Growth and nitrate reductase activity in *Juniperus oxycedrus* subjected to organic amendments and inoculation with arbuscular mycorrhizae

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Summary

The effectiveness of reforestation programs on degraded soils in the Mediterranean region is frequently limited by a low soil availability and a poor plant uptake and assimilation of nutrients. While organic amendments can improve the nutrient supply, inoculation with mycorrhizal fungi can enhance plant nutrient uptake. A pot experiment was conducted in 2004 to study the influence of inoculation with an arbuscular mycorrhizal (AM) fungus (*Glomus intraradices* Schenck & Smith) or with a mixture of three AM fungi (*G. intraradices*, *G. deserticola* Trappe, Bloss. & Menge, and *G. mosseae* (Nicol & Gerd.) Gerd. & Trappe) and of an addition of composted sewage sludge or *Aspergillus niger*–treated dry-olive-cake residue on plant growth, nutrient uptake, mycorrhizal colonization, and nitrate reductase (NR) activity in shoot and roots of *Juniperus oxycedrus* L. Six months after planting, the inoculation of the seedlings with *G. intraradices* or a mixture of three AM fungi was the most effective treatment for stimulating growth of *J. oxycedrus*. There were no differences between the two mycorrhizal treatments. All treatments increased plant growth and foliar N and P contents compared to the control plants. Mycorrhizal inoculation and organic amendments, particularly fermented dry olive cake, increased significantly the NR activity in roots.

Key words: arbuscular mycorrhiza / dry olive cake / inoculation / nitrate reductase / sewage sludge

1 Introduction

The drought-tolerant *Juniperus oxycedrus* L. shows promise for the reforestation of degraded lands in Mediterranean regions. However, the performance of this shrub is frequently limited by a low soil availability and a poor plant uptake and assimilation of nutrients. The establishment of a mycorrhizal symbiosis can improve the performance of shrub species in semiarid ecosystems (*Caravaca* et al., 2003a). Arbuscular mycorrhizae (AM) can contribute substantially to the P and N nutrition of plants. In addition, an increase in nitrate reductase (NR) activity has been shown in both roots and shoots of AM-infected plants under induced water deficit (*Caravaca* et al., 2003b). The identification of efficient AM strains is a prerequisite to inoculation programs, since nutrient uptake and metabolism in mycorrhizal plants depend on the mycorrhizal endophyte (*Azcón* and *Tobar*, 1998). The quality and productivity of degraded soils can be improved by organic amendments (*Roldán* et al., 1996). The beneficial effects of organic amendments include provision of plant nutrients, increased humus content and thereby increased water-holding capacity, improved soil structure, and increased microbiological activity, which, in turn, favor the establishment and viability of a stable plant cover (*Caravaca* et al., 2003c). Negative effects of such amendments greatly depend on their content of heavy metals and xenobiotica. For example, the use of dry olive cake has been shown to have a detrimental effect on seed germination, plant growth, and microbial activity and phytotoxic effects of olive-mill residues, due to their phenol, organic-acid, and fatty-acid contents have been reported (*Linares* et al., 2003). Efforts to decrease the environmental impact of olive-mill wastes include biological fermentation with filamentous fungi, such as *Aspergillus niger* (*Vassilev* et al., 1995), or white-rot fungi (*Linares* et al., 2003). Such biosystems, involving agrowastes and microorganisms, have been used for rock-phosphate (RP) solubilization and improvement of crop plant growth and nutrition in agricultural soils (*Vassilev* et al., 1998). Other organic amendments, such as sewage sludge, should be composted before they are applied to soil in order to achieve biological transformations of the organic matter and avoid the presence of those organic substances, which can be considered phytotoxic (*Gliotti* et al., 1997). However, there are no data on the effects of dry olive cake or sewage sludge, or their interactions, with respect to the performance of mycorrhizal shrub species in revegetation programs. Besides plant-growth parameters, the efficient uptake of nutrients and their assimilation, particularly that of nitrate-N, are of interest in this context.

The objective of this study was to compare the effectiveness of mycorrhizal inoculation with an AM fungus or with a mixture of three AM fungi and/or the addition of composted sewage sludge or *A. niger*–treated olive-cake residue, with respect to nitrate reductase activity in root and shoot, mycorrhizal colonization, plant growth, and nutrient uptake in *Juniperus oxycedrus* L.

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2 Materials and methods

2.1 Growing conditions and substrates

A pot experiment was carried out in the nursery of the University of Murcia (SE Spain) in 2004. During the 6-month experimental period, the temperature ranged from 11°C to 34°C, and the relative humidity was between 40% and 80%. Midday photosynthetically active radiation (PAR) averaged 260 $\mu E\ m^{-2}\ s^{-1}$.

An agricultural soil that had been used to cultivate citrus fruits was collected near Murcia. The main characteristics of the soil used were: pH (H_2O, 1:5) 8.89; electrical conductivity 0.18 dS m^{-1}; organic C 1.80%; total N 2.01 g kg^{-1}; available P 70 $\mu g\ g^{-1}$; extractable K 440 $\mu g\ g^{-1}$; cation-exchange capacity 15 cmol kg^{-1}.

The compost used in this experiment was produced from a mixture of wood shavings and an aerobically digested sewage sludge, at a rate of 1:1 (v:v). The sewage sludge was obtained from a water-treatment plant in Murcia. During the first 2 months of the composting process, the waste heaps were turned in open air nine times, while during the following 2 months (maturation stage), the compost was allowed to stabilize on boards. Dry olive cake, a lignocellulosic material obtained from an olive-mill located in Granada (Spain), was dried at 60°C and then ground to pass a 2 mm–pore screen. Portions of 15 g of dry olive cake were mixed with 40 mL of Czapek solution (agar 15.0 g L^{-1}; K_2HPO_4 1.0 g L^{-1}; $FeSO_4 \cdot 7\ H_2O$ 0.01 g L^{-1}; KCl 0.5 g L^{-1}; $MgSO_4 \cdot 7\ H_2O$ 0.5 g L^{-1}; $NaNO_3$ 3.0 g L^{-1}; sucrose 30.0 g L^{-1}; pH = 7.3) for static fermentation in 250 mL Erlenmeyer flasks. Rock phosphate (Morocco fluorapatite, 12.8% P, 1 mm mesh), was added at a rate of 0.75 g per flask. This medium was sterilized by autoclaving at 120°C for 30 min. A spore suspension of *Aspergillus niger* NB2 (1.2×10^7) was spread carefully over the surface of the medium. The mixture was allowed to ferment at 30°C for 20 d without shaking. The characteristics of the composted sewage sludge and the dry olive cake after fermentation were determined by standard methods (*Page* et al., 1982) and are shown in Tab. 1.

Table 1: Chemical characteristics of the composted sewage sludge and the fermented dry olive cake.

	Composted sewage sludge	**Fermented dry olive cake**
pH (H_2O, 1:5)	6.1 ± 0.0*	4.0 ± 0.0
Electrical conductivity (1:5, $\mu S\ cm^{-1}$)	3095 ± 48	1231 ± 6
Organic C ($g\ kg^{-1}$)	380 ± 4	222 ± 1
Water-soluble C ($\mu g\ g^{-1}$)	7245 ± 22	1146 ± 9
Total N ($g\ kg^{-1}$)	14.5 ± 0.1	6.2 ± 0.1
Total P ($g\ kg^{-1}$)	4.5 ± 0.1	3.8 ± 0.1
Fe ($\mu g\ g^{-1}$)	6562 ± 165	–
Cu ($\mu g\ g^{-1}$)	212 ± 8	–
Zn ($\mu g\ g^{-1}$)	588 ± 30	–
Ni ($\mu g\ g^{-1}$)	44 ± 3	–
B ($\mu g\ g^{-1}$)	85 ± 2	–
Cd ($\mu g\ g^{-1}$)	9 ± 1	–
Pb ($\mu g\ g^{-1}$)	180 ± 28	–

* Mean ± standard error (N = 6).

2.2 Plant materials

The plant used for the experiment, *J. oxycedrus*, is a low-growing tree reaching a height of 3–4 m, which often grows as a shrub. This shrub has a typical Mediterranean distribution and is well-adapted to drought conditions. However, knowledge of revegetation strategies involving *J. oxycedrus* is still very limited. Seeds of *J. oxycedrus* were grown for 8 months in peat and cocopeat (1:1, v:v) substrate under nursery conditions without fertilizer addition. At the time of planting, average shoot dry weight was about 0.81 g.

The mycorrhizal fungi used in the experiment, *Glomus intraradices* Schenck & Smith (EEZ 1), *Glomus deserticola* Trappe, Bloss. & Menge (EEZ 45), and *Glomus mosseae* (Nicol & Gerd.) Gerd. & Trappe (EEZ 43), were obtained from the collection of the experimental field station of Zaidín, Granada. The acronym EEZ refers to Estación Experimental del Zaidín. The AM fungal inoculum consisted of a mixture of rhizospheric soil from trap cultures (*Sorghum* sp.) containing spores, hyphae, and mycorrhizal root fragments.

2.3 Experimental design and layout

The experiment was a mesocosm assay, laid out as a completely randomized two-factorial design with five replications (45 pots). The first factor (organic amendments) comprised an nonamended control, the addition of composted sewage sludge at a rate of 5% (w/w), and the application of fermented dry-olive-cake residue at a rate of 5% (w/w) to the soil. The second factor (inoculation) comprised an noninoculated control, inoculation of *J. oxycedrus* plants with *G. intraradices,* and inoculation with a mixture of *G. intraradices, G. deserticola*, and *G. mosseae.*

In 600 mL pots, 400 g of soil were placed. In early February 2004, the amendments (composted sewage sludge or fermented dry-olive-cake residue) were mixed manually with the experimental soil. The corresponding arbuscular mycorrhizal inoculum was applied at a rate of 5% (v/v). The same amount of an autoclaved mixture of the inocula was added to control plants, supplemented with a filtrate (<20 μm) of the culture to provide the microbial populations accompanying the mycorrhizal fungi. One *J. oxycedrus* seedling was transplanted per pot, and the plants were well watered with tap water during the 6 months of the growth period.

2.4 Plant analyses

Basal stem diameter and height of plants were measured, and the fresh and dry (105°C, 5 h) mass of shoots and roots were recorded 6 months after treatment application. Plant tissues were ground prior to chemical analysis. Foliar concentration of N and P was determined after digestion in nitric perchloric acid (5:3) for 6 h, by colorimetry according to *Murphy* and *Riley* (1962). Foliar P was determined colorimetrically according to *Murphy* and *Riley* (1962), foliar N was determined after Kjeldahl digestion.

The percentage of root length colonized by arbuscular mycorrhizal fungi was calculated by the gridline intersect method (*Giovannetti* and *Mosse*, 1980) after staining with trypan blue (*Phillips* and *Hayman*, 1970).

Nitrate reductase activity was assayed *in vivo* by measuring NO_2^- production in tissue that had been vacuum infiltrated with buffered NO_3^- solutions (*Downs* et al., 1993). The roots and shoots from the seedlings were collected in the morning between 8:30 and 11:00 solar time. Roots and shoots of *J. oxycedrus* were cut into 5 mm sections. Approximately 300 mg of tissue were placed into tubes containing 2 mL of an incubation medium consisting of 0.05 M tris-HCl at pH 7.8 and 0.25 M KNO_3. The tubes were sealed and kept in the dark at 30°C for 1h. The nitrite released into the medium was determined after incubation by treating 1 mL of the aliquots with 1 mL of 1% sulfanilamide in 1 M HCl and 1 mL of 0.01% N-1-napthyl-ethylenediamine hydrochloride (NNEDA). After 15 min, the optical density was measured at 540 nm with a Beckman spectrophotometer.

2.5 Statistical analyses

Data were log-transformed to achieve normality. Additions of composted sewage sludge or fermented dry-olive-cake residues, mycorrhizal inoculation and their interaction effects on measured variables were tested by a two-way analysis of variance, and comparisons among means were made using the least-significant-difference (LSD) test, calculated at $p < 0.05$. Statistical procedures were carried out with the software package SPSS 10.0 for Windows.

3 Results

3.1 Growth and mycorrhizal infection of *J. oxycedrus*

The effect of the application of organic amendments to the soil and the inoculation of *J. oxycedrus* with mycorrhizae, sole or in combination, on plant-growth characteristics and fungal colonization of roots was compared. Six months after treatment application, the addition of composted sewage sludge and of fermented dry olive cake as well as the mycorrhizal inoculation had significantly increased shoot and root dry weights, height, and basal diameter of *J. oxycedrus* seedlings compared to the nonamended and noninoculated control (Tabs. 2 and 3). The AM inoculation increased shoot biomass more effectively than the addition of organic amendments alone, and there were no significant differences between the two mycorrhizal-inoculation treatments. Combining mycorrhizal inoculation with the addition of composted sewage sludge or fermented dry olive cake increased the growth of seedlings more than with each treatment applied separately.

All treatments increased foliar contents of N and P over the control plants (Tabs. 2 and 3). As observed for the growth

Table 2: Growth parameters, leaf N and P content, mycorrhizal colonization of roots, and nitrate reductase activity of *J. oxycedrus* seedlings in response to composted sewage sludge and addition of fermented dry olive cake and mycorrhizal inoculation 6 months after treatment application (n = 5).

	C	S	D	G	M	SG	SM	DG	DM
Height (cm)	19* (1)	26 (1)	24 (1)	27 (1)	24 (1)	28 (1)	29 (1)	29 (1)	23 (1)
Basal diameter (mm)	2.8 (0.0)	3.5 (0.0)	3.3 (0.1)	3.6 (0.1)	3.4 (0.1)	3.4 (0.1)	3.1 (0.1)	3.1 (0.1)	3.2 (0.0)
Shoot dry matter (g plant^{-1})	2.64 (0.07)	3.39 (0.06)	3.44 (0.04)	3.84 (0.15)	4.21 (0.17)	4.19 (0.09)	4.32 (0.18)	4.46 (0.04)	3.96 (0.06)
Root dry matter (g plant^{-1})	1.46 (0.13)	2.65 (0.12)	2.12 (0.10)	2.71 (0.11)	2.30 (0.12)	2.65 (0.11)	2.41 (0.15)	2.52 (0.05)	2.05 (0.10)
Nitrogen (mg plant^{-1})	15.4 (0.9)	30.1 (1.0)	31.0 (0.8)	35.2 (0.9)	36.1 (1.2)	35.4 (1.1)	34.4 (1.3)	34.5 (1.0)	35.6 (1.2)
Phosphorus (mg plant^{-1})	0.90 (0.11)	2.80 (0.25)	3.00 (0.19)	3.52 (0.20)	3.40 (0.23)	3.41 (0.18)	3.25 (0.17)	3.33 (0.20)	3.40 (0.21)
Mycorrhizal colonization (%)	18 (1)	5 (1)	10 (2)	47 (2)	63 (1)	37 (2)	33 (1)	53 (2)	56 (1)
Shoot NR activity (nmol NO_2^- (g FW)$^{-1}$ h^{-1})	3.58 (0.12)	2.77 (0.07)	7.08 (0.73)	3.89 (0.45)	1.98 (0.07)	4.95 (0.39)	3.01 (0.21)	4.53 (0.39)	6.94 (0.81)
Root NR activity (nmol NO_2^- (g FW)$^{-1}$ h^{-1})	6.54 (0.13)	12.93 (0.52)	14.75 (1.36)	11.19 (0.96)	10.65 (0.19)	11.65 (0.87)	11.10 (0.71)	12.32 (0.38)	31.94 (1.28)

C = control; S = composted-sewage-sludge addition; D = addition of fermented dry olive cake; G = inoculation with *G. intraradices*; M = inoculation with a mixture of three AM fungi; SG = composted-sewage-sludge addition and inoculation with *G. intraradices*; SM = composted-sewage-sludge addition and inoculation with a mixture of three AM fungi; DG = addition of fermented dry olive cake and inoculation with *G. intraradices*; DM = addition of fermented dry olive cake and inoculation with a mixture of three AM fungi.
NR: nitrate reductase; FW: fresh weight.

Table 3: Two-factor ANOVA (composted-sewage-sludge addition and addition of fermented dry olive cake and mycorrhizal-inoculation treatments) for selected performance parameters of *J. oxycedrus* seedlings 6 months after treatment application.

	Amendment (A)	Mycorrhiza (M)	Interaction (A × M)
Height	<0.001	<0.001	0.008
Basal diameter	0.063	0.002	<0.001
Shoot	0.001	<0.001	0.029
Root	0.009	0.002	0.003
Foliar N	<0.001	<0.001	0.322
Foliar P	<0.001	<0.001	0.185
Colonization	0.010	<0.001	0.846
Shoot nitrate reductase	0.047	0.373	0.339
Root nitrate reductase	0.001	0.041	0.180

parameters, mycorrhizal inoculation was the most effective treatment to increase foliar N and P contents of shoots.

Mycorrhizal inoculation resulted in significantly higher percentages of root colonization by the fungus than in noninoculated plants, with highest colonization in case of the mixture of three AM fungi (Tab. 2). The application of composted sewage sludge or fermented dry olive cake reduced root colonization, irrespective of the inoculation treatment.

3.2 Nitrate reductase activity

Irrespective of treatment, nitrate reductase (NR) activity was lower in shoots than in roots of *J. oxycedrus* (Tab. 2). Only treatments involving the application dry olive cake resulted in a significant increase of shoot NR activity . This effect was largest when dry-olive-cake application was combined with a mixed inoculation of mycorrhizal fungi (Tabs. 2 and 3). In roots, all but the control treatments tended to increase NR activity. However, this effect was significant only in the case of dry olive cake, irrespective of the AM-inoculation treatment. There was a positive interaction between the addition of fermented dry olive cake and the inoculation with the mixture of three AM fungi. The root NR activity of seedlings inoculated with a mixture of mycorrhizal fungi and grown in the soil amended with fermented dry olive cake was about five times higher than that of control plants.

4 Discussion

A successful reforestation of degraded land may require the addition of nutrients and organic matter to the soil. Two widely available organic nutrient sources in the Mediterranean region comprise sewage sludge and olive residues. Furthermore, there is a need to enhance the capacity of the reforestation species to acquire and assimilate nutrients. This can effectively be achieved by inoculation with appropriate mycorrhiza strains. The combined effects of organic soil amendments and mycorrhizal inoculation is seen to substantially improve the performance of reforestation species such as *J. oxycedrus.*

Published research on the use of organic soil amendments in eroded soils shows that organic amendments can improve a range of soil-physical parameters and increase soil nutrient content and availability, particularly of N and P (*Navas* et al., 1999; *Cox* et al., 2001). The results of the present study demonstrate the viability of applying fermented dry-olive-cake residues in the presence of rock phosphates and composted sewage sludge in order to improve nutrient supply and hence the growth of *J. oxycedrus.* Plants grown in soil amended with fermented dry olive cake or composted sewage sludge had higher nutrient (N and P) contents in their tissues than plants grown in the nonamended soil. During the course of *A. niger* fermentation, the rock phosphate is solubilized, increasing the level of bio-available P in the dry olive cake (*Vassilev* et al., 1998). The absence of a negative effect of the fermented residue on the growth of *J. oxycedrus* suggests that phytotoxic substances in dry olive cake are reduced during the fermentation process. The two organic amendments produced similar increases in biomass yield, in spite of the different amount and nature of their organic matter and different contribution of N and P nutrients to soil. For example, the dissolved organic carbon or water-soluble C, which has been proposed as a parameter of compost maturity, was higher in the composted sewage sludge than in the fermented dry olive cake.

The two mycorrhizal-inoculation treatments showed the same effectiveness with respect to improving the performance of *J. oxycedrus*, and both inoculation treatments were more effective with respect to increasing shoot biomass than the addition of organic amendments alone. The fact that the foliar N and P contents of plants inoculated with AM fungi were higher than those of noninoculated plants grown in the soil amended with fermented dry olive cake or composted sewage sludge reaffirms the key role of mycorrhizae in sustaining the plant cover under conditions of nutrient deficiency, as well as showing the potential of including mycorrhizal inoculation to guarantee plant performance in revegetation programs. On the other hand, the benefit of the combined treatment on growth of *J. oxycedrus* seedlings was not additive and was similar to that of each individual treatment.

Juniperus species prefer the uptake of nitrate over that of ammonium. Nitrate is also the dominant inorganic N form in aerobic organic substrates such as sewage-sludge compost or dried olive cake. However, nitrate needs to be enzymatically reduced to translate nitrate-N uptake into N assimilation for proteins or chlorophyll synthesis. The enzyme responsible for this reduction, nitrate reductase, is induced by high nitrate supply (*Kandlbinder* et al., 2000). The fact that plants growing in the amended soil showed higher NR activity than control plants could be due to higher soil nitrate supply, arising from the fermented dry olive cake or composted sewage sludge. The reduction process is highly energy-demanding and hence frequently limited by P availability. Combining the NR activation through nitrate addition *via* organic amendments with improved P uptake trough mycorrhizal fungi is seen to enhance the N assimilation of *J. oxycedrus.* It is worth noting the synergistic effect between the fermented dry olive cake and inoculation with a mixture of three AM fungi for increasing NR activity in roots of *J. oxycedrus.*

In addition to the effect of mycorrhizal activity on plant N acquisition, some studies have shown an increased NR activity both in the shoot (*Azcón* and *Tobar*, 1998) and in the root (*Caravaca* et al., 2003b) of mycorrhizal plants. This can be seen as an indicator for NO_3^- assimilation, indicating an increase in N metabolism in AM plants. In our study, the presence of AM fungi in the roots positively affected NR activity in the roots of *J. oxycedrus,* but there were no significant differences between the two mycorrhizal treatments. Some authors speculated that the increase in NR activity of mycorrhizal plants can be related to this enzyme's requirement for phosphate, which may complex with the molybdenum of the enzyme, thereby facilitating NO_3^- reduction (*Ruíz-Lozano* and *Azcón*, 1996). As indicated above, inoculated plants had higher foliar P contents than noninoculated plants. Apart from a mycorrhizal effect on the NR activity of the host plants, it has been shown that AM fungi possess NR activity (*Kaldorf* et al., 1998).

In conclusion, the mycorrhizal inoculation effectively stimulated the growth of *J. oxycedrus.* Both microbial-treated dry olive cake and composted sewage sludge enhanced nutrient uptake and N assimilation. The combined use of both organic amendments and mycorrhizal inoculation appears effective to stimulate *J. oxycedrus*-seedling growth for use in reforestation programs.

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References

Azcón, R., Tobar, R. M. (1998): Activity of nitrate reductase and glutamine synthetase in shoot and root of mycorrhizal *Allium cepa*. Effect of drought stress. *Plant Sci.* 133, 1–8.

Caravaca, F., Barea, J. M., Palenzuela, J., Figueroa, D., Alguacil, M. M., Roldán, A. (2003a): Establishment of shrub species in a degraded semiarid site after inoculation with native or allochthonous arbuscular mycorrhizal fungi. *Appl. Soil Ecol.* 22, 103–111.

Caravaca, F., Alguacil, M. M., Díaz, G., Roldán, A. (2003b): Use of nitrate reductase activity for assessing the effectiveness of mycorrhizal symbiosis in *Dorycnium pentaphyllum* under induced water deficit Comm. *Soil Sci. Plant. Anal.* 34, 2291–2302.

Caravaca, F., Figueroa, D., Alguacil, M. M., Roldán, A. (2003c): Application of composted urban residue enhanced the performance of afforested shrub species in a degraded semiarid land. *Bioresour. Technol.* 90, 65–70.

Cox, D., Bezdicek, D., Fauci, M. (2001): Effects of compost, coal ash, and straw amendments on restoring the quality of eroded Palouse soil. *Biol. Fertil. Soils* 33, 365–372.

Downs, M. R., Nadelhoffer, K. J., Melillo, J. M., Aber, J. D. (1993): Foliar and fine root nitrate reductase activity in seedlings of four forest tree species in relation to nitrogen availability. *Trees* 7, 233–236.

Giovannetti, M., Mosse, B. (1980): An evaluation of techniques for measuring vesicular-arbuscular mycorrhizal infection in roots. *New Phytol.* 84, 489–499.

Gliotti, C., Giusquiani, P. L., Businelli, D., Machioni, A. (1997): Composition changes of dissolved organic matter in a soil amended with municipal waste compost. *Soil Sci.* 162, 919–926.

Kaldorf, M., Schmelzer, E., Bothe, H. (1998): Expression of maize and fungal nitrate reductase genes in arbuscular mycorrhiza. *Mol. Plant. Microbiol. Interac.* 11, 439–448.

Kandlbinder, A., Weiner, H., Kaiser, W. M. (2000): Nitrate reductase from leaves of Ricinus (*Ricinus communis* L.) and spinach (*Spinacia olerecea* L.) have different regulatory properties. *J. Exp. Bot.* 51, 1099–1105.

Linares, A., Caba, J. M., Ligero, F., de la Rubia, T., Martínez, J. (2003): Detoxification of semisolid olive-mill wastes and pine-chip mixtures using *Phanerochaete flavido-alba. Chemosphere* 51, 887–891.

Murphy, J., Riley, J. P. (1962): A modified single solution method for determination of phosphate in natural waters. *Anal. Chim. Acta* 27, 31–36.

Navas, A., Machín, J., Navas, B. (1999): Use of biosolids to restore the natural vegetation cover on degraded soils in the badlands of Zaragoza (NE Spain). *Bioresour. Technol.* 69, 199–205.

Page, A. L., Miller, R. H., Keeney, O. R. (1982): Methods of soil analysis. American Society of Agronomy and Soil Science Society of America, Madison, Wisconsin.

Phillips, J. M., Hayman, D. S. (1970): Improved procedures for clearing roots and staining parasitic and vesicular-arbuscular mycorrhizal fungi for rapid assessment of infection. *Trans. Br. Mycol. Soc.* 55, 158–161.

Roldán, A., Albaladejo, J., Thornes, J. (1996): Aggregate stability changes in a semiarid soil after treatment with different organic amendments. *Arid Soil Res. Rehab.* 10, 139–148.

Ruíz-Lozano, J. M., Azcón, R. (1996): Mycorrhizal colonization and drought stress as factors affecting nitrate reductase activity in lettuce plants. *Agric. Ecosyst. Environ.* 60, 175–181.

Vassilev, N., Baca, M. T., Vassileva, M., Franco, I., Azcón, R. (1995): Rock phosphate solubilization by *Aspergillus niger* grown on sugar-beet waste medium. *Appl. Microbiol. Biot.* 44, 546–549.

Vassilev, N., Baca, M. T., Vassileva, M., Franco, I., De Nobili, M. (1998): Mineralization of three agro-industrial wastes by an acid-producing strain of *Aspergillus niger*, in De Bertoldi, M., Sequi, P., Lenunes, B., Papi, T.: The Science of Composting. Chapman and Hall London, pp. 1375–1379.

