field and, averagely, the herbage biomass accumulation resulted not being affected by the year.

4.1. Impact of Poplars on Forage Yield

Regarding the total AGB accumulation, our results showed that averagely sulla was about +20% more productive than ryegrass. In literature variable results depending more on the species than the family of the forage crop are documented. Overall, in Mediterranean area, forage grasses show greater yield than legumes. For example, contrary to our findings, Atis and Acikalin [58], studying in Eastern Turkey cool season forages, i.e., grass pea (*Lathyrus sativus* L.) and forage wheat (*Triticum aestivum* L.), found that wheat was more productive than grass pea (7.85 vs. 3.76 t d.m. ha⁻¹). A previous study from Hassan et al. [59], carried out in Egypt under arid conditions, showed summer grasses, such as sudangrass (*Sorghum × drummondii* Steud.) and pearl millet (*Pennisetum glaucum* L.), highlighting higher total biomass yield in comparison to cowpea (*Vigna unguiculata* L.) (20.6 and 23.5 vs. 8.3 t ha⁻¹). Saia et al. [60], in South of Italy, comparing Mediterranean forage legumes with annual ryegrass found that snail medick (*Medicago scutellata* L.) was the most productive species, with a biomass yield significantly higher than that of the N-fertilized ryegrass, whereas hairy vetch (*Vicia villosa* Roth.) had the lowest biomass yield, which was not different from that of the unfertilized ryegrass. Anyway, very little is known about the adaptation of these forage crops to agroforestry. Indeed, our findings also showed an interaction between system and crop, highlighting as sulla yielded 35% more than ryegrass in the system with trees (SIPAST), whereas for the system without (PAST) no differences between the crops were observed. This reduction in ryegrass yield in SIPAST was probably due to the competition for resources with trees, above all water and soil nutrients. Actually, we assume that during the first season of vegetation, roots of both herbaceous and tree intercrops explore the same shallow soil profile. Anyway, the forage yield of ryegrass in SIPAST did not result significantly lower than the one of ryegrass in PAST, suggesting ryegrass could be a suitable species for introduction in young AFS. Sulla may be even less affected by competition thanks to biological N₂-fixation and the subsidiary production of N. Our results about ryegrass were confirmed by findings of Pardon et al. [61], achieved in a temperate agroforestry with walnut trees (*Juglans regia* L.) in South of Canada. In this study, dealing with the effect of different age of trees on various crops, the authors found that the forage maize (*Zea mays* L.) production was no different in young tree plots with respect to the outer maize rows. Considering legume crops, the results of Querné et al. [15] on alfalfa (*Medicago sativa* L.) grown in mature alley cropping system with hybrid walnut (*Juglans nigra × regia* cv NG23) in southern France, showed that the yield was negatively correlated especially with increasing shade levels, ranging between 0.42 and 0.68 kg DM m⁻² from the tree row to the middle of the alley. Similar results about alfalfa were found by Mantino et al. [27] in an olive tree (*Olea europea* L.) AFS in central Italy and by McGraw et al. [62] in a black walnut (*Juglans nigra* L.) alley cropping in North America, demonstrating that the most limiting factor for legume yield was light availability. Indeed, in absence of other factors of competition, several studies conducted

also in artificial shading conditions applied to pot and plot trials, demonstrated that an increasing level of shade might cause legume yield depletion [21–25]. Hence, based on these observations, sulla could be considered a suitable forage crop in a system in transition to AFS, but presumably only when the light does not represent a limiting factor for the legume growth.

4.2. Tree-Crop N Dynamics

Concerning the percentage of N content in the shoot and root biomass, as expected, sulla showed averagely +52% content in comparison to ryegrass due to the BNF, thus cascading on a very different N yield (19.5 vs. 6.84 g N m⁻²) of the forage crop. Additionally, no effect of the system nor of the area of sampling was highlighted. Other authors like Huss-Danell et al. [63] and Carranca et al. [41] confirmed this higher N content in legume biomass compared to the grass species.

Concerning the source of N identified in the tissues of the legume crop, in our work, the range of the values of %Ndfa of sulla were comparable with values reported in literature for subtropical [64] and Mediterranean [65], rainfed conditions, but were different between the two years of the study. In the first year, the percentage of N₂-fixation was indeed higher, on average, compared to the second one (~90% vs. ~70%). We infer that this difference was likely due to the different rainfall distribution occurred among the two years. The elevated precipitation observed in the first winter season might have caused a sensible N leaching, that probably stimulated, in turn, a greater N₂-fixing activity in sulla. It is well known that low level of mineral N in the soil is one of the major factors behind the activation of the genes regulating N₂-fixation in legumes [19,20].

Our results about $\delta^{15}\text{N}$ in the shoots of sulla showed only in the field 1 a significant effect of the presence of intercropped trees. A lower value (−90%) of $\delta^{15}\text{N}$ was found in PAST compared to SIPAST, but the %Ndfa was not similarly affected anyway (averagely 90% for both systems). In turn, root $\delta^{15}\text{N}$ and %Ndfa showed contrasting results in the two field experiments: (i) in the field 1 a similar trend with respect to the shoot biomass was observed, as, indeed, $\delta^{15}\text{N}$ was affected by cropping system and a reduction of $\delta^{15}\text{N}$ (−57%) and a +35% increment for %Ndfa turned out in PAST; (ii) conversely, in the field 2 a reverse trend was highlighted, i.e., the cropping system affected $\delta^{15}\text{N}$, that was −37% lower in SIPAST than PAST, whereas in turn %Ndfa was +10% in SIPAST, as far as these differences were not so relevant in quantitative terms. Considering the amount of N-fixed accumulated (a parameter more related to the AGB production), averaged over the two fields, we found a significant interaction S × Ar that showed for SIPAST-1-m an amount of N-fixed in sulla 35% higher than PAST-1-m, while no difference was observed with respect to SIPAST-4-m and PAST-4-m. Hence, it can be inferred that near the trees, sulla invest more energy in N₂-fixation, due to the competition with the tree roots for soil N, that became less available for sulla roots. The increased BNF and N availability allowed also a greater AGB production in this phase, where sulla likely did not suffer from the low level of tree shade.

To our knowledge, this work is the first one investigating the relationship between non N₂-fixing trees and legume crops during the first year to transition towards AFS. However, several studies have reported results from more mature systems showing contrasting results. The work of Goh et al. [66] was the only one studying that issue in a young AFS, where the 3-year-old *Pinus radiata* trees were intercropped with several grass and legume forage crops. The authors found that the position of pastures from trees did not significantly affect %Ndfa and the amount of Nfixed, suggesting that trees had little effect on N₂-fixing ability of legumes. Querné et al. [15] in Southern France, studying the effect of hybrid walnut trees on N₂-fixation of intercropped alfalfa, found that walnut trees had a positive effect on the part %Ndfa since alfalfa shoot $\delta^{15}\text{N}$ decreased with the distance from the tree row, suggesting that some belowground interspecific interactions may negatively affect BNF activity in case of close proximity to the trees. In fact, atmospheric N fixation by legumes is known to be strongly limited by high soil N availability [18]. Isaac et al. [20], in a 25-year old AFS with several species, intercropped with soybean (*Glycine max* (L.) Merr.), found

that even if nodule biomass was lower at the tree-crop interface, an increment in %Ndfa for soybean intercropped with trees occurred with respect to the sole crop. They hypothesized that competition for soil nutrient resources could stimulate a major rate of N₂-fixation close to the trees, while when competitive effects are reduced, namely at greater distances from the tree row, they observed a decreasing %Ndfa, indicating a possible preference of the legume for soil N sources. Still Isaac et al. [20] found that Nfixed in soybean associated with trees was comparable at both the 1- and 4-m tree-crop distances, although %Ndfa consistently declined.

Considering the support of legume crops to the tree growth, our results showed that during the first season no difference in the increment of height and diameter of the poplars was found between ryegrass and sulla plots. Our hypothesis that legumes can advantage poplar growth in the shortly early phases after the plantation was not confirmed by literature, anyway. Indeed, Thomas et al. [35] studying the growth dynamics of fast-growing tree species intercropped with alfalfa, found that shortly after planting, due to belowground competition for nutrient resources the intercropped poplars highlighted a delayed growth rate compared to the poplars in pure stand. Similar results as decrease or no effect of the legume crops were reported by Gakis et al. [67], Powell and Bork [68] and Rivest et al. [31]. On the other hand, as reported by other authors [32,33], we achieved to observe in the tree biomass, especially in field 2, a relevant percentage of N derived from the N₂-fixation (about 60%). The amount of %Ndft found in our work did not correlate with the tree growth, and it is likely to be linked to the greater proportion of Nfixed in tree-crop soil interface. We argue that, being the dominant form around their roots, poplars simply absorbed mostly the mineral N originating from that fixed by the legume with respect to the N already present in the soil. This is because legumes release nitrogen rhizodeposits with a low C/N ratio, that is mainly immobilized as microbial residues and forms then a labile pool of organic matter, which provides the main pool for remineralization of N [69]. However, this study is still preliminary and further investigations are needed to assess the validity of this hypothesis, also considering other tree and legume crop combinations.

4.3. Nitrogen Soil Dynamics

In this work, we also investigated the possibility that trees, already during the first season after planting, might show the capability to reduce the amount of soil mineral N between the ditch and the crop, allowing a major nitrate catching effect. We expected this phenomenon was likely to occur especially when poplars were intercropped with sulla, and that a N decreasing trend from crop towards the ditch occurred. In field 1, STN did not show any difference, while in the field 2 a +10% of total N content in SIPAST at 0–30 cm depth in spring and the same increment for both depths in autumn were observed. This result was partially confirmed by Carranca et al. [41], who found in a mature AFS as the STN was depleted apart from the trees compared to the area beneath. This is a valuable result but with limited impact on interpretability since STN is mostly formed by organic N and thus is slowly reactive to agronomic practices. In this sense, studying the level of nitrates in the soil could be more informative on sudden changes due to crop conditions.

In our study, limited only to field 2, NO₃⁻ content resulted to be significantly affected by the crop; indeed, the nitrate content in sulla plots was averagely +40% with respect to ryegrass in spring at 0–30 cm depth and in autumn at both depths. The interaction crop × area affected NO₃⁻ content at 0–30 cm depth only in spring, but in both fields: (i) in field 1 an increasing value for RYE-DITCH (+63%) was observed with respect to RYE-SWARD, but for both treatment combinations no difference was observed respect to SUL-DITCH and SUL-SWARD, with these latter not differing from each other and accounting on average for 5 mg NO₃⁻ kg⁻¹; (ii) in field 2, SUL-SWARD showed the highest value of NO₃⁻ (+70%) compared to all other crop × area levels. In fact, as explained by Hernández-Esteban [16] in a study based on legume-rich pasture in a typical Spanish dehesa, legumes increase N acquisition through the N₂-fixation, accumulating a massive of N and increasing soil N availability, as well as, grasses absorb preferably soil mineral N,

especially nitrate [70]. Instead, the higher amount of nitrates that we observed at DITCH position (for both SUL and RYE crop treatments) in the field 1 could be explained by the weather conditions during the season 2020–2021. In fact, as mentioned above, in the first year the sowing was performed late due to high soil moisture, and then after the sowing high precipitation were registered during the winter as well. The high rainfall has determined a waterlogging situation during the crop emergence and a slow growth during the winter season. Huge rainfall combined with low crop uptake might have likely caused relevant nitrate leaching in early spring. Similarly, poplars, that were planted in February in a flooded soil, probably had started slowly the roots growth and thus, at that time, they were unable to catch nitrates leached in the shallower layer, where most of their young roots were concentrated. Our hypothesis is supported by the lack of significant differences between RYE and SUL plots, revealing in the first year generally high nitrate concentration irrespective of the crop species (if N₂-fixing or not).

To evaluate if the trees can buffer N content between ditches and crops already during the first season of growing, we investigated, limitedly to SIPAST plots, 3 areas of sampling. Expectedly, STN did not highlight interesting trends, and this fact was in line with finding of Andrianarisoa et al. [71], who observed that STN in AFS did not change in the soil even after 14 years after hybrid walnuts planting. NO₃[−] resulted affected instead by the area of sampling: (i) in field 1, although only at 0–30 cm depth in spring, DITCH showed a +50% of content compared to TREE, while the SWARD did not result different from the others; (ii) in field 2 instead, it was possible to observe a decreasing nitrate content from the SWARD to the DITCH position in spring at both depths (on average 6.0, 3.5 and 2.0 mg NO₃[−] kg^{−1} for SWARD, TREE and DITCH respectively). Similarly, in autumn, SWARD resulted in 50% greater nitrate concentration than TREE and DITCH at both depths. In fact, as reported by several authors, trees act as nutrient lifts since deep tree roots can uptake N deposited or leached in deeper layers (namely, below the herbaceous crop rooting zone), resulting in reducing N leaching losses [13,71], and limiting the potential risk of pollution of water bodies.

In our work, the interaction crop × area showed the following: (i) in field 1, nitrate content was affected by the interaction only in spring at 0–30 cm, where average soil concentrations in SUL-SWARD and RYE-DITCH were 56% greater than SUL-TREE, RYE-TREE and RYE-SWARD, and moreover, SUL-TREE was 50% lower than SUL DITCH, confirming the scavenging role of the trees especially beside SUL plots; (ii) in field 2, NO₃[−] soil concentration was affected by crop × area at 30–60 cm depth, where SUL-SWARD resulted in the highest content (averagely +67%) compared to the other levels of crop × area (not significantly only with respect to RYE-SWARD). This latter result seems to suggest that in the deepest horizons the roots of poplars could be a barrier to reduce the N loss already in the first year after tree planting. Similar results were found by Andrianarisoa et al. [71] in a 14-year old hybrid walnut tree AGF study, showing as the soil mineral nitrogen (mainly in NO₃[−] form) progressively decreased from the middle of the alley to the tree trunk especially at 0–1 m depth. According to Mulia and Dupraz [72], the relocation of the tree rooting zone in deeper layers was induced partially by competition with the crop roots. Also, Gómez-Rey et al. [73], studying N accumulation and availability in pastures established with high proportion of legumes (improved pasture) in an oak system in Southern Portugal, they found that oak trees could prevent the N-leaching of four times compared to the open field, in case of higher inorganic-N production.

Overall, our results highlight the agri-environmental advantages of the AFS adoption, already during the first year after the tree plantation. Trees may play a pivotal role in reducing N losses within the agroecosystem and improving the N cycling earlier in the transitional phase. Despite this, as reported by Paris et al. [12], farmers in Italy are not so confident addressing the AF transition. On one hand, this is because so far AGF has been considered too challenging in terms of efforts needed (e.g., multidisciplinary knowledge and expertise, high labor demand, high investments), and on the other hand the incomes can be actualized only over long time, thus amplifying the distance between costs and

benefits in the short term. Although the well known tree potential in supplying ecosystem services, as reported by Kim and Isaac [13], to date policy instruments to support farmers towards the transition to AFS had been lacking. However, according with the second pillar of the new Common Agricultural Policy (CAP) 2023–2027 (https://agriculture.ec.europa.eu/common-agricultural-policy/cap-overview/cap-2023-27_it (accessed on 15 June 2023)) and as reported on the website of AIAF (Italian Association of Agroforestry) (<http://www.agroforestry.it/focus-psn-2023/> (accessed on 15 June 2023)), the national strategic plan set for Italy has introduced some actions to support the adoption of the AFS, specifically: (i) a max contribution of 5000 or 4000 € ha⁻¹ for silvoarable and silvopastoral systems, respectively, to compensate for tree plantation costs; (ii) a second action will be implemented to cover the costs for maintaining the AGF from the second to the fifth year after tree plantation, with a contribution between 800 and 1800 € ha⁻¹ per year. The introduction of this kind of measures could be considered a first step to increase farmers' interest regarding this kind of agricultural system and to facilitate its adoption across Europe.

5. Conclusions

The aim of this study was to measure the agronomic performance of ryegrass and sulla when introduced in a very young age poplars AFS, as well as to unravel the N-fixing capability of sulla. Moreover, the eventual N-transfer by sulla to poplars and the effect on poplar growth were evaluated. Finally, we tried to assess if the poplars in a system in transition to AF could reduce the nitrate leaching, just during the first season after the plantation, in a Mediterranean area and in rainfed conditions. Averaged over other factors, herbage productivity was higher for sulla and in the silvopastoral system, while ryegrass did not result being less productive in plots without trees. The N₂-fixation analysis showed contrasting results due probably to the winter rainfall differences among the two years of the trial, but the amount of N₂-fixed that is linked with biomass production showed greater values near the tree-crop interface. Furthermore, it was possible to observe a N-transfer from the inner crop towards poplar rows. Poplars did not show differences in growth between ryegrass and sulla association, anyway. Despite the very young age of poplars, a decreasing nitrate content from the center of the plots to the ditch, with a lower peak especially in the tree-crop sampling area, was detected. These outcomes showed that sulla in a young phase of trees could be a suitable forage for Mediterranean AFS especially because it can't be affected by shade in those conditions, and that poplars are able already during the first year after planting to be a buffer for nitrate leaching. However, this study was a very preliminary assessment of the dynamics relative to the transition towards AFS and further investigations are needed to: (i) investigate other Mediterranean grass and legume forage crops to confirm our general hypotheses, (ii) studying the complete biennial (or more) cycle of forage crops in young AFS; (iii) investigate more in depth the soil microflora dynamics and root nodulation; iv) assess also the nutrient flux in the soil circulant water solutions and their availability for the crops.

Supplementary Materials: The following supporting information can be downloaded at: <https://www.mdpi.com/article/10.3390/agronomy13071761/s1>, Table S1: *p* values of the factor effects of averaged values of the two fields on total ABG, N₂yield, measured on sulla and ryegrass, and on N₂fixed measured on sulla.; Table S2: *p* values of the factor effects, on the N%, δ 15N and N₂dfa%, measured on shoots and roots of sulla in the Field 1 and Field 2; Table S3: *p* values of the effect of Crop on the increment of poplar trunk height (HINC), diameter at 130 cm (DCINC) and diameter at the foot (DFINC) in the SIPAST system in Field 1 and Field 2; Table S4: *p* values of the factor effects, on the STN and soil NO₃⁻ measured on soil sampled at 0–30 and 30–60 cm of depth in spring and autumn in the Field 1 and Field 2; Table S5: average value of STN and soil NO₃⁻ measured on soil sampled at 0–30 and 30–60 cm of depth in spring and autumn in Field 1 and Field 2; Table S6: *p* values of the factor effects on the STN and soil NO₃⁻ measured on soil sampled at 0–30 and 30–60 cm of depth in spring and autumn in the Field 1 and Field 2 only for the system SIPAST; Table S7: average

value of STN and soil NO_3^- measured on soil sampled at 0–30 and 30–60 cm of depth in spring and autumn in the Field 1 and Field 2 only in the system SIPAST.

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