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Research Article

Assessing the Influence of Summer Organic Fertilization Combined with Nitrogen Inhibitor on a Short Rotation Woody Crop in Mediterranean Environment

Anita Maienza,[1,2] Giovanni Mughini,[3] Luca Salvati,[1] Anna Benedetti,[1] and Maria Teresa Dell'Abate[1]

[1] *National Research Centre for Agriculture, Research Center for the Soil-Plant System (CRA-RPS), Via della Navicella 2-4, 00184 Roma, Italy*
[2] *National Research Council, Institute of Biometeorology (IBIMET-CNR), Via Caproni 8, 50145 Firenze, Italy*
[3] *National Research Centre for Agriculture, Research Unit for Intensive Wood Production, Via Valle della Quistione 27, 00166 Roma, Italy*

Correspondence should be addressed to Anita Maienza; anitamaienza@gmail.com

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The European Union Directive 91/676/EEC, known as Nitrates Directive, has dictated basic agronomic principles regarding the use of animal manure source as well as livestock and waste waters from small food companies. The use of nitrification inhibitors together with animal effluents as organic fertilizers could be beneficial for nutrient recycling, plant productivity, and greenhouse gas emission and could offer economic advantages as alternative to conventional fertilizers especially in the Mediterranean region. The aim of the present study was to investigate differences in plant productivity between bovine effluent treatments with (or without) addition of a nitrification inhibitor (3,4 DMPP) in a short rotation woody crop system. Results of the field experiment carried out in a Mediterranean dry environment indicated that the proposed strategy could improve tree growth with indirect, beneficial effects for agroforestry systems.

1. Introduction

Short rotation woody crops (SRWC) include fast growing species (e.g., poplar, willow, eucalyptus, and black locust) capable of producing large amounts of biomass for energy purpose in a relatively short time. Lake Albacutya provenance of *Eucalyptus camaldulensis* Dehnh is one of the best choices for SRWC in Mediterranean environments [1, 2]; however in low quality soils with dry periods the species is able to cover the soil but with low yields [3]. Some eucalypt clones have been selected as more productive than *E. camaldulensis*, and for two of these clones (Viglio and Velino) the European Union plant patent has been requested [3, 4].

Two cultivation models are suggested for eucalyptus SRWC in Italy: (i) 5,000–5,500 plants/ha^{-1} for 3-4 rotation cycles *f* 2-3 years each and (ii) 1,100–1,600 plants/ha^{-1} for 2 rotation cycles instead of 5-6 age.

Eucalyptus SRWC with rotation age of 2-3 years each requires higher amount of water and nutrients than conventional Eucalyptus crop. In Brazil SRWC Eucalyptus plantation 2.5 years of age (5,000–6,000 plants/ha^{-1} and 2/3 years of rotation age) is estimated to absorb in the vegetal tissues high amounts of N,P, K, and Ca from soil [5] three times higher than a conventional plantation (600 to 1,600 p ha^{-1} and eight years of rotation age). Moreover water contribution was estimated to be four times higher [5]. In conventional *E. globulus* Labill. plantation in Spain and Brazil, the quantity of water to produce 1 kg of dry biomass is evaluated in 306 and 344 liters, respectively [3, 6].

The use of irrigation and fertilization to satisfy SRWC request is not feasible because it is usually uneconomic [7–9]. As a consequence, fertilization with manure could be an attractive solution to recycle sewage waste and supply nutrients, by increasing fertilization potential.

Animal manure recycling could increase soil organic matter and nutrient content and plant production and preserve water used for irrigation [10]. However one of the earliest pieces of EU legislation aimed at controlling pollution and improving water quality, the European Directive (91/676/EEC) enforced in law in 1991, rules basic agronomic practices regarding the use of animal manure source, livestock, and waste water from small food companies. In fact, the agricultural use of nitrates in organic and chemical fertilizers has been the source of 50% of total nitrogen discharge into surface water [11–13] in Europe.

The application of livestock waste to soil needs appropriate treatments in order to preserve soil and ground water from nitrate pollution [12, 13] and greenhouse gas emissions. In the last years attention has been paid to the use of nitrification inhibitors mixed with effluent with the aim of minimizing the environmental problems related to animal manure disposal [11, 14, 15].

The nitrification inhibitor 3,4-dimethylpyrazole phosphate (3,4 DMPP) delays the bacterial oxidation of ammonia to nitrite by depressing the activity of *Nitrosomonas* bacteria in the soil (first step of nitrification) for a short time (30 days), whereas the second step of nitrification appears to be not influenced [15]. Thus, losses due to nitrate leaching decreased with nitrification steps. A number of studies [16, 17] reported significant reduction in NO_3^- formation in soil and N_2O gaseous loss has also been reduced due to the addition of nitrification inhibitors.

The objective of the present study was to analyze differences in Eucalyptus plant growth in a Mediterranean SRWC after summer treatments with bovine effluent with (or without) addition of 3,4 DMPP as nitrification inhibitor, in order to establish whether 3,4 DMPP in addition to effluents can offer advantage in agroforestry. An additional aim of the study was to test the 3,4 DMPP effect on NH_3 volatilization from soil in order to highlight possible environmental advantages of such a fertilization strategy. To the best of our knowledge the present study is the first investigation on these issues carried out in a Mediterranean SRWC.

2. Materials and Methods

The field experiment was conducted in an SRWC Eucalyptus plantation located in Central Italy (Rome), in a farm of Agricultural Research Council (CRA-PLF) (41° 54′ 33.55″ N,12° 21′ 37.83″ E) during June 2011. The soil is a *Luvisols* with a sandy loam texture (sand 63%, clay 21%, and silt 16%) and pH 7.0. The soil is poor in nutrient with concentrations of organic carbon (TOC) 1.0%, total nitrogen (N_{tot}) 0.06%, and available phosphorus (P) 8 mg kg^{-1} and a high capacity of cation exchange (23 mEq 100 g^{-1} soil). Soil fertility index calculated using soil chemical and biochemical variables (Table 1) falls in class of "stress" [9]. Eucalyptus pure species (*E. gomphocephala* DC, *E. bridgesiana* R.T. Baker, and *E. camaldulensis*) and a number of hybrid clones of *E. camaldulensis* × *E. bicostata* Maiden, Blakely & Simmons, occur in the experimental SRWC. Plant density per hectare amounts to 5,000 p ha^{-1} with 20 plants per plot in row. Plantation was harvested when the plants were three years old and the experiment was carried out during the first growing season after the harvesting. A completely randomized block design with three replicates was used for each treatment. Treatment was carried out with bovine effluent with a N_{tot} content by 0.32% (N–NH_4^+ 0.17%, N–NO_3 0.0025%, and N–NO_2 0.0004%) and TOC 5.7%. Three field tests were carried out: (i) control nonfertilized (C); (ii) bovine effluent only (Ef); (iii) bovine effluent with the addition of 3,4 DMPP (Ef + DMPP). Treatments were done once in June with tanker furrowing plowing with 170 kg N ha^{-1}; 3,4 DMPP was added at the rate of 1% of N_{tot}. A pure species (*E. camaldulensis*) and a hybrid clone Viglio were selected for the experiment. Ef and Ef + DMPP were put into the ground 40 cm depth at 50 cm distance from plants. NH_3 volatilization from soil was monitored after the treatment with a simple chamber method for field determination (Drager-Tube Method, DTM) [11]. Two measurements per day were carried out, once at morning (7 am) and once at midday (1 pm) with 3 replicates for each plot. Values of NH_3 concentrations on the Drager indicator tubes have a coefficient of variation between 10 and 15%, as indicated by the manufacturer. A meteorological station was put downwind of the field measurement pole. Temperature and rainfall measurements were taken according to international standards.

Table 1: Chemical and biochemical parameters of control soil, samples were collected at (0–30 cm). Data were calculated on averge of six field replicates.

Soil parameters	Average soil	SE
Sand (%)	63	2.3
Silt (%)	16	0.01
Clay (%)	21	0.5
N_{tot} (%)	0.06	0.01
TOC (%)	1.06	0.02
pH	7	0.2
qCO_2	0.17	0.01
Cumulative C-CO_2 evolved over 28 days (mg kg^{-1} soil)	282.11	10.5
Respiration measured at 28 days (mg C-CO_2 kg^{-1} soil)	0.4	0.02
C_{mic} (mg-C kg^{-1} soil)	95.53	2.8

Plant growth analysis was carried out at the end of the first growing season (November 2011) using Basal Area (BA) to establish plants growth on the first growing season. Diameter to the breast height (DBH) for each experimental plant was measured with a digital caliber to 130 cm from the soil and each DBH converted in BA [$BA = \pi(DBH/2)^2$] and BA total value for each plot was calculated considering 20 neighboring plants. DBH and BA are standard characters

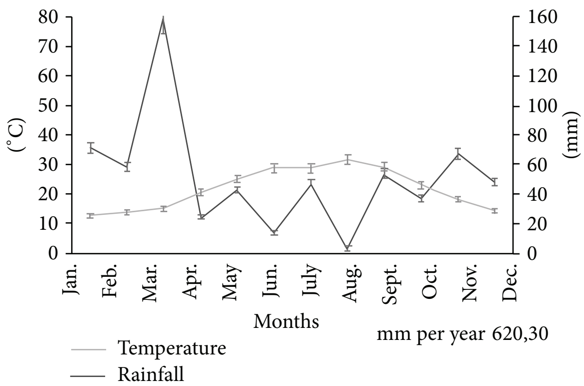

Figure 1: Bagnouls-Gaussen plot. Air temperature and rainfall data were collected by a meteorological station during the summer season 2011 in CRA-PLF experimental site.

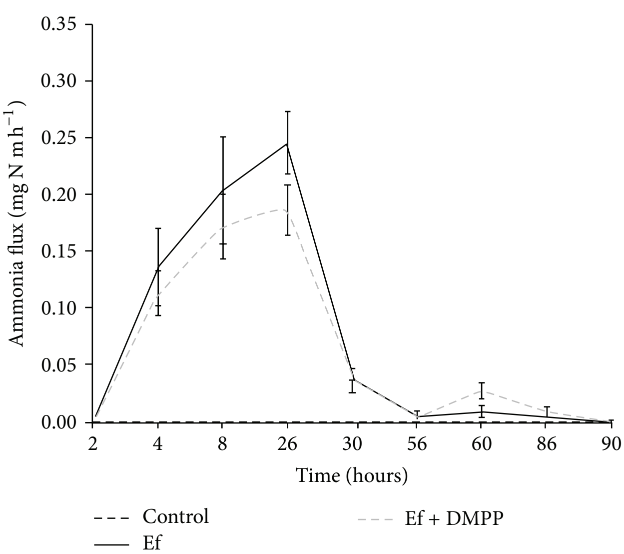

Figure 2: Ammonia volatilization detected by chambers method tube. Manual measurements were done at the beginning of the treatment and for 92 hs after. Control is the soil with any treatment, Ef is bovine manure effluent only, and Ef + DMPP is combination of bovine manure effluent and 3,4 DMPP.

used to evaluate plant growth for Eucalypts as well as for also in other forest trees [18]. Kruskal-Wallis H and Mann-Whitney U nonparametric inference testing at $P < 0.05$ was applied to BA values with the purpose of verifying differences in the median of the examined groups.

3. Results and Discussion

Bagnouls-Gaussen climate diagram (Figure 1) highlighted a drought period from April to October, indicating that plants had been under water stress for a seven-month period, due to lack of ground water. The ammonia flux from soil was detected for 92 hours after the treatments and this loss of flux should be also due to the beneficial effect of the disposal

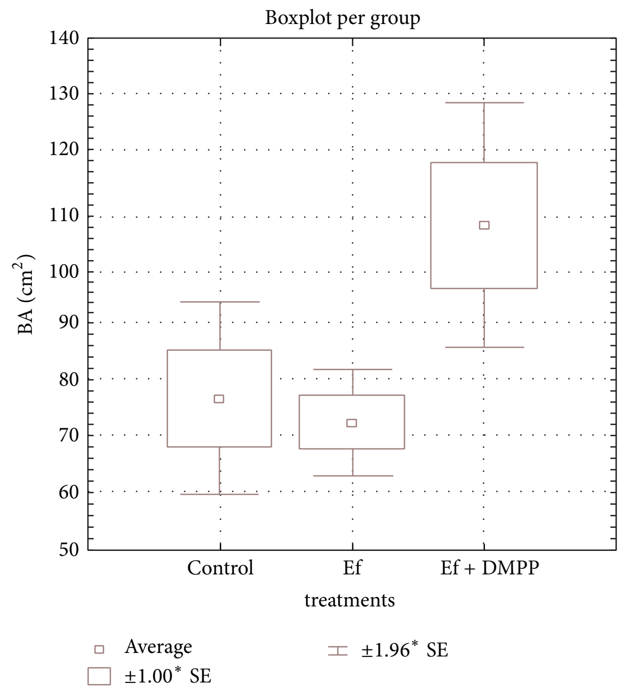

Figure 3: Viglio clone growths measured by Basal Area BA $= \pi(\mathrm{DBH}/2)^2$ by groups. Control is Viglio without treatment, Ef is Viglio treated with bovine manure effluent, and Ef + DMPP is Viglio grown with effluent combined with 3,4 DMPP.

procedure, that is, the burial of fertilizers into the soil. The Ef + DMPP treatment moderately reduces NH_3 emissions from soil during 52 hours, while a significant reduction (Mann-Whitney U, $P < 0.05$) compared with Ef treatment was observed at 26 hours (Figure 2).

Plant growth estimates showed high variability of the BA values for *E. camaldulensis* with nonsignificant effects of treatment (Mann-Whitney U, $P < 0.05$). These results are probably due at high genetic variability of *E. camaldulensis* (in this case each plant is a different genotype) and no sufficient number of replications. The Viglio clone grew significantly (Kruskal-Wallis H test, $P < 0.05$) after treatments (BA values, Table 2) and showed the best growth performance in Ef + DMPP treatment which resulted also as being more productive than *E. camaldulensis* according to Mughini [4] and Facciotto et al. [3].

The Viglio clone BA was compared by fertilization treatment (Figure 3). Ef treatment revealed being ineffective possibly due to water stress and restricted nutrient availability during the experiment; otherwise Ef + 3,4 DMPP treatment showed a clear increase (Mann-Whitney U test, $P < 0.05$). The different responses of Viglio clone to treatments are related to nutrient soil content, as temporarily determined by manure application: lower ammonia losses and longer availability of ammonium to the plants due to delayed oxidation to nitrate acted by 3,4 DMPP should be the main processes affecting the different nutrients uptake. In fact, in Eucalyptus plantations nitrogen in the soil is dominated by

Table 2: Basal area (BA) in cm^2 of *E. camaldulensis* and *E. camaldulensis* × *E. bicostata* (Viglio clone). Data were collected on 20 trees for each plot replicates, standard errors are also reported.

	Treatment	Total BA/plots (mean of 20 trees) (cm^2)	Average	SE
E. camaldulensis	C	105.89	76.50	16.74
		75.69		
		47.91		
	Ef	68.03	61.74	13.78
		81.83		
		35.36		
	Ef + DMPP	55.14	67.32	13.83
		51.91		
		94.91		
Viglio clone	C	60.65	76.82	8.74
		79.18		
		90.64		
	Ef	63.20	72.56	4.77
		75.67		
		78.82		
	Ef + DMPP	95.37	107.30	11.02
		129.32		
		97.22		
Average			85.56	6.95

ammonia [14, 19] and ammonium is absorbed faster (30–40 times) than the nitric form [19]. In this context, the use of 3,4 DMPP in Ef [11, 15, 20] supports the ammonium availability for the plants during dry season longer and more efficiently than Ef alone.

4. Conclusions

Results of this study support the development of a strategy of organic fertilization in Mediterranean regions based on combination of 3,4 DMPP and animal manure. The main effects were to improve plant growth in Eucalyptus Viglio clone SRWC in a Mediterranean dry environment after the first growing season and to reduce ammonia emissions from soil in the first 26 hours after the fertilization, especially important in summer, when gaseous emissions are enhanced by high temperature. The climate conditions of summer 2011 and low soil fertility of the field could have conditioned the effect on growth of the treatments with Ef alone. Further investigations on biomass productivity, nitrate leaching, and soil ecology can provide more information about the efficiency and the sustainability of this practice.

Conflict of Interests

The authors declare that there is no conflict of interests regarding the publication of this paper.

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