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*in*

**Leone V. (ed.), Lovreglio R. (ed.).**
**Proceedings of the international workshop MEDPINE 3: conservation, regeneration and restoration of Mediterranean pines and their ecosystems**

**Bari : CIHEAM**
**Options Méditerranéennes : Série A. Séminaires Méditerranéens; n. 75**

**2007**
pages 167-173

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# THE CONTRIBUTION OF BACTERIA AND FUNGI TO SOIL BIOLOGICAL ACTIVITY IN A *PINUS PINEA* WOOD ON VESUVIUS MOUNT

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***Abstract***

*Streptomycin and cycloheximide were applied alone or in combination to the upper soil layer (0-5 cm) of a Pinus pinea forest to determine their effects on $CO_2$ evolution dynamics, enzyme activities, such as dehydrogenase and hydrolase, microbial biomass N, N-mineralization and N-nitrification.*

*The biocide-treated soil had less respiration than the control. Consistently with the pattern of microbial respiration, microbial biomass N as well as N-$NH_4^+$ and N-$NO_3^-$ contents, had a lower level in the treated samples than the control samples.*

*The contribution of fungi to soil respiration was greater than bacteria in every sample in every season.*

*Both dehydrogenase and hydrolase were more active during the winter and spring than in summer and autumn.*

## INTRODUCTION

Fungi and bacteria are the two main soil microorganism groups playing an important role in the decomposition of organic matter. Nevertheless, their relative activity depends on the type of ecosystem and on environmental conditions. The differentiation of fungal from bacterial activity has been carried out using selective biocides, such as the bactericide streptomycin and the fungicide cycloheximide [1] [2], that specifically inhibit protein synthesis by acting on bacterial (70s) and fungal (80s) ribosomes, respectively [3] [4]. The aim of this study was to determine the effect of streptomycin and cycloeximide on respiration rate, enzyme activities, microbial biomass N, N-mineralization and N-nitrification in the upper soil layer (0-5 cm) of a *Pinus pinea* forest.

## STUDY SITE

The site had 86% tree cover and was located along a gentle slope on the Southeast mountainside of Vesuvius. Trees were about 40 years old at sampling time and planted on lapillus from the last eruption (1944). Climatic and site data are reported in the Table 1.

Table 1. Climatic and site data of the experimental plot

| Canopy cover | *Pinus pinea L.* |
|---|---|
| Lat./Long. | 40°49'N<br>14°28'E |
| Altitude (m a.s.l.) | 250 |
| Annual mean temperature (°C) | 13.2 |
| Mean temperature of the coldest month (°C) | 5.9 |
| Annual mean precipitation (mm) | 960 |
| Lang aridity index (P/T) | 73 |

The soil is characterized by a low respiration rate (Table 2), especially when compared to other forests as well as *Fagus sylvatica* on Monte Taburno and of *P. laricio* at Sila [5] or of *Q. ilex* on Mount Vesuvius or

of Bosco di San Silvestro (CE) [6] and in a maquis in Castel Volturno Nature Reserve [7]. Nevertheless, this rate was comparable to that of *P. pinea* wood in Castel Volturno Reserve [7].

Table 2. The main soil characteristics of the experimental plot

| Humus | *Moder* |
|---|---|
| Organic layer (cm) | 2-4 |
| pH | 6 |
| Water holding capacity (g $H_2O$ $^{-1}$100 g d.w.$^{-1}$) | 14.9 |
| Corg | 3.54 |
| $CO_2$ evolution (mg g d.w.$^{-1}$ $d^{-1}$) | 0.18 |
| Cmic (mg g d.w.$^{-1}$) | 1.1 |
| q $CO_2$ (mg C-$CO_2$ mg Cmic $^{-1}$ $d^{-1}$) | 2.87 |
| CEM (mg C-$CO_2$ g $C^{-1}$ $d^{-1}$) | 13.1 |

Soil respiration was positively correlated to soil water content and to organic matter content and shows a seasonal pattern with the highest values in autumn and winter and the lowest in summer and spring [5].

The metabolic rate of soil microflora ($qCO_2$), an index of metabolic efficiency of microorganism communities [8], was high compared to other ecosystems [9] [10] suggesting the limited ability of microbial biomass to retain energy and to maintain metabolic balance. The CEM, coefficient of endogenous mineralization, represents the rate of organic carbon fraction, which is mineralized to $CO_2$. This provides important information on organic matter mineralization and the soil's potential to accumulate or to lose carbon. The CEM values increases in soil under stress (fire, crop-rotation, etc.) [11] [12]. When compared with other ecosystems (Castel Volturno Nature Reserve ) in non-stress conditions, it showed a high value [10].

## MATERIAL AND METHODS

The upper layer of soil (0-5cm) was collected in the four seasons from 30 microsites within the experimental plot of 2500$m^2$. To evaluate the contribution of bacteria and fungi to soil biological activity, the sieved soil (Ø 2mm) at 60% of holding capacity was amended with glucose (5 mg/g d.w.) and the biocides such as the bactericide streptomycin sulphate (3 mg/g d.w.) or the fungicide cycloheximide (2 mg/g d.w.) applied singly or in combination and kept at 25℃ for 20 hours. Samples only with glucose were the controls.

Soil respiration was measured as $CO_2$ change according to Froment [13] by a double titrating with hydrochloridic acid 0.05 N. Dehydrogenase and hydrolase activities were measured according to Von Mersi & Schinner [14] and Schnürer & Rosswall [15], respectively.

Microbial biomass N was determined by the fumigation-extraction method as described by Vance et al. [16] and Brookes et al. [17] and was determined by the expression $B_n = 1.85 \times E_n$ where $E_n$ is N extracted by $K_2SO_4$ from fumigated soil less that extracted from unfumigated soil, and 1.85 is the proportionality factor [18].

The N-$NH_4^+$ and N-$NO_3^-$ were determined with thymol colorimetric method [19] and with brucina colorimetric method [20].

For the last parameters, microbial biomass N, N-$NH_4^+$ and N-$NO_3^-$, the data concern only to the winter and summer.

The bacteria/fungi activity ratio was calculated according to Anderson and Domsch [21] by considering the percentage of effective inhibition.

## RESULTS AND DISCUSSION

Figure 1 shows the $CO_2$ evolution rate from samples treated and untreated with biocides. Biocides not only did not totally reduce the activity of their target groups as reported in the literature [1] [2] but their percentage of inhibition was low and varied with the seasons, probably because a different sensitivity of microbial community. By considering the samples amended with both biocides, we observed an inhibition of respiration higher in summer and autumn (~70%) than in winter and spring (~30%). The contribution of fungi to soil respiration always exceeded that of bacteria even with seasonal pattern (Table 3). In summer, in fact, the contribution of fungi is very low, probably because the aridity strongly limited the growth of fungal populations.

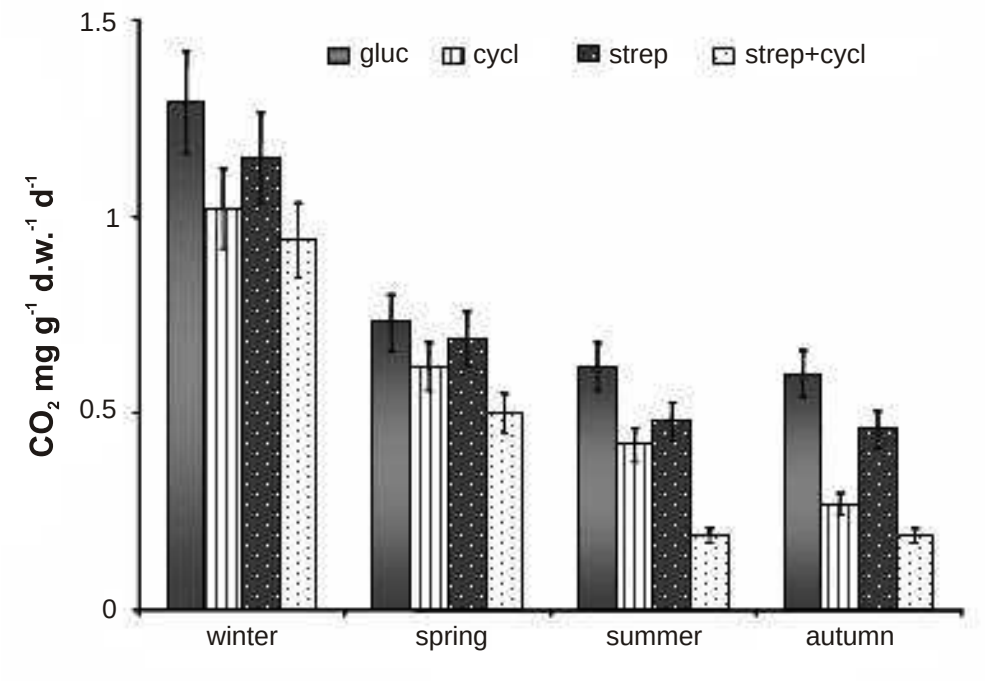


Figure 1. $CO_2$ change from soil control and samples amended with streptomycin and/or cycloheximide during the four seasons.

Table 3. The percentage of bacterial and fungal respiration to total.

| | % bacterial respiration | % fungal respiration |
|---|---|---|
| Winter | 34 | 66 |
| Spring | 27 | 73 |
| Summer | 41 | 59 |
| Autumn | 30 | 70 |

Figure 2 shows the potential activity of dehydrogenase and of hydrolase in the four seasons. Both dehydrogenase and hydrolase were more active during the winter and spring and less active in summer and autumn. By considering dehydrogenase activity, the inhibition appeared, similarly to respiration, lower in winter and spring (59% and 35%, respectively) than in summer and autumn (64% and 85%, respectively).

The contribution of fungi to dehydrogenase activity appeared greater than bacteria in winter and autumn but lower in spring and summer (Table 4). On the contrary, by considering hydrolase activity, the contribution of fungi was greater than bacteria at every sampling time, despite less difference in summer and autumn (Table 4). As for the effect of biocides, in this case the inhibition was very low (from 7% to 25%) with the only exception in autumn (53%).

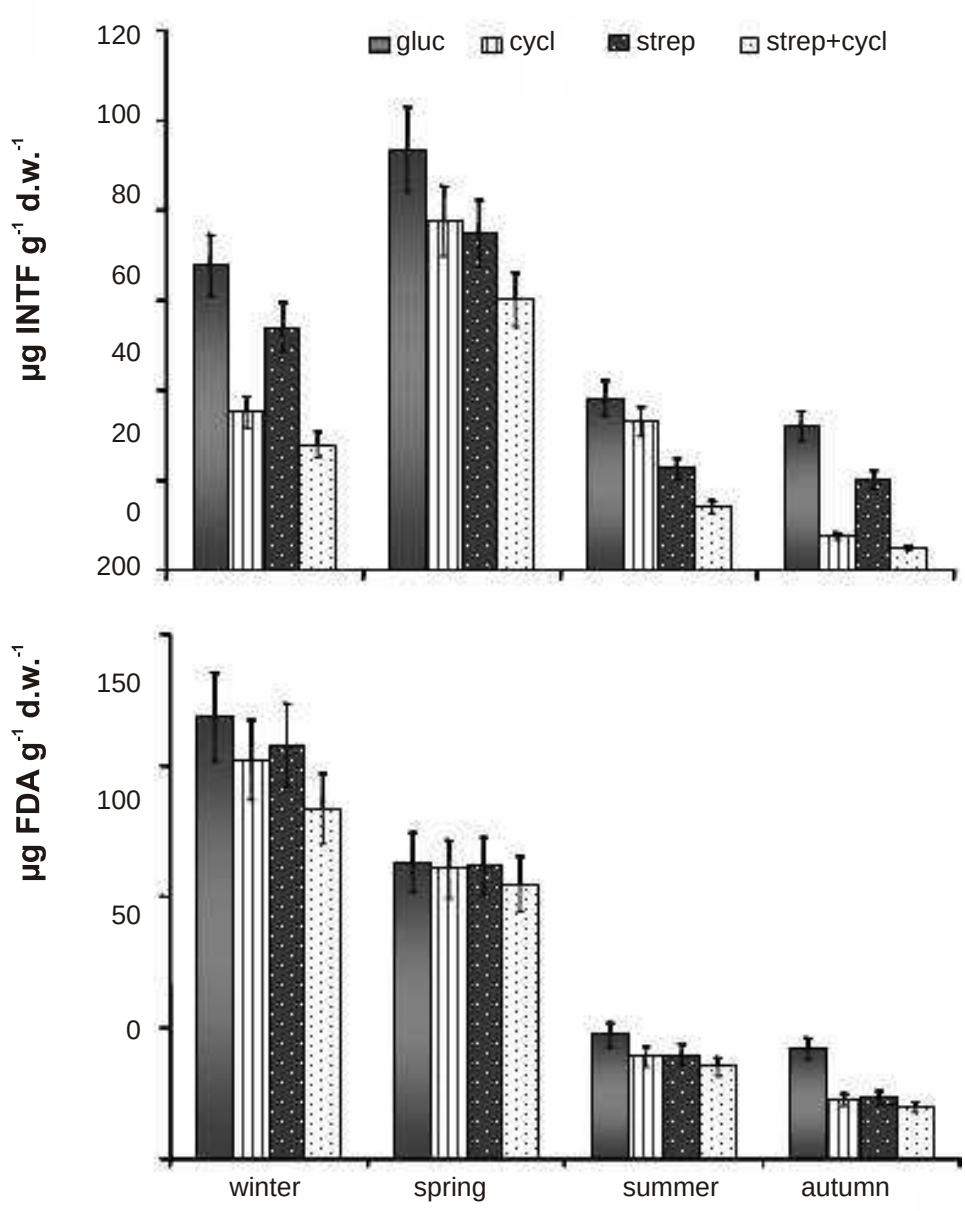


Figure 2. Dehydrogenase (up) and hydrolase (down) activities in soil control and samples amended with streptomycin and/or cicloheximide during the four seasons.

Table 4. The percentage of bacterial and fungal enzymatic activities to total.

| | % bacterial dehydr. activity | % fungal dehydr. activity | % bacterial hydr. activity | % fungal hydr. activity |
|---|---|---|---|---|
| Winter | 30 | 70 | 39 | 61 |
| Spring | 54 | 46 | 39 | 61 |
| Summer | 75 | 25 | 48 | 52 |
| Autumn | 33 | 67 | 48 | 51 |

Microbial biomass N showed an increase compared to the control, both in winter and summer; the biocide combinations, instead, markedly depressed microbial biomass N (Fig.3). Similar results were found by Landi *et al.* [2] in a forest soil of Viterbo (Italy) where a reduction of microbial biomass C and N was observed during the first day of incubation.

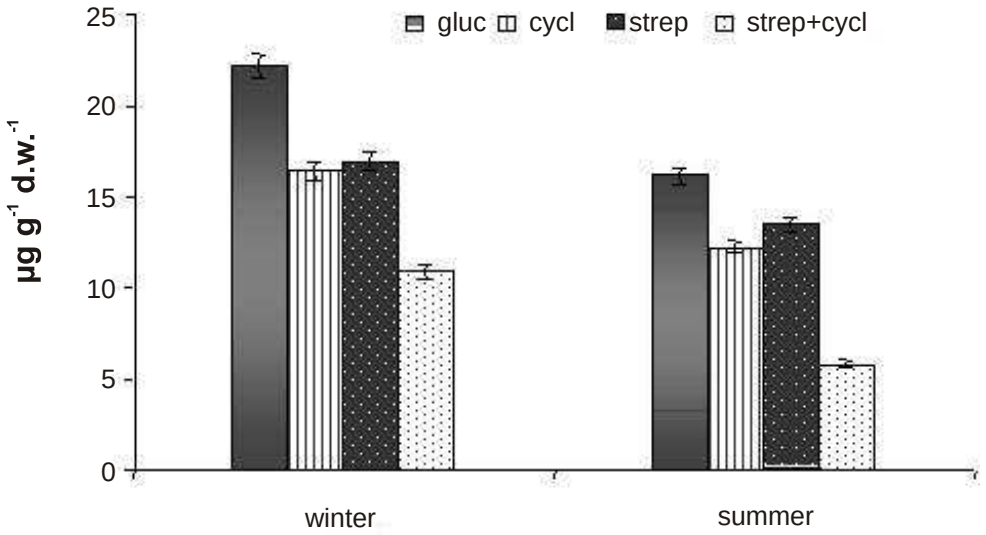


Figure 3. Microbial biomass N from soil control and samples amended with streptomycin and/or cicloheximide during summer and winter.

When considering the samples amended with the inhibitors applied singly or in combination we observed a similar microbial biomass N decline in winter and summer (Table.5).

Table 5. Microbial biomass N content in soil samples treated with the inhibitors in % of the control.

| | Strep. | Cycl. | Strep. + Cycl. |
|---|---|---|---|
| Winter | 81 | 67 | 33 |
| Summer | 79 | 46 | 32 |

The inhibitors underlined a reduced $N\text{-}NH_4^+$ and $N\text{-}NO_3^-$ production (Fig.4). As mentioned above for the other parameters, the greatest inhibition was observed in the samples with both biocides. The $N\text{-}NH_4^+$ and $N\text{-}NO_3^-$ contents in the samples treated singly or in combination with the biocides were reduced at 56-72% and 44-64% respectively compared to the control.

No significant differences were observed between samples treated with streptomycin and cycloheximide, suggesting that the contribution of bacteria and fungi to the N-mineralization and nitrification was similar (~50%).

The decrease of $N\text{-}NO_3^-$ in the samples treated with cycloheximide could be the result of the inhibition of the heterotrophic nitrification principally by fungi. The decrease of $N\text{-}NO_3^-$ in the samples treated with cycloheximide could be the result of the inhibition of the heterotrophic nitrification principally by fungi [22] and also demonstrated in forest soils [23] [24].

The increase of $N\text{-}NH_4^+$ and $N\text{-}NO_3^-$ concentration in the surviving microorganisms could depend on the mineralization of the compounds released by microbial cells killed by the antibiotics. This was also found by other authors [2].

Although both inhibitors may be used as C and N sources by numerous microbial species, it is unlikely that soil microrganisms can degrade the antibiotics-soon after addition, and to transform N in $NH_4^+$ or $NO_3^-$ [2].

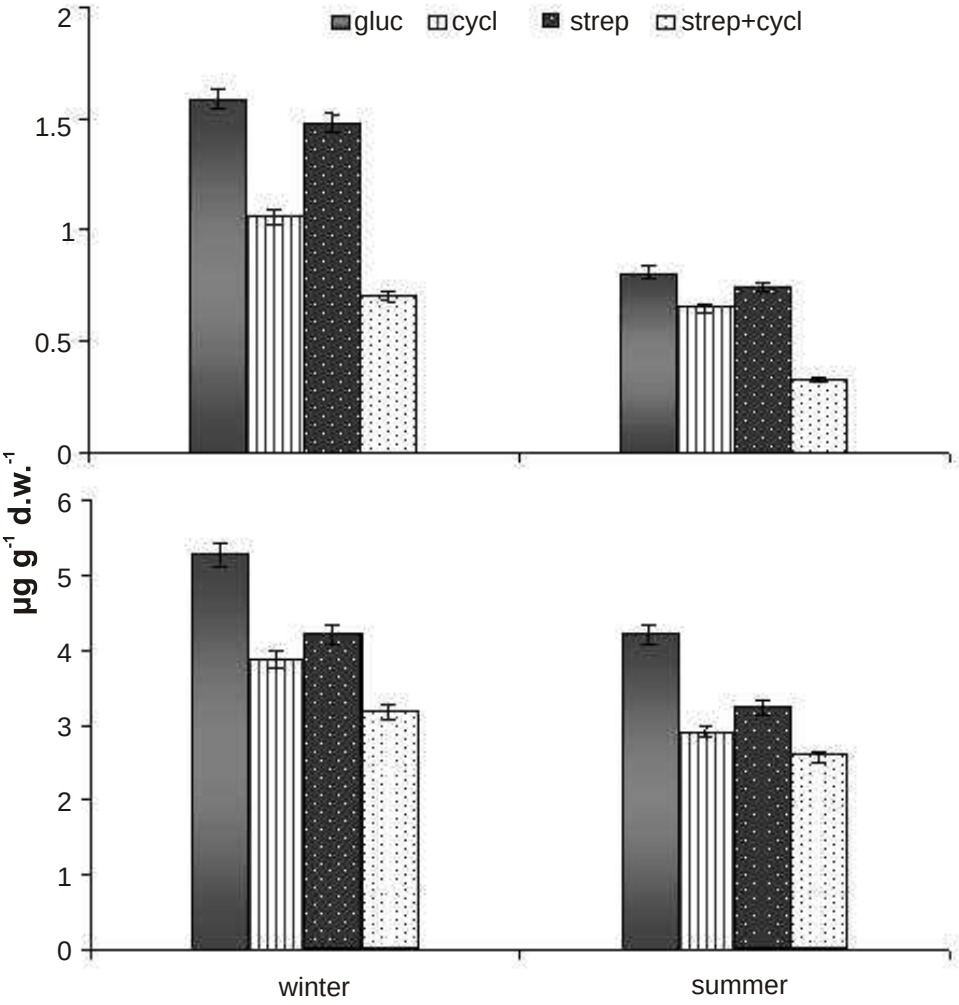


Fig. 4. $N\text{-}NO_3^-$ (up) and $N\text{-}NH_4^+$ (down) contents in soil control and samples amended with streptomycin and/or cycloheximide during summer and winter.

## CONCLUSION

The study of the contribution of bacteria and fungi to soil biological activity in a *Pinus pinea* forest on Vesuvius mount has shown that:

biocides not only did not totally reduce the respiration activity of their target groups, but their percentage of inhibition was low and varied with the seasons, probably from a different sensitivity of the microbial community;
both dehydrogenase and hydrolase activities showed higher activity values during the winter and spring and lower values in summer and autumn;
microbial biomass N, in the samples with streptomycin or cycloheximide, increased compared to the control, but the biocide combinations markedly depressed them;
the inhibitors underlined reduced $N\text{-}NH_4^+$ and $N\text{-}NO_3^-$ productions. As with the other parameters, the greatest inhibition was observed in the samples with both biocides.

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