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Mid-Term Effects of Forest Thinning on N Mineralization in a Semi-Arid Aleppo Pine Forest

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Abstract: In order to assess the sustainability of silvicultural treatments in semiarid forests, it is necessary to know how they affect the nutrient dynamics in the forest. The objective of this paper is to study the effects of silvicultural treatments on the net N mineralization and the available mineral N content in the soil after 13 years following forest clearings. The treatments were carried out following a randomized block design, with four treatments and two blocks. The distance between the two blocks was less than 3 km; they were located in Chelva (CH) and Tuéjar (TU) in Valencia, Spain. Within each block, four experimental clearing treatments were carried out in 1998: T0 control; and T60, T75 and T100 where 60%, 75% and 100 of basal area was eliminated, respectively. Nitrogen dynamics were measured using the resin tube technique, with disturbed samples due to the high stoniness of the plots. Thirteen years after the experimental clearings, T100, T75 and T60 treatments showed a twofold increase in the net mineralization and nitrification rates with respect to T0 in both blocks (TU and CH). Within the plots, the highest mineralization was found in sites with no plant cover followed by those covered by undergrowth. These results can be explained in terms of the different litterfall qualities, which in turn are the result of the proportion of material originating from *Pinus halepensis* Mill. vs. more decomposable undergrowth residues.

Keywords: Mediterranean forest; forest management; *P. halepensis*; nitrogen cycling; soil nitrogen

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1. Introduction

The Aleppo pine (*Pinus halepensis* Mill.) is one of the most important conifers in the Mediterranean basin because of its ability to adapt to a wide range of environmental conditions, covering an area of 2,500,000 ha [1,2]. In Spain, this species is widespread due to its use in reforestations in order to protect the soil in semi-arid areas [3,4]. In the case of the Valencian Community, *P. halepensis* forests account for 72% of the wooded area [5]. Its status as a pioneer species has also contributed to its expansion by colonizing burned areas and abandoned agricultural fields [6]. In Europe, large parts of the Mediterranean forest stands are facing to lack of management due to the low profitability of forest products. The lack of economic value attributed to some forest functions, such as the protection they provide against erosion and floods, or their role as a C fixing agent in the face of climate change [7,8] is another reason for their low management.

In order to assess the sustainability of silvicultural treatments, it is necessary to know how they affect the nutrient dynamics in forests. Silvicultural intervention causes changes in biogeochemical cycles, changes that can lead to the long-term loss of forest fertility and productivity [9–11]. The extraction of wood causes a reduction in the nutrient reserves of the ecosystem, and the modification of the canopy directly affects the nutrient inputs to the soil by modifying the quantity and quality of litterfall [12]. Thinning alters the early decomposition rate and nutrient immobilization release pattern of foliar litter in Mediterranean stands [13–15]. The reduced soil C/N ratio induced by thinning also accelerates the decomposition of soil organic matter [16]. Changes in the microclimate may also occur, which influence the rates of litter decomposition [17] and of the N mineralization in the soil [18]. In addition, root absorption is reduced and this can favour the loss of nutrients from the ecosystem by leaching, particularly nitrate [19]. Assessing the impact of silvicultural treatments on the biogeochemical cycles in forest ecosystems is complex, due not only to the diversity of pools and the flows involved, but also to the different time scales in which they operate. Therefore, holistic approach is necessary, which includes short-term studies of the impact of treatments combined with the long-term analysis on nutrient reserves [9,11]. Mediterranean forests are particularly sensitive due to the low nutrient content of their soils [20]. In low-productivity ecosystems with a low nutrient availability, the primary production is controlled by nutrients and especially, N, availability [21].

Among the effects of silvicultural treatments on nutrient cycling, it is essential to assess the changes in soil N mineralization considering that this element is often the most limiting for forest growth [22]. Several authors have provided examples of studies on the effects of forest management on soil N mineralization [23–27]. Fewer studies exist for Mediterranean forests [18,28]. In addition, the elimination of vegetation might lead to unwanted nitrate leaching, which affects water quality [19,29–31]. After clearcutting, N mineralization rates are expected to increase [18,23,32–34]. This effect could be due to: (i) increases in soil temperature and humidity; (ii) the increased availability of labile organic matter from debris and dead roots; (iii) and the reduced overall immobilization rates due to lower amounts of litterfall and root exudates [12,18,23,32]. It is generally assumed that increased mineralization rates are transient, mostly disappearing after 2–5 years after the treatment [12,35]. This response seems to last longer in conifers in comparison to broadleaved forests [27], but most studies report results up to 10 years after clearing [36]. Nevertheless, Kranabetter and Coates [37] found no important differences after 10 years while Frazer et al. [18] reported higher mineralization rates after 17 years. Prescot [23] suggested that the remaining basal area remaining is not an accurate predictor of mineralization rates after a clearing. In fact, in some cases the observed decreases in the net N mineralization were related to increases in microbial immobilization [36], to soil acidification [26], or to changes in the forest undergrowth composition and decomposer communities [38]. One interpretation is that there might be sensitive and non-sensitive ecosystems in relation to the possible changes in N mineralization induced by forest clearings [39]. The intensity of clearing seems to be of little relevance to the changes in N mineralization, while clearcutting induces different changes in N mineralization [40–43].

This study is centred in a dense Aleppo pine forest that is over 55 years old, whose origin is the regeneration of abandoned agricultural fields. Different silvicultural treatments were designed and executed in experimental plots in 1998. The silvicultural treatments tested aimed to transform the monospecific homogeneous mass of pine forests into a mixed mass of *P. halepensis* and *Q. ilex* L. subsp. *ballota* (Desf.) Samp. [44]. Our hypothesis is that the stand density control could be an adequate management practice to improve the nitrogen availability in unmanaged pine forests with a high stand density. The objective of this paper is to study the effects of silvicultural treatments on the net N mineralization and the available mineral N content in the soil 13 years following the clearings. To analyze the importance of spatial variability [36,45], special attention is also given to the effects of litter layer depth and undergrowth cover within the experimental plots where the measurements were carried out.

2. Materials and Methods

2.1. Study Area and Silvicultural Treatments

The experimental site is located in the southwestern foothills of the Iberian mountain range, Valencia Province, Spain, close to Alto de la Montalbana (39°49′26.00″ N; 1°05′47.01″ W; 960 masl). Jurassic materials with some sediments from the Triassic and Cretacic reliefs [46] dominate the local geology. Soils are rendzinic leptosols developed over calcareous rocks and with intrusions of albic luvisols and calcareous regosols [47]. The climate is typically Mediterranean with very hot and dry summers and a dampened monoxeric-meso-Mediterranean climate, according to FAO-UNESCO classification. Mean annual temperature is 12.5 °C and mean annual precipitation is 457 mm (1960–1990). The vegetation is a dense Aleppo pine (*Pinus halepensis* Mill.) plantation over 55 years old, mostly planted on abandoned agricultural fields. Undergrowth is dominated by *Juniperus phoenicea* L., *Juniperus oxycedrus* L., and *Quercus coccifera* L., indicator species belonging to the plant community Rhamno lycioidis-Quercetum cocciferae [48]. In the spring of 1998, an experimental study began to compare different silvicultural systems in this *P. halepensis* stand. The main goal of these silvicultural systems was to convert the stand into a mixed forest of *P. halepensis* and *Q. ilex* L.to increase its biological diversity and resilience. Treatments were carried out following a randomized block design, with four treatments and three blocks (Figure 1). The distance among the three blocks was less than 3 km. In each block, four experimental square plots (30 m × 30 m with a 7.5 width along each edge as a buffer zone) were selected, one per treatment. Treatments were: (i) T0—untreated control reference; (ii) T60—moderate shelterwood with 60% of mean basal area removed; (iii) T75—strong shelterwood with 75% of mean basal area removed; (iv) T100—clearcutting (100% of mean basal area removed). For this study, two blocks were selected. They were located in Tuéjar and Chelva, hereafter TU and CH, corresponding to block II "Tuéjar right" and block III "Chelva" in [13]. A third block was discarded in this work due to its high stoniness. Both blocks had a similar slope (<5%), canopy and climatic characteristics, similar pH (8–8.5), but contrasting soil texture, whereas soil from TU had clay loam texture and soils from CH had sandy loam texture.

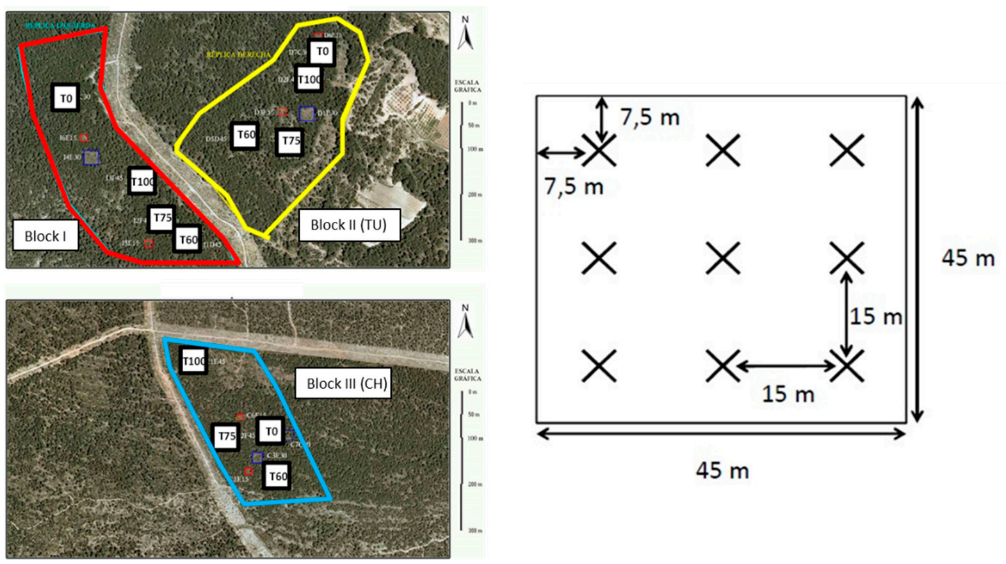

Figure 1. Spatial distribution of silvicultural treatments in each block and distribution of points selected for nitrogen mineralization in each plot with the perimeter treatment area included.

2.2. *Experimental Design and Field Sampling*

Nine points were selected in each plot following a regular grid with a spacing of 15 m between points (Figure 1). For the initial sampling, in each one of the nine selected points, the thickness of the organic horizon was measured, whereas the total C and N were analyzed in five points per plot (the center and the corners of the square).

Nitrogen dynamics were measured using the resin tubes technique [49], with modifications adapted to our conditions. The technique consisted of incubating soil samples in the field in vertical tubes that had ion exchange resin bags attached to the lower openings. The resin intercepted the mineral nitrogen (NO_3^- + NH_4^+) that could be lost by leaching. For sequential samplings, in the field incubation experiment carried out every two months, a new point was selected near the previous ones (~0.5 m distance), depending on the stoniness. In total, six sequential bimonthly incubations were performed throughout a year

(August 2011–July 2012). Annual net N mineralization and nitrification were calculated as the sum of net N mineralization and nitrification over the six incubation periods. To take the soil samples, the litter layer was removed, and the tubes were filled with soil from which the larger coarse elements were previously removed (disturbed soil). Once the tubes were installed in the mineral soil, they were covered with the layer of litter previously removed at each point, to simulate the initial cover. At each measuring point, a soil sample was taken. An aliquot of the sample was used to fill a tube with the coupled resin that was placed back in the place where the sample was taken. The rest of the sample was transported to the laboratory to analyze, as soon as possible, its mineral nitrogen content (hereafter, initial content).

After an incubation period of two months, the tube with resins was collected and transported to the laboratory for analysis of the mineral N content of the soil (final content) and of the resin. The net nitrification was obtained as the sum of the final nitrate N plus the nitrate N content in the resin, subtracting the initial nitrate N. Similarly, net mineralization was obtained as the sum of final mineral nitrogen plus resin mineral nitrogen minus initial mineral nitrogen. The PVC pipes had a length of 15 cm, an external diameter of 5 cm and a thickness of 2.5 mm, and were bevelled at the bottom to facilitate insertion into the ground. The resin bags were constructed using rubber rings (O-rings) attached to nylon fabric by silicone. Each bag was prepared with 4 g of 50% mixture of cationic (Dowex 50W-X8) and anionic (Dowex 1-X8) resin.

Due to the high stoniness of the plots, a modification of the original resin-core technique of DiStefano and Gholz [49] was carried out and consisted of filling the tubes with disturbed soils. In order to quantify the effect of the sample alteration on the mineralization, an additional unaltered sample was prepared in each plot. Thus, one point out of the nine was selected in each plot (at the point where stoniness was lower) and an unaltered sample was installed adjacent to the altered one. The undisturbed sample was obtained by inserting the tube into the soil using a mallet, carefully removing it and replacing the 2 cm layer of soil from the bottom by the resin bag. After that, the tube was reinserted in its original place.

The DiStefano and Gholz [49] design included the installation of another resin bag at the top of the tube to intercept the mineral nitrogen inputs. However, as our study was carried out in an ecosystem with water limitations, the upper bag was not installed to avoid the interferences of resin bag in the evaporation process. To measure these inputs, passive collectors with ion exchange resins were used [50]. These collectors were installed at a height of 30 cm above the soil surface and picked up the throughfall and dry deposition, using a 19 cm diameter funnel. Connected to the funnel was a cartridge containing 11 g of the same resin mixture used in the mineralization tubes. To intercept the solid object, fiberglass wool was placed inside the funnels. Fourteen collectors were installed: 7 in the TU block and 7 in the CH block. The funnels were distributed between the centre of each one of the plots, one on the periphery under a tree, another under scrub, and the last in a cleared area (firebreak). The results were presented based on the vegetation cover of each point. The frequency of collection of the resin cartridges was bimonthly, coinciding with the samplings of resin-cores.

As the biological and chemical characteristics of the soil seem to be more related with the type of vegetation cover in this type of ecosystem [51], each one of the nine areas per plot was classified in one of the following coverage categories: uncovered soil (B), with total absence of vegetation cover; shrub (S), soil covered with shrubby vegetation; tree (T), point at a distance of less than 3 m from an adult Aleppo pine; and tree with shrub (TS), shrubby vegetation at a distance less than 3 m from an adult Aleppo pine.

Soil temperature (5 and 10 cm depth) was measured in all the plots with soil temperature probes (RT-1, Decagon Devices, Pullman, WA, USA) attached to dataloggers (EM50, Decagon Devices, Pullman, WA, USA), which recorded data at hourly intervals. The average bimonthly temperature values were obtained.

2.3. Chemical Analysis

The soil samples were transported to the laboratory in a portable refrigerator where they were sieved, maintaining their moisture content, with a 4 mm diameter sieve. In the incubated samples, the stoniness, the total content of soil in the tube, and the gravimetric humidity were determined. The soil samples were kept refrigerated until the extraction of mineral nitrogen that was carried out in a period of less than 48 h. Mineral N was extracted shaking 10 g of each sample with 100 mL of 2M KCl for 1 h. Soil extracts were analyzed for N-NO_3^- and N-NH_4^+ in a flow injection analyzer (FIAStar 5000, Foss Tecator, Höganäs, Sweden).

In the case of resins, they were transported and kept refrigerated until mineral nitrogen was extracted. For the resin bags in the tubes, N-NO_3^- and N-NH_4^+ were extracted sequentially with three extractions of 33 mL of 2M KCl lasting 1 h each. In the resin cartridges from the atmospheric deposition collectors, the extractions were made sequentially with two extractions of 50 mL of 2M KCl stirring for one hour. These extracts were also analyzed for ammonium and nitrate content using the FIA analyzer.

A subsample of each one of the initial soil samples was crushed and sieved for 500 µm. Total C and total N were determined using a total analyzer (Flash EA 1112 series-Leco Truspec) in the 500 µm soil fraction. In order to obtain the total organic carbon (Corg), the inorganic C measured by the calcimetermethod, was discounted from the total carbon.

2.4. Statistical Analysis

Initial ammonia and nitrate N data analysis was performed by means of repeated measures ANOVA, with the silvicultural treatment as an inter-subject factor and time as an intra-subject factor. One-way ANOVA was applied to the annual net mineralization and nitrification data, obtained as the sum of the six bimonthly periods measured, with the silvicultural treatment as a factor. To study in more detail the effect of silvicultural treatment on the dynamics of initial ammonium, initial nitrate, net mineralization and net nitrification, a one-way ANOVA was performed for each measurement date. The effect of plant cover on annual net mineralization and nitrification was evaluated with two-way ANOVA (silvicultural treatment and block as factors). Finally, the relationship between the thickness of organic horizon and the annual net mineralization and nitrification was studied using linear regression analysis. Linear regressions were also used to evaluate the effect of sample alteration on the annual net mineralization and nitrification values. When the ANOVAs indicated significant differences between silvicultural treatments, the Tukey's HSD post hoc test was used. If Levene's test indicated unequal variances of transformed data, then Tamhane's T2 post hoc test was used. All the statistical analyses were performed with SPSS v. 16.

3. Results

3.1. Effects of Experimental Thinning on N Dynamics, Net Mineralization, Nitrification and the Soil Total C and N

Overall, the silvicultural treatments studied here produced increments in net N mineralization and nitrification rates when compared to the control (Table 1). The net N annual mean mineralization measured in the treatments T60, T75 and T100 increased by a factor of 2.31 in comparison to T0. Similar results were obtained for the net annual nitrification rates. These observations were found in both experimental blocks, TU and CH (Table 1). The total soil C and N at the surface soil layer were also found to increase as a function of the thinning intensity, although other factors such as inter-plot variability seemed to be as important.

Considering the mean soil NH_4^+ and NO_3^- contents, the thinning treatments showed no statistically significant differences, except the NH_4^+ content in CH (Table 1). Similarly, the annual dynamics showed non-significant variations with the intensity of thinning (Figure 2). A peak in NH_4^+ can be observed in both blocks in winter while no seasonal trends were observed for NO_3^-.

Table 1. Effects of silvicultural treatments on total organic C and N, net cumulative mineralization and nitrification for each block (one-way ANOVA), and effects of silvicultural treatments on ammonia and nitrate for each block (repeated measured ANOVA).

Block	Treatment	Corg (%)	N (%)	C/N (g g^{-1})	Average Content N-NH_4^+ (mg kg^{-1})	Average Content N-NO_3^- (mg kg^{-1})	Annual Mineralization (mg N kg^{-1})	Annual Nitrification (mg N kg^{-1})
TU	T0	**5.4 ± 0.5 ab**	**0.26 ± 0.05 b**	20.8 ± 2.2	1.48 ± 1.06	0.43 ± 0.47	**22.8 ± 12.2 b**	**14.2 ± 6.5 b**
	T60	**5.5 ± 1.4 ab**	**0.30 ± 0.07 b**	18.4 ± 3.4	1.60 ± 1.70	0.32 ± 1.00	**44.4 ± 21.8 ab**	**22.7 ± 11.8 ab**
	T75	**3.8 ± 0.7 b**	**0.22 ± 0.03 b**	17.2 ± 3.7	1.38 ± 1.38	0.32 ± 0.51	**50.8 ± 23.9 a**	**33.3 ± 18.2 a**
	T100	**7.2 ± 1.4 a**	**0.43 ± 0.11 a**	17.2 ± 3.3	1.90 ± 2.05	0.43 ± 0.66	**44.1 ± 15.8 ab**	**33.1 ± 17.0 a**
	p-value	**0.001**	**0.002**	0.268	0.432	0.840	**0.021**	**0.000**
CH	T0	**1.1 ± 0.7 b**	**0.03 ± 0.02 b**	69.3 ± 62.3	**1.45 ± 0.91 b**	0.38 ± 0.42	**17.9 ± 12.4 b**	**15.0 ± 11.6 b**
	T60	**3.3 ± 1.0 a**	**0.15 ± 0.03 a**	20.4 ± 3.2	**2.46 ± 2.08 a**	0.67 ± 1.06	**50.3 ± 15.7 a**	**36.8 ± 15.5 a**
	T75	**1.7 ± 0.8 b**	**0.07 ± 0.05 b**	28.9 ± 9.9	**2.43 ± 1.29 a**	0.33 ± 0.37	**44.0 ± 21.9 a**	**33.2 ± 18.4 a**
	T100	**1.5 ± 0.3 b**	**0.07 ± 0.01 b**	20.8 ± 2.1	**1.91 ± 1.44 ab**	0.62 ± 0.70	**48.7 ± 26.0 a**	**35.5 ± 22.4 a**
	p-value	**0.002**	**0.000**	0.079	**0.014**	0.313	**0.000**	**0.000**

$p < 0.05$ is indicated in bold; lower case letters denote significant differences (Tukey's HSD post hoc test) for the factor silvicultural treatment.

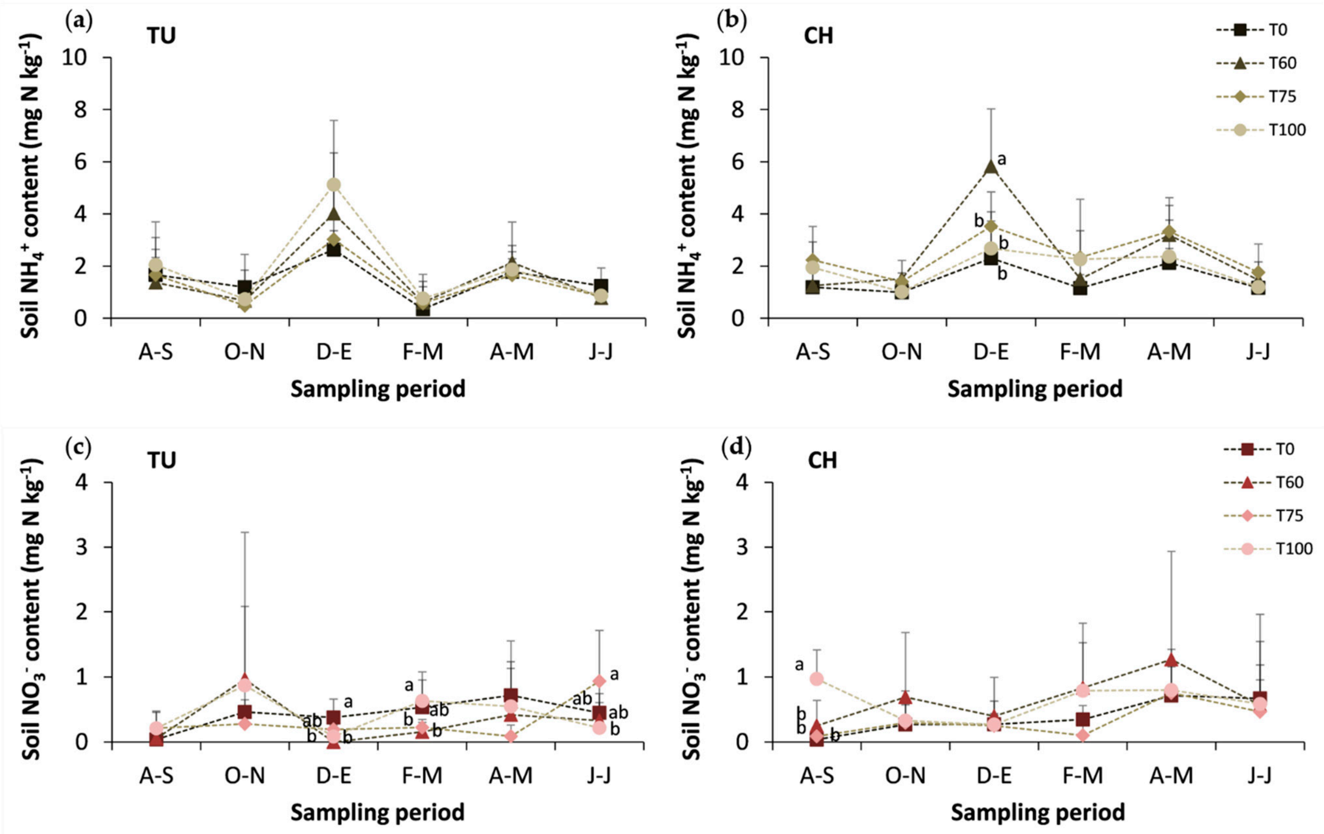

Figure 2. Temporal evolution of initial content in soil of (**a**) ammonium in Tuéjar, (**b**) ammonium in Chelva, (**c**) nitrate in Tuéjar and (**d**) nitrate in Chelva as a function of silvicultural treatments.

When the bimonthly dynamics of nitrogen mineralization and nitrification were followed (Figure 3) the lowest values were found in the control T0, and this tendency prevailed throughout the year in most incubations. The highest rates of mineralization and nitrification corresponded to the latter summer period and the lowest rates were in December–January, reaching even values of N net immobilization (Figure 3). The behavior of the soil humidity as measured at the end of each incubation showed differences between the blocks with maximum values in T100 in TU and T60 in CH.

The partial thinning treatments, T60 and T75, did not affect soil moisture with respect to controls, but the treatment T100 reduced the average soil moisture in the periods studied. The absence of differences between the treatments T0, T60 and T75 can be explained by the increase in the tree canopy and the development of the shrub along the time elapsed since the treatments were carried out. These results (lower humidity in T100 treatment) seem to be in line with the results of Raz-Yaseef et al. [52], as well as with the observations of Prévosto et al. [53]. The lower humidity recorded in the T100 plots may be due to a greater direct evaporation from the soil surface, due to the reduction in coverage.

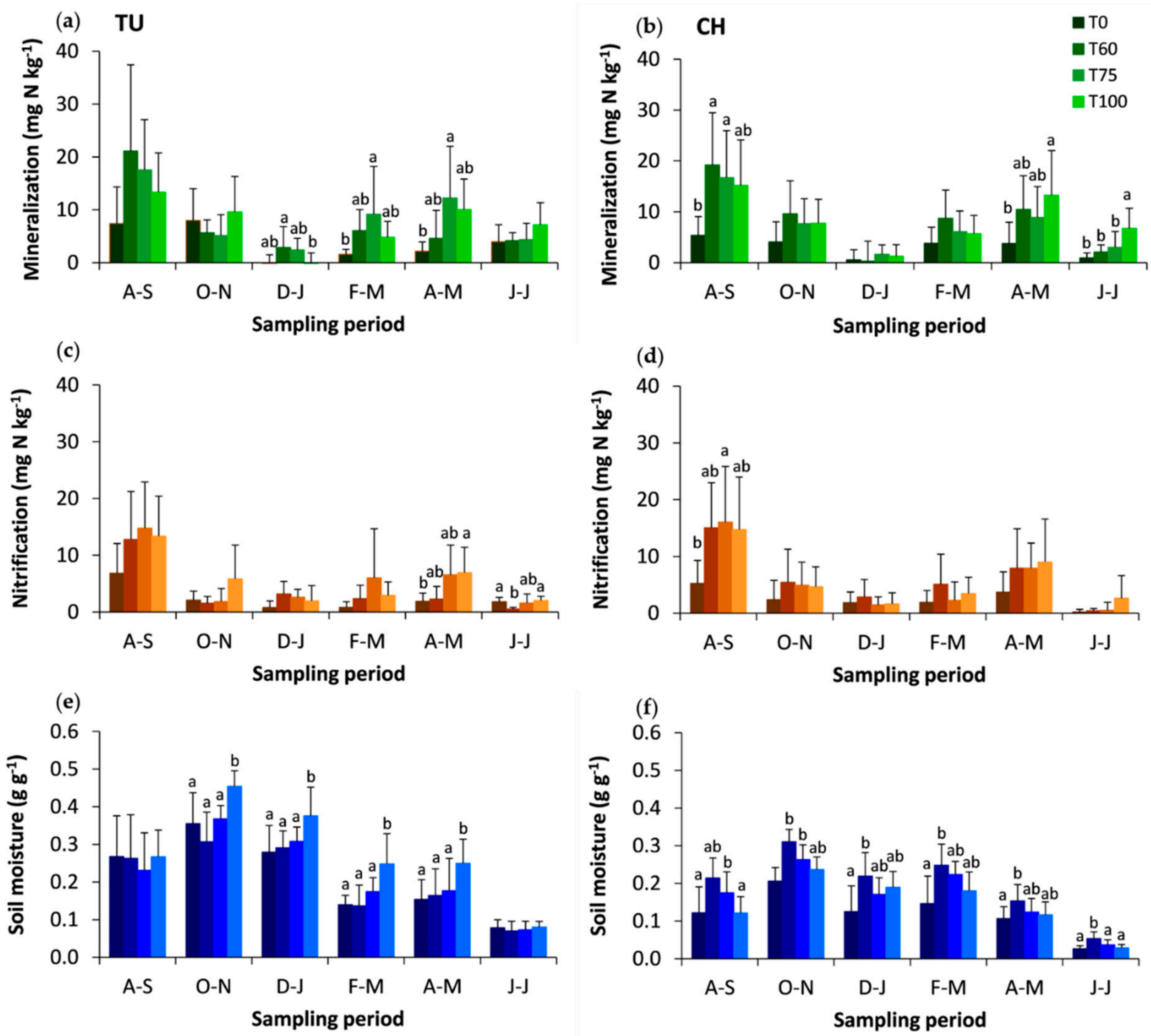

Figure 3. The evolution with time of net N mineralization in Tuéjar (**a**) and Chelva (**b**), net nitrification in Tuéjar (**c**) and Chelva (**d**), as well as soil moisture in Tuéjar (**e**) and Chelva (**f**) in the incubation vessels at the end of the incubation; values for all treatments and blocks. Lower case letters denote significant differences (Tukey's HSD post hoc test) for the factor silvicultural treatment in each sampling period.

Thirteen years after the clearing treatment, significant differences in the organic layer depth among the clearing treatments were found in TU (Table 2), with greater values in the control and a decrease in the organic layer depth according to the intensity of clearing. The same effect was found in CH but the differences were not statistically significant.

Table 2. Organic layer depth for each silvicultural treatment.

Block	Treatment	Organic Layer Depth (mm)
TU	T0	**6.9 ± 3.5 b**
	T60	**4.0 ± 2.9 ab**
	T75	**2.5 ± 3.2 a**
	T100	**1.8 ± 2.7 a**
	p-value	**0.007**

Table 2. *Cont.*

Block	Treatment	Organic Layer Depth (mm)
CH	T0	3.1 ± 1.9
	T60	2.9 ± 2.7
	T75	1.8 ± 1.5
	T100	1.4 ± 1.2
	p-value	NS

$p < 0.05$ is indicated in bold. Lower case letters denote significant differences (Tukey's HSD post hoc test) for the factor silvicultural treatment. NS, non significant differences.

3.2. Effects of Experimental Thinning on Soil Temperature

The analysis of the effect of the silvicultural treatments on the soil temperature (Figure 4) showed that the T100 treatment modified the dynamics of the soil temperature, increasing the thermal amplitude with greater mean temperatures in the warmest periods and lower mean temperatures in the colder periods. The 75 partial-cut treatment reduced the mean temperature in the cold period whereas the T60 partial cut treatment did not cause significant changes, possibly for the ten years that had elapsed since the performance of the treatments. The T100 treatment had modified the three strata that can interfere the radiation balance in mineral soil: the tree canopy, the shrub and the organic horizon. Thus, the temperature changes induced by this treatment can be explained by the absence of the tree canopy or the reduction in the litter layer thickness. The tree canopy acts as a barrier against incident radiation [54] limiting summer temperature increases. Otherwise, the litter layer is a thermal insulator that buffers the extreme values of the soil temperature [54–56]. In this work, it is not possible to separate the relative importance of each of these two strata to explain the differences in the soil temperature caused by T100. A great heterogeneity was observed in the T100 with many gaps in the organic horizon; for this reason, it is expected that the soil temperature presents a high spatial variability after the removal of the tree cover [57].

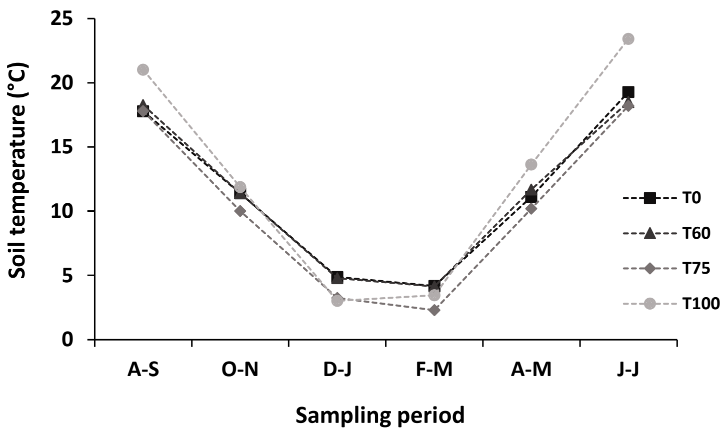

Figure 4. Average soil temperature (0–10 cm) in each sampling period and for each treatment.

3.3. Effects of Tree Cover and Organic Horizon on the Net Mineralization and Nitrification Rates and on Mineral Nitrogen Inputs to Soil

The undergrowth induced a significant effect on the net mineralization and nitrification rates (Figure 5). Both rates were found to be highest in sites with scant undergrowth: open sites (B) and shrubs (S), while the lowest values corresponded to sites under trees (T). Since the distribution of the undergrowth follows closely the thinning treatments, these results seem to be coherent with the ones reported in the previous section. The lower mineralization and nitrification rates corresponding to the treatment T0 could be related to the greater frequency of sites where the influence of adult pines was prevalent. On the other hand, the thickness of the organic soil horizon showed weak linear correlations with the net annual N mineralization ($R^2 = 0.12$, $p = 0.002$ Figure 6, left), as well as with the net annual nitrification ($R^2 = 0.18$, $p < 0.001$, Figure 6, right). In both cases the slope is negative and significantly different from zero (−2.66 with $p = 0.002$ for net mineralization, and −2.60 with $p < 0.001$ for net nitrification). In reference to the input of mineral N by throughfall (Table 3), the highest values were recorded for the collectors below vegetation, which included adult trees (T, TS).

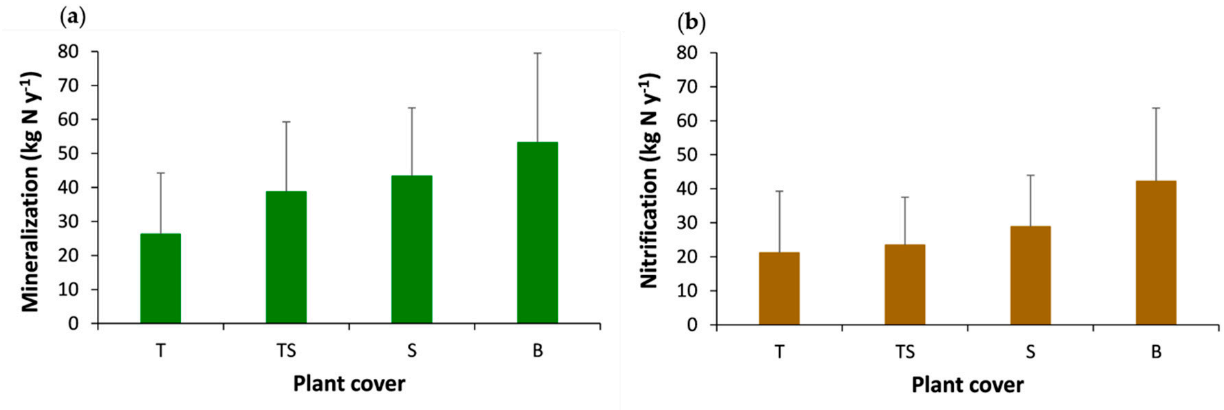

Figure 5. Effect of plant cover on net annual N mineralization (**a**) and net annual nitrification (**b**).

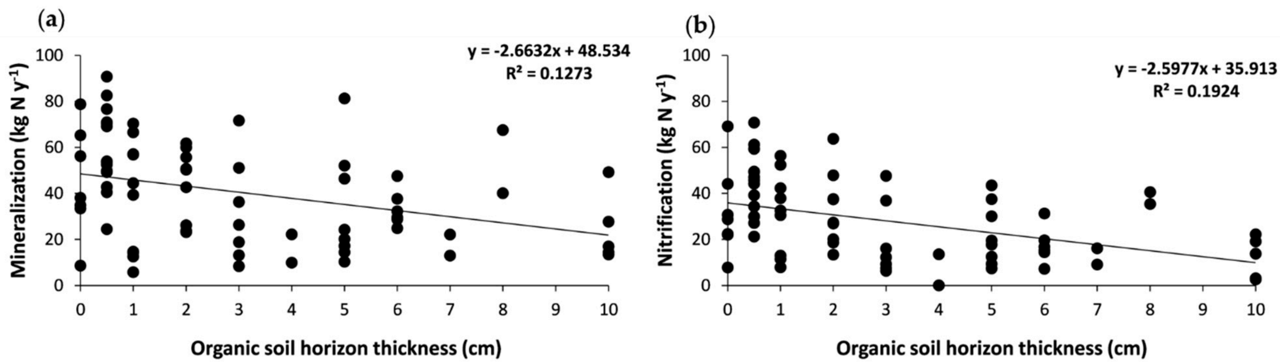

Figure 6. Effect of the organic soil horizon thickness on net annual N mineralization (**a**) and net annual nitrification (**b**).

Table 3. Annual input to the soil via throughfall and dry deposition of ammonium and nitrate, as well as the total mineral N input as a function of plant cover.

Plant Cover	NH_4^+ (kg N ha^{-1} y^{-1})	NO_3^- (kg N ha^{-1} y^{-1})	Mineral N (kg N ha^{-1} y^{-1})
B	1.89 ± 1.37	0.82 ± 0.19	2.71 ± 1.53
S	1.12 ± 0.12	0.70 ± 0.24	1.82 ± 0.84
T	3.98 ± 3.32	3.20 ± 2.07	7.18 ± 5.39
TS	3.48 ± 1.33	3.82 ± 1.86	7.30 ± 2.73

3.4. Net Mineralization and Nitrification Rates in Disturbed and Undisturbed Samples

Figure 7 shows the relationships between the disturbed and undisturbed soil samples for the net N mineralization and nitrification. In general, the alteration of the sample increased both rates. Despite the high dispersion of data in both cases, the altered samples explained 66% of variance of mineralization in the undisturbed samples and 74% in the case of nitrification.

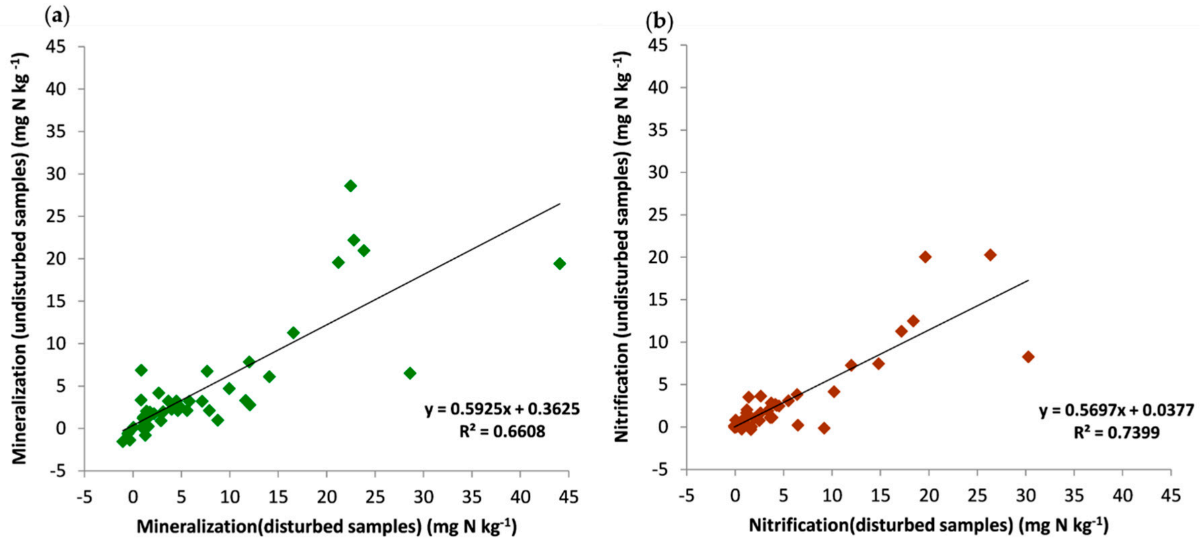

Figure 7. Linear relationships between disturbed and undisturbed samples for bimonthly N mineralization (**a**) and nitrification rates (**b**).

4. Discussion

The dynamics of the microclimatic variables (temperature and soil moisture) is still modified thirteen years after treatment by the thinning treatments. In the plot subjected to cutting, the incident radiation has increased on the soil surface, causing an increase in the annual temperature (especially due to the summer period). Additionally, both litter moisture and mineral soil moisture were lower in the T100 plots in the summer periods. In summary, under the Mediterranean climate, T100 treatment caused microclimatic conditions on the soil surface, a priori less favorable for biological activity (microbial activity, the survival of vegetation, and the development of seedlings) than in the rest of the plots in the critical period: the summer drought. These adverse conditions persisted a decade after the interventions, and it is expected that the effect was even more intense in the years immediately after the felling. Maestre et al. [58] found that the pine cover substantially reduced radiation and caused slight increases in soil moisture, but this did not facilitate the establishment of scrub under the cover. Galiana et al. [59], analyzed the vegetation structure in these stands in summer 2016, the shrub cover being significantly lower in the T0 plots. The understory species composition also differed with a prevalence of *Quercus coccifera* in T100, T75 and T60 plots and *Juniperus oxycedrus* in T0 plots.

We studied N mineralization using the resin-tube technique with disturbed soil due to the high stoniness in our plots. As in other studies [60], our values are higher than those of the undisturbed soil. Due to the aeration of the samples and the breakage of soil aggregates, the values obtained in this study are closer to the potential mineralization rates, i.e., those obtained under ideal laboratory conditions, than the net mineralization rate under real conditions [28].

Furthermore, when we compared soil humidity within the tubes with the values obtained for soils in the vicinity of the tubes, we found that the former was higher at the end of the incubation. For example, in the first incubation period (August–September) when the highest mineralization rates were found (Figure 2), the soil humidity at the end of incubation was 0.11 and 0.09 g g^{-1} higher than in the surrounding soil in TU and CH, respectively (results not shown). This could be due to several reasons, including the lack of transpiration caused by the elimination of roots, the absence of lateral continuity, since the tube walls were not perforated, or the hindrance of the vertical continuity imposed by the resin bags. The latter case seems to be important as we found a higher water content in the lower section of the tubes. We could conclude that our study was not able to reproduce the real dynamics of the soil moisture, which would have contributed to a higher microbial activity with stronger effects, especially over nitrification. Due to these facts, in our experiments the annual rate of mineralization seems to be more controlled by temperature limitations than by soil water shortages. This could also explain why we observed the highest values in summer in contrast to the dynamics found by Rapp et al. [28] in the Mediterranean, where the highest values corresponded to spring. Nevertheless, other authors found high mineralization rates during the dry summer conditions in the Mediterranean [61]. Some caution should also be taken when extrapolating these results to other conditions, since N mineralization was only studied in two areas. Additionally, in the treatment of T60 in block CH, the soil had a higher clay content (28%) in contrast to T100 (12%) and T0 (13%). Due to this, the higher clay content in the T60 plot was likely the cause of its higher C and N contents compared with the other plots in CH. This makes the comparison between plots T0 and T60 difficult, since we cannot attribute the effects of silvicultural treatments, which might be shadowed by differences in soil texture.

Thirteen years after the clearings, the N mineralization rates were higher in treatment T100 when compared to the controls. A similar result was reported by Frazer et al. [18] after the clearings were carried out 17 years earlier under Mediterranean conditions. All possible explanations for this, as expressed above, could be the possible cause of the observed values for T100: (i) changes in soil microclimate; (ii) a reduction in C input due to a decreased litterfall rate and root exudates; (iii) and changes in the microbial substrates. In reference to the microclimate, we observed a higher soil temperature in this plot which could be associated with higher mineralization rates and microbial activity [62]. Soil humidity differences between T100 and the control plots seemed to be unrelated to the increases in mineralization rates. For example, in the first sampling period, the final soil humidity was very similar for treatments T0 and T100 while the mineralization was higher in the latter (Figure 3). On the other hand, the reduction in the C input via the litterfall observed in T100 can also be related to increases in net mineralization. This reduction in C input by litterfall includes both the aboveground components and the death and recycling of fine roots, as well as the root exudates causing a reduction in microbial biomass, and hence an overall immobilization of N [12,23]. Lastly, the substrate could have been modified in treatments T100 due to: (i) an increase in undergrowth and grasses and/or (ii) the decomposition of dead roots. The litterfall from the undergrowth in those plots seems to be more labile than *P. halepensis* needles, thus providing a better substrate than *P. halepensis* needles. Almagro and Martínez-Mena [63] found that the leaves of *Rosmarinus officinalis* L. decomposed faster than *P. halepensis* needles; they concluded that this was due to a better quality (bio-degradability) of C in the leaves of the former species. Additionally, the growth of some N-fixing species, such as *Genista scorpius* L. (DC.) and *Ulex parviflorus* Pourr., could have contributed to an increase in their litter bio-availability, and hence the mineralization rate in T100. On the other hand, the remaining dead fine roots would have disappeared after 13 years since the treatment. Nonetheless, Ludovici et al. [64] found that 10 years after a clearing in a *Pinus taeda* L. forest, 40% of the lateral roots were still present at 1m around the stumps. In our case, it is still possible that a fraction of the remaining roots would constitute an extra source of nutrients. When sampling sites, the decomposing above-ground areas of the trees were avoided to minimize the effects of their contribution to mineralization.

When analyzing the results obtained for the plots where successive uniform clearings were performed, an important question arises: Why are N dynamics in the treatments, T60 and T75, similar to that of the clearcutting treatment T100 but different to the controls? Our results are somewhat surprising but not unique. Kim et al. [40] found similar results in a *Quercus rubra* forest two years after the treatments, but not during the first year. They assumed that this could be due to the decomposition of dead, fine roots. Taken together our results suggest that the quality of the substrate, as opposed to changes in the microclimate or lower C inputs, is the factor that best explains why the treatments, T60 and T75, showed similar results to T100 but not to the controls. Several observations seem to support this hypothesis: (i) there are significant differences in the aboveground litter fall C input between T60 and T75 when compared to T100 [13]; (ii) mineralization is a little sensitive to the thickness of the organic horizon, a variable that affects the microclimate in mineral soil; (iii) the soil temperature in T100 plots is significantly higher than in T0, T60 and T75; and (iv) the frequency of plant cover in the arbustive strata in treatments T60 and T75 is closer to T100 than in T0 [59]. This type of plant cover showed higher mineralization rates than the other typed; (v) the C/N ratio is higher in soils of the control plots than in the other treatments, although these differences were non-significant. In summary, our results showed that all silvicultural treatments led to an increase in microbial activity related of the changes in the substrate, this process is magnified in areas under B and S plant cover even though there was less N input due to the throughfall reaching the soil (Table 2). The two undergrowth categories B and S usually present a low proportion of *P. halepensis* needles in the organic horizon. The increase in the mineralization rate seems to be independent of the silvicultural treatments indicating that, in this ecosystem, the mineralization responded more to the quality of the litterfall than to its total amount, as was reported by Scott and Binkley [65] and Ferrari [66]. The latter author emphasized the importance of spatial heterogeneity in the relationship between the litterfall and N mineralization. Considering the plot level scale, our results suggest the possible existence of a threshold value above which the increments in the proportion of undergrowth litterfall produced no further effect on N mineralization. In our T60 treatment this hypothetical threshold could have been overtaken. However, our work does not allow us to draw definitive conclusions regarding this issue. Further experiments, especially in relation to the role of decaying cut trees' dead roots, are necessary to clarify this point. Additionally, a better understanding of the role of N-fixing undergrowth species in N mineralization is needed.

Despite the differences found in the rates of net N mineralization and nitrification, the amounts of ammonium and nitrate in the soil were not clearly found to be related to the silvicultural treatments, except in the case of ammonium in CH. In TU, the lack of differences in the ammonium content could be ascribed to root absorption while nitrate could also be lost by leaching, especially in the case of the CH block, where the soil was of a sandy loam texture. In undisturbed soil samples, the proportion of the leached nitrate intercepted by the resin was 8.7% for TU and 25.5% for CH (results not shown). It is possible that a proportion of nitrate produced by mineralization in the treatment plots could have been lost by leaching, leading to a lesser expression of the differences in the concentrations at a 0–15 cm depth.

The results of this study, and the previous work [13], suggest that in a *Pinus halepensis* forest under a Mediterranean climate, the availability of nitrogen from the trees of the treated plots has increased compared to the trees of the untreated forest. Our study found: (1) an increase in the mineralization of soil N in treated plots, (2) lower concentrations of Mg in the needles in the summer peak of litterfall [13], and (3) higher concentrations of N and P in the summer peak of litterfall, with the N concentration in litterfall [13] being 5 mg g^{-1}, close to the maximum potential reabsorption limit of 3 mg g^{-1} proposed by Killingbeck [67]. Sardans et al. [68] also found the increase in N and P availability was followed by a reduction in the Mg concentration of litterfall. Several authors also found that the reduction in the basal area decreased the foliar content of Mg [14] or both K and Mg [15].

The soil composition (indicated by the block factor) influenced some important aspects, such as soil humidity, nutrients concentration in litterfall and the nutrients released via the decomposition of the needles. However, the overall results suggested that the differences between the soils, with different textures and nutrient contents, had little influence on the responses of the forest to the intensity of felling. The reason for this was that in mature forests, the main supply of nitrogen comes from the biogeochemical cycle of the ecosystem (litterfall and decomposition) and not so much from the external geochemical cycle [10]. The effects of silvicultural interventions on soil nitrogen reserves appear in the long term (decades) and depend on other factors, such as the intensity of treatment [10,11].

Thirteen years after the experimental clearings, T100, T75 and T60 treatments showed a twofold increase in net mineralization and nitrification rates with respect to T0 in both blocks (TU and CH). Unexpectedly, the results of partial clearings were closer to those of clear-cutting (T100) and to the controls (T0). Within the plots, the mineralization was higher in sites with no plant cover and in those under with undergrowth lower than 1 m. Both observations (between plots and within plots) can be explained in terms of the different litterfall qualities, which in turn are the result of the proportion of material originating from *P. halepensis* or more decomposable undergrowth residues. It is noteworthy that soil humidity was higher as a result of using disturbed soil samples to fill the tubes. In addition, the mineralization rates measured should be taken as potential rates due to the use of disturbed soil samples. Finally, the available NH_4^+-N content in the soil was increased by the silvicultural treatments in CH but not in TU. No differences were found in the contents of NO_3^-- N in either block which could be due to root absorption and leaching.

5. Conclusions

Most studies on the nutrient cycles in forests tend to be limited to the tree stratum, which implies an underestimation of the true amount of nutrients movement in the ecosystem. The undergrowth must have played an important role, both in the absence of differences in the climatic variables associated with the clearings and in limiting the negative effects of the T100 treatments. This can be seen in the trend of increasing N mineralization rates in the soil associated with the presence of the undergrowth, compared with the soil with an exclusive presence of *P. halepensis*. Thus, due to the recalcitrant chemical characteristics of *P. halepensis* needles, it is possible conclude that pine clearings favour shrub development, which plays a more important role as a nutrient booster than in other forest ecosystems dominated by tree species with more labile leaves.

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Data Availability Statement: The data presented in this study are available on request from the corresponding author.

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