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The mycorrhizal status and colonization of 26 tree species growing in urban and rural environments

Luke D. Bainard · John N. Klironomos · Andrew M. Gordon

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Abstract Urban environments are highly disturbed and fragmented ecosystems that commonly have lower mycorrhizal fungal species richness and diversity compared to rural or natural ecosystems. In this study, we assessed whether the mycorrhizal status and colonization of trees are influenced by the overall environment (rural vs. urban) they are growing in. Soil cores were collected from the rhizosphere of trees growing in urban and rural environments around southern Ontario. Roots were extracted from the soil cores to determine whether the trees were colonized by arbuscular mycorrhizal fungi, ectomycorrhizal fungi, or both, and to quantify the percent colonization of each type of mycorrhizal fungi. All 26 tree species were colonized by arbuscular mycorrhizal fungi, and seven tree species were dually colonized by arbuscular mycorrhizal and ectomycorrhizal fungi. Overall, arbuscular mycorrhizal and ectomycorrhizal fungal colonization was significantly ($p<0.001$) lower in trees growing in urban compared to rural environments. It is not clear what 'urban' factors are responsible for the reduction in mycorrhizal fungal colonization; more research is needed to determine whether inoculating urban trees with mycorrhizal fungi would increase colonization levels and growth of the trees.

Keywords Urban ecology · Arbuscular mycorrhizal fungi · Ectomycorrhizal fungi

L. D. Bainard (✉)
Department of Integrative Biology, University of Guelph,
50 Stone Road E,
Guelph, ON N1G 2W1, Canada
e-mail: lbainard@uoguelph.ca

J. N. Klironomos
Biology and Physical Geography Unit,
University of British Columbia,
3333 University Way,
Kelowna, BC V1V 1V7, Canada

A. M. Gordon
School of Environmental Sciences, University of Guelph,
50 Stone Road E,
Guelph, ON N1G 2W1, Canada

Introduction

Urban environments account for only 2% of the earth's land surface (Grimm et al. 2000), but yet over half the world's population live in urban areas, and this number is projected to increase to two thirds by 2050 (UN-HABITAT 2006). As a result, urbanization and the associated land transformation processes are only expected to increase. Urban environments are characterized as being highly disturbed and fragmented ecosystems (McDonnell and Pickett 1990), consisting primarily of buildings and sealed surfaces, and patches of managed, ruderal, and natural vegetation (McKinney 2002). These varied and patchy habitats create a highly heterogeneous environment, which enables especially high establishment rates of exotic plant species and reduced number of native species as compared to rural or forest areas (Kowarik 1995). In addition, the substrates found in urban environments are commonly much modified from their original states (Rebele 1994).

Urban expansion can have a strong impact on the physical and biological components of the soil environment. Urban soils have a modified structure due to the mixing and homogenization of soil horizons, reducing the stratification of the soil (Craul 1992), and leading to high spatial heterogeneity throughout individual soil layers (DeKimpe and Morel 2000). Compaction of the soil also occurs due to the breakdown of structure or aggregation as the mechanical action involved in the mixing breaks down some of the

smaller aggregates (Craul 1992). Other common characteristics of urban soils include lack of aeration, higher pH, formation of a surface crust on bare soils, high concentration of various pollutants, and enhanced variability in nutrient content (Craul 1985).

These physical modifications have a strong influence on the biota that inhabits these soils. In general, urban soils compared to rural soils have reduced species diversity, total biomass, and number of organisms (Harris 1991). For example, soil fungal species richness (Lawrynowicz 1982) and abundance (Pouyat et al. 1994) were lower in the urban core compared to the rural surroundings. There is also evidence that anthropogenic activities that result in pollution (Cairney and Meharg 1999), nitrogen deposition (Egerton-Warburton and Allen 2000), and disturbance (Reeves et al. 1979) can have a strong influence on mycorrhizal fungi.

There are only a limited number of studies that have assessed the impact of urban environments on mycorrhizal fungi. Differences in arbuscular mycorrhizal fungal community structure between urban and rural environments have been observed in desert ecosystems (Stabler et al. 2001; Cousins et al. 2003), where lower colonization levels were found in trees growing in urban desert sites compared to rural desert sites (Stabler et al. 2001). In addition, Wiseman and Wells (2005) found that *Acer rubrum* L. trees in urban sites had significantly lower arbuscular mycorrhizal colonization compared to forested sites. A similar trend for ectomycorrhizal fungi has been observed with higher colonization and species richness of ectomycorrhizal fungi found in rural oak forests (Baxter et al. 1999) and evergreen broad-leaved forests (Ochimaru and Fukuda 2007) when compared to urban forest stands.

Based on these limited number of studies, it appears that urban environments tend to have a lower abundance and different mycorrhizal community composition compared to rural or more natural environments. However, very few tree species have been studied in this regard, and it is thus difficult to generalize across the wide variety of tree species that grow in urban environments. The objective of this study was to compare the mycorrhizal colonization of 26 tree species that are found in urban environments in southern Ontario, Canada, to those found in rural environments.

Materials and methods

Tree species

The mycorrhizal colonization of 26 tree species in urban and rural environments was assessed for the following species, *Acer negundo* L., *Acer nigrum* F. Michx., *Acer pensylvanicum* L., *Acer platanoides* L., *Acer rubrum* L., *Acer saccharinum* L., *Acer saccharum* Marshall, *Aesculus hippocastanum* L., *Betula alleghaniensis* Britton, *Betula papyrifera* Marshall, *Cercis canadensis* L., *Fraxinus americana* L., *Fraxinus nigra* Marshall, *Gleditsia triacanthos* L., *Juglans nigra* L., *Juniperus virginiana* L., *Populus deltoides* W. Bartram ex Marshall, *Populus grandidentata* Michx., *Populus tremuloides* Michx., *Prunus pensylvanica* L. f., *Prunus serotina Ehrh.*, *Prunus virginiana* L., *Quercus palustris* Munchh., *Quercus rubra* L., *Robinia pseudoacacia* L., and *Thuja occidentalis* L.

Study sites

For each species, five locations were identified in an urban environment and five locations in a rural environment, each pair of environments separated by a minimum of 5 km. Urban environments included city parks, streetscapes, or private residential property in ten cities located in southern Ontario, Canada (Brampton, Brantford, Burlington, Cambridge, Guelph, Kitchener, Milton, Mississauga, Oakville, and Toronto). Trees sampled in urban environments were likely nursery-produced landscape trees. Rural environments included forested areas (>20 ha each) throughout southern Ontario, Canada. A tree core was taken the previous year from each tree sampled in the study, and only trees that ranged from 20 to 25 years of age were included in the analysis.

At each location, a soil core (6×15 cm) was collected using a bulb planter from beneath three individual trees for each species. To limit any seasonal variation in mycorrhizal colonization, all soil cores were collected from May 26 to June 21. Soils were similar in nature, generally of the Brunisol and Luvisol soil series, and sandy-loam in texture. We acknowledge that soils from urban areas may have been differentially impacted depending upon cultural history in the area. The soil cores from the three individual trees were considered subsamples, and data within a location was pooled. The independent units were the tree species among locations. Soil cores were stored in plastic bags at 4°C prior to processing (approx. 2–3 weeks). Roots were extracted by washing soil cores using a high pressure hose, and cleaned with tap water. From each soil core, a subsample of healthy, fine roots was divided in half and stored in 50% ethanol prior to assessment for arbuscular mycorrhizal and ectomycorrhizal colonization.

Mycorrhizal colonization

Roots were examined for ectomycorrhizal colonization by placing the roots on a tray and counting the number of root tips with mycorrhizal structures. Percent ectomycorrhizal colonization was determined as the proportion of root tips colonized by ectomycorrhizal fungi. In addition, cross sections were prepared (cleared with KOH, and then stained

with Chlorazol Black E) for a subset of ectomycorrhizas to confirm the presence of a hartig net.

Arbuscular mycorrhizal colonization was assessed by clearing (with 10% KOH) and staining (with 0.05% Chlorazol Black E) (Brundrett 1994) a random subset of roots from each sample and mounting on microscope slides. Percent arbuscular mycorrhizal colonization was determined by scoring 150 locations along the root segments for the presence of mycorrhizal structures (McGonigle et al. 1990). The presence of arbuscules, vesicles, and hyphae was also recorded.

Statistical analyses

Urban and rural mycorrhizal colonization means were compared for each tree species individually and for the tree species pooled together using Student's *t* test. The data were square root transformed prior to data analysis in order to meet the assumptions of Student's *t* test, but the untransformed values are presented in the figures. A type I error rate of α=0.05 was used to determine if mycorrhizal colonization was significantly different between trees growing in urban and rural environments. All statistical analyses were performed with SPSS 16.0 (SPSS Inc. 2008).

Results

All 26 tree species were colonized by arbuscular mycorrhizal fungi, and seven tree species were dually colonized by arbuscular mycorrhizal and ectomycorrhizal fungi. Percent arbuscular mycorrhizal colonization ranged from 2.4 to 53.4% (Fig. 1), and ectomycorrhizal colonization from 14.4 to 50.8% (Fig. 2). Tree species that formed a tripartite association with arbuscular mycorrhizal and ectomycorrhizal fungi (*Betula lutea*, *B. papyrifera*, *P. deltoides*, *P. grandidentata*, *P. tremuloides*, *Q. palustris*, *Q. rubra*) had the lowest levels of arbuscular mycorrhizal colonization. The mycorrhizal status of all 26 tree species was not affected by the environment. All 26 tree species, regardless of their urban–rural location, possessed both arbuscules and vesicles associated with arbuscular mycorrhizal fungi. All tree species that formed a tripartite association, regardless of their location, were colonized by ectomycorrhizal fungi.

Overall, trees growing in the urban environments had a reduced arbuscular mycorrhizal colonization compared to trees growing in rural environments. Ten of the tree species growing in urban environments had a significantly lower arbuscular mycorrhizal colonization than in rural environments. *P. deltoides,* which forms a tripartite association, was the only tree species that had a significantly higher arbuscular mycorrhizal colonization in urban environments. When all the tree species were pooled together (Fig. 3), arbuscular mycorrhizal colonization in urban environments was significantly lower (37%, $p<0.001$) compared to rural environments. In contrast, when the tree species that formed a tripartite association were pooled together, arbuscular mycorrhizal colonization was not significantly different ($p>0.05$) between the two environments.

Tree species that formed a tripartite association had lower ectomycorrhizal colonization in urban environments compared to trees growing in rural environments. Two of the seven tree species (*P. deltoides* and *P. tremuloides*)

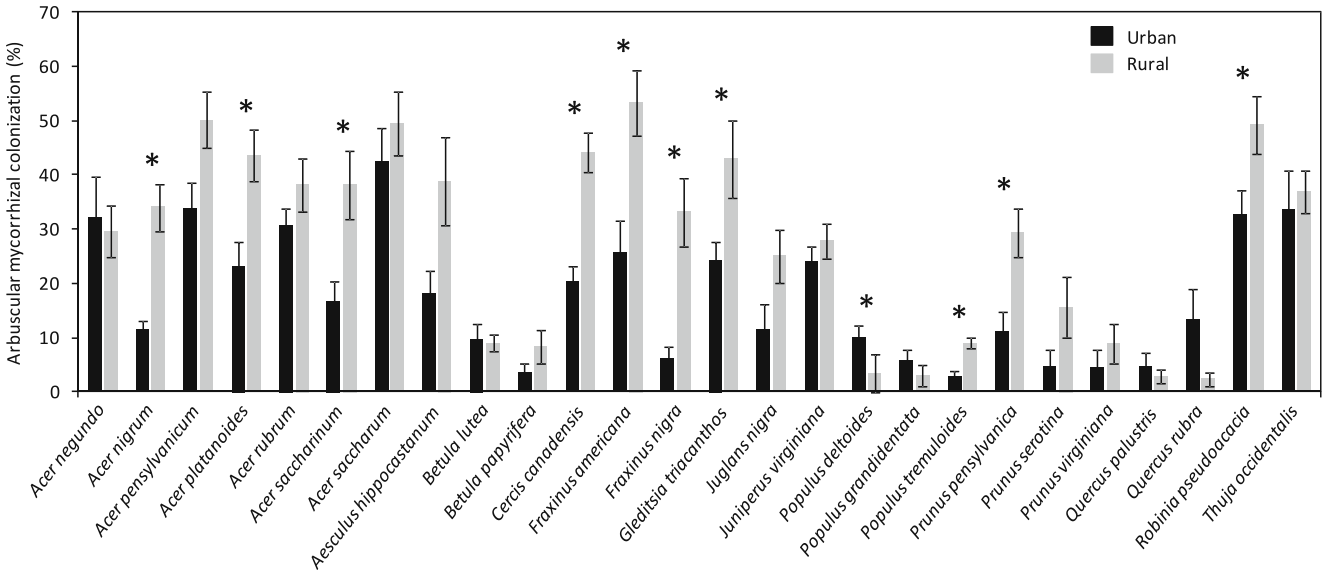

Fig. 1 Arbuscular mycorrhizal colonization of trees growing in urban and rural environments. *Bars with asterisks* above them indicate a significant difference between the urban and rural environments (p=0.05)

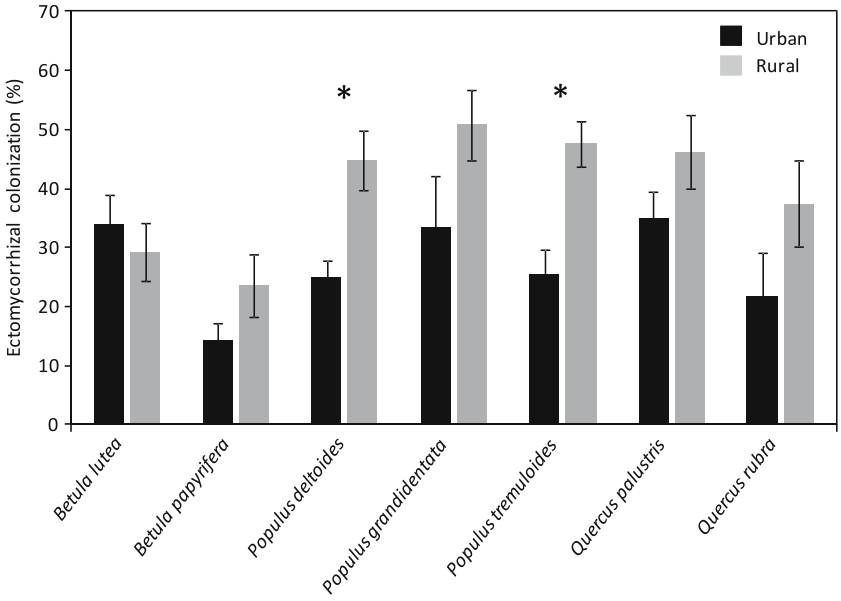

Fig. 2 Ectomycorrhizal colonization of trees growing in urban and rural environments. *Bars with asterisks* above them indicate a significant difference between urban and rural environments (p=0.05)

growing in urban environments had significantly lower ectomycorrhizal colonization than in rural environments. When the tree species that formed a tripartite association were pooled together (Fig. 3), ectomycorrhizal colonization was significantly lower (33%, p<0.001) in urban environments compared to rural environments.

Discussion

The results from this study provide a survey of the mycorrhizal status of 26 tree species growing in urban and rural environments. The mycorrhizal status (presence or absence of mycorrhizal structures) of all the tree species was unaffected by the environment. This provided evidence that mycorrhizal fungi were present, and in sufficient abundance in the urban soils to form arbuscular mycorrhizal, ectomycorrhizal, or dual symbioses with the tree species surveyed in this study.

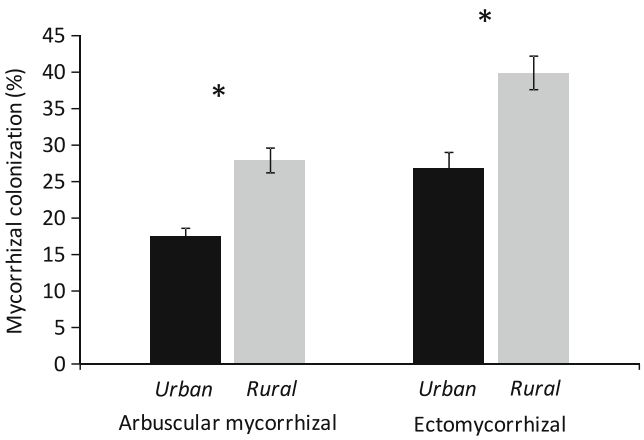

Fig. 3 Mycorrhizal colonization of all tree species pooled together growing in urban and rural environments. *Bars with asterisks* above them indicate a significant difference between urban and rural environments (p=0.05)

The reduced arbuscular mycorrhizal and ectomycorrhizal colonization levels found in trees growing in urban areas suggest that these environments have a lower mycorrhizal fungal propagule abundance or infectivity compared to the less human impacted, rural environments. A similar trend has been observed in a number of other studies for arbuscular mycorrhizal (Stabler et al. 2001; Wiseman and Wells 2005) and ectomycorrhizal fungi (Baxter et al. 1999; Ochimaru and Fukuda 2007). The lower mycorrhizal colonization could be due to the highly disturbed and modified (pH, nutrient content, pollutants, lack of aeration, etc.) nature of urban soils. For example, Jasper et al. (1991) found that following the disturbance of forest soil, the arbuscular mycorrhizal infectivity, or levels of colonization was reduced by almost 50% compared to undisturbed soil. Ectomycorrhizal infectivity and diversity have also been shown to be affected by disturbance. Boerner et al. (1996) found that ectomycorrhizal colonization and diversity were inversely related to time since site disturbance. However, other studies looking at different forms of disturbance such as clear-cutting, have found that while these types of disturbances had an adverse effect on species richness and altered species composition, the ectomycorrhizal infectivity or colonization was not affected (Byrd et al. 2000; Jones et al. 2003).

The lack of or limited number of mycorrhizal host species in urban environments may also be a contributing factor to the reduced mycorrhizal colonization found in this study. The fragmented and disturbed nature of urban

environments limits the number of plant species that associate with ectomycorrhizal fungi. In addition, disturbed soils tend to be inhabited by a high proportion of non-arbuscular mycorrhizal plant species (Reeves et al. 1979), which may help to exasperate or maintain the low mycorrhizal infectivity of the soil. Alternatively, the reduced mycorrhizal colonization of urban trees may be a function of higher environmental stress compared to trees growing in rural environments rather than differences in mycorrhizal abundance or infectivity. Previous studies have shown that mycorrhizal associations can be negatively influenced by environmental stresses, regardless of mycorrhizal abundance (Gehring and Whitham 1992; Klironomos and Allen 1995).

Seven of the 26 tree species in this study formed a tripartite association with arbuscular mycorrhizal and ectomycorrhizal fungi. Tree species that form this type of association are predominantly colonized by arbuscular mycorrhizal fungi during early stages of growth, but colonization declines as trees mature and become more heavily colonized by ectomycorrhizal fungi (Bellei et al. 1992; Egerton-Warburton and Allen 2001). All trees sampled in this study were mature individuals (20–25 years of age) and as expected were well colonized by ectomycorrhizal fungi and had low arbuscular mycorrhizal fungal colonization. Overall, tree species that formed a tripartite association had reduced ectomycorrhizal colonization in the urban environments, but arbuscular mycorrhizal colonization was not significantly different between the environments. Previous studies have shown that abiotic factors such as soil moisture and elevation can alter ectomycorrhizal colonization of tripartite forming tree species (Gehring et al. 2006; Pagano and Scotti 2008; Querejeta et al. 2009). In addition, Weijtmans et al. (2007) found that ectomycorrhizal colonization was reduced in mature *Leptospermum scoparium* J.R. et G. Forst. trees growing in grassland ecosystems that lacked ectomycorrhizal associating species, compared to trees in adjoining forests. The physical modifications of urban soils and reduced number of ectomycorrhizal associating species in urban environments may play a role in the reduced ectomycorrhizal fungal colonization of tree species that form tripartite associations. In turn, these trees may rely more on arbuscular mycorrhizal fungi in urban environments than in rural environments.

The lower levels of mycorrhizal colonization found in urban trees could potentially have an adverse effect on trees growing in urban areas. Inoculating trees, either established or newly planted, with mycorrhizal fungi may increase mycorrhizal colonization levels and possibly the growth and survival of trees in urban environments. A number of studies have attempted to assess the impact of inoculating urban trees with commercial mycorrhizal inoculants with varying results (Garbaye and Churin 1996; Gilman 2001; Ferrini and Nicese 2002; Appleton et al. 2003; Rao et al. 2006). Although urban trees had lower levels of colonization, all trees were well colonized by mycorrhizal fungi in both environments. Further research is needed to determine whether inoculating urban trees with mycorrhizal fungi would increase colonization levels and subsequently the overall growth of trees in urban environments.

In conclusion, this study revealed that in general, arbuscular mycorrhizal and ectomycorrhizal colonization of trees growing in urban environments is lower compared to rural environments. It is not clear what factors are specifically responsible for the reduction in mycorrhizal fungal colonization, although they are likely related to changes due to urbanization as previously discussed. Despite the lower levels of colonization, all tree species were well colonized by mycorrhizal fungi in the urban environments indicating that inoculation may not be necessary, though more research is needed.

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References

Appleton B, Koci J, French S, Lestyan M, Harris R (2003) Mycorrhizal fungal inoculation of established street trees. J Arboric 29:107–110

Baxter JW, Pickett STA, Carreiro MM, Dighton J (1999) Ectomycorrhizal diversity and community structure in oak forest stands exposed to contrasting anthropogenic impacts. Can J Bot 77:771–782

Bellei MM, Garbaye J, Gil M (1992) Mycorrhizal succession in young *Eucalyptus viminalis* plantations in Santa Catarina (southern Brazil). Forest Ecol Manag 54:205–213

Boerner REJ, DeMars BG, Leicht PN (1996) Spatial patterns of mycorrhizal infectiveness of soils long a successional chronosequence. Mycorrhiza 6:79–90

Brundrett M (1994) Clearing and staining mycorrhizal roots. In: Brundrett M, Melville L, Peterson L (eds) Practical methods in mycorrhizal research. Mycologue Publications, Waterloo, pp 42–46

Byrd KB, Parker VT, Vogler DR, Cullings KW (2000) The influence of clear-cutting on ectomycorrhizal fungus diversity in a lodgepole pine (*Pinus contorta*) stand, Yellowstone National Park, Wyoming, and Gallatin National Forest, Montana. Can J Bot 78:149–156

Cairney JWG, Meharg AA (1999) Influences of anthropogenic pollution on mycorrhizal fungal communities. Environ Pollut 106:169–182

Cousins JR, Hope D, Gries C, Stutz JC (2003) Preliminary assessment of arbuscular mycorrhizal fungal diversity and community structure in an urban ecosystem. Mycorrhiza 13:319–326

Craul PJ (1985) A description of urban soils and their desired characteristics. J Arboric 11:330–339

Craul PJ (1992) Urban soil in landscape design. Wiley, New York

DeKimpe CR, Morel JL (2000) Urban soil management: a growing concern. Soil Sci 165:31–40

Egerton-Warburton LM, Allen EB (2000) Shifts in arbuscular mycorrhizal communities along an anthropogenic nitrogen deposition gradient. Ecol Appl 10:484–496

Egerton-Warburton LM, Allen EB (2001) Endo- and ectomycorrhizas in *Quercus agrifolia* Nee. (Fagaceae): patterns of root colonization and effects on seedling growth. Mycorrhiza 11:283–290

Ferrini F, Nicese FP (2002) Response of English oak (*Quercus robur* L.) trees to biostimulants application in the urban environment. J Arboric 28:70–75

Garbaye J, Churin JL (1996) Effect of ectomycorrhizal inoculation at planting on growth and foliage quality of *Tilia tomentosa*. J Arboric 22:29–34

Gehring CA, Whitham TG (1992) Reduced mycorrhizae on *Juniperus monosperma* with mistletoe: the influence of environmental stress and tree gender on a plant parasite and a plant-fungal mutualism. Oecologia 89:298–303

Gehring CA, Mueller RC, Whitham TG (2006) Environmental and genetic effects on the formation of ectomycorrhizal and arbuscular mycorrhizal associations in cottonwoods. Oecologia 149:158–164

Gilman EF (2001) Effect of nursery production method, irrigation, and inoculation with mycorrhizae-forming fungi on establishment of *Quercus virginiana*. J Arboric 27:30–39

Grimm NB, Grove JM, Pickett STA, Redman CL (2000) Integrated approaches to long-term studies of urban ecological systems. Biosci 50:571–584

Harris JA (1991) The biology of soils in urban areas. In: Bullock P, Gregory PJ (eds) Soils in the urban environment. Blackwell Scientific Publications, Oxford, pp 139–152

Jasper DA, Abbott LK, Robson AD (1991) The effect of soil disturbance on vesicular–arbuscular mycorrhizal fungi in soils from different vegetation types. New Phytol 118:471–476

Jones MD, Durall DM, Cairney JWG (2003) Ectomycorrhizal fungal communities in young forest stands regenerating after clearcut logging. New Phytol 157:399–422

Klironomos JN, Allen MF (1995) UV-B mediated changes on below-ground communities associated with the roots of *Acer saccharum*. Funct Ecol 9:823–930

Kowarik I (1995) On the role of alien species in urban flora and vegetation. In: Pysek P, Prach K, Rejmanek M, Wade M (eds) Plant invasions—general aspects and special problems. SPB Academic Publishing, Amsterdam, pp 85–103

Lawrynowicz M (1982) Macro-fungal flora of Lodz. In: Bornkamm R, Lee JA, Seaward MRD (eds) Urban, ecology, the second European ecological symposium. Blackwell Scientific Publications, Oxford, pp 41–47

McDonnell MJ, Pickett STA (1990) Ecosystem structure and function along urban–rural gradients: an unexploited opportunity for ecology. Ecol 71:1232–1237

McGonigle TP, Miller MH, Evans DG, Fairchild GL, Swan JA (1990) A new method which gives an objective measure of colonization of roots by vesicular–arbuscular mycorrhizal fungi. New Phytol 115:495–501

McKinney ML (2002) Urbanization, biodiversity, and conservation. Biosci 52:883–890

Ochimaru T, Fukuda K (2007) Changes in fungal communities in evergreen broad-leaved forests across a gradient of urban to rural areas in Japan. Can J For Res 37:247–258

Pagano MC, Scotti MR (2008) Arbuscular and ectomycorrhizal colonization of two *Eucalyptus* species in semiarid Brazil. Mycoscience 49:379–384

Pouyat RV, Parmelee RW, Carreiro MM (1994) Environmental effects of forest soil-invertebrate and fungal densities in oak stands along an urban–rural land use gradient. Pedobiol 38:385–399

Querejeta JI, Egerton-Warburton LM, Allen MF (2009) Topographic position modulates the mycorrhizal response of oak trees to interannual rainfall variability. Ecology 90:649–662

Rao B, Marx DH, Jeffers B (2006) Response of oaks and elm to soil inoculations with mycorrhizal fungi and rhizobacteria in a nursery. Arboric Urban For 32:62–66

Rebele F (1994) Urban ecology and special features of urban ecosystems. Glob Ecol Biogeogr Lett 4:173–187

Reeves FB, Wagner D, Moorman T, Kiel J (1979) The role of endomycorrhizae in revegetation practices in the semi-arid west. I. A comparison of incidence of mycorrhizae in severely disturbed vs. natural environments. Am J Bot 66:6–13

Stabler LB, Martin CA, Stutz JC (2001) Effect of urban expansion on arbuscular mycorrhizal fungal mediation of landscape tree growth. J Arboric 27:193–202

UN HABITAT (2006) State of the world's cities 2006/7. United Nations, New York

Weijtmans K, Davis M, Clinton P, Kuyper TW, Greenfield L (2007) Occurrence of arbuscular mycorrhiza and ectomycorrhiza on *Leptospermum scoparium* from Rakaia catchment, Canterbury. New Zeal J Ecol 31:255–260

Wiseman PE, Wells C (2005) Soil inoculum potential and arbuscular mycorrhizal colonization of *Acer rubrum* in forested and developed landscapes. J Arboric 31:296–302