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Using soil microbial inoculations to enhance substrate performance on extensive green roofs

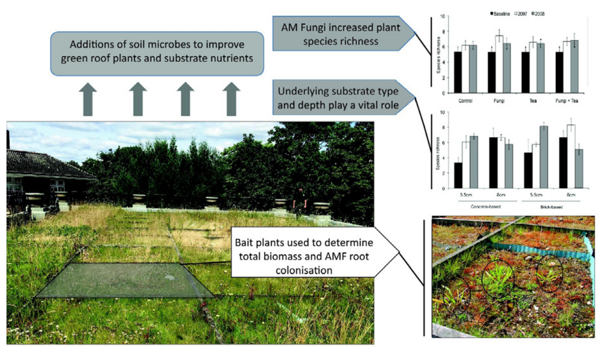
Chloe J. Molineux [a,*], Alan C. Gange [b], Darryl J. Newport [a]

[a] Sustainability Research Institute, University of East London, Docklands Campus, 4-6 University Way, London E16 2RD, United Kingdom
[b] School of Biological Sciences, Royal Holloway University of London, Egham, Surrey TW20 0EX, United Kingdom

HIGHLIGHTS

- AM Fungi and 'Compost Tea' improved plant performance measurements and AMF root colonisation, compared to controls.
- Substrate nutrient levels were extremely limited, but N and P were increased with treatments in brick-based substrates.
- Plant sp. richness improved with AM fungal inoculants but was also affected significantly by depth over time.

GRAPHICAL ABSTRACT



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ABSTRACT

Green roofs are increasing in popularity in the urban environment for their contribution to green infrastructure; but their role for biodiversity is not often a design priority. Maximising biodiversity will impact positively on ecosystem services and is therefore fundamental for achieving the greatest benefits from green roofs. Extensive green roofs are lightweight systems generally constructed with a specialised growing medium that tends to be biologically limited and as such can be a harsh habitat for plants to thrive in. Thus, this investigation aimed to enhance the soil functioning with inoculations of soil microbes to increase plant diversity, improve vegetation health/performance and maximise access to soil nutrients. Manipulations included the addition of mycorrhizal fungi and a microbial mixture ('compost tea') to green roof rootzones, composed mainly of crushed brick or crushed concrete. The study revealed that growing media type and depth play a vital role in the microbial ecology of green roofs, with complex relationships between depth and type of substrate and the type of microbial inoculant applied, with no clear pattern being observed. For bait plant measurements (heights, leaf numbers, root/shoot biomass, leaf nutrients), a compost tea may have positive effects on plant performance when grown in substrates of shallower depths (5.5 cm), even one year after inoculums are applied. Results from the species richness surveys show that diversity was significantly increased with the application of an AM fungal treatment and that overall, results suggest that brick-based substrate blends are most effective for vegetation performance as are deeper depths (although this varied with time). Microbial inoculations of green roof habitats appeared to be sustainable; they need only be done once for benefits to still been seen in subsequent years where treatments are added independently (not in combination). They seem to be a novel and viable method of enhancing rooftop conditions.

* Corresponding author.
E-mail address: chloe_molineux@yahoo.com (C.J. Molineux).

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1. Introduction

Extensive green roofs are those with a shallow rootzone – generally between 5–15 cm in depth, and often fall into three main types: *Sedum* systems, wildflower systems and biodiverse roofs. From an ecological perspective, biodiverse roofs that mimic brownfield habitat are of great interest and importance in our urban landscapes (Schadek et al., 2009). With increasing construction in our cities it is vital to create wildlife spaces to mitigate associated negative effects. Biodiverse green roofs therefore offer great potential, if designed appropriately (Lundholm, 2015), to offer regional biodiversity at roof level (Connop et al., 2016). The issue is that many green roofs are constructed with a lack of knowledge about how to maximise biodiversity (Kadas, 2002). *Sedum* systems are selected by architects for their proven hardiness to rooftop conditions (Monterusso et al., 2005) and the aesthetic value of instant greening (Molineux et al., 2009). Biodiverse roofs are becoming more popular in cities like London, however these are often extremely homogenous – with the same substrate type and depth (Heim and Lundholm, 2014) over the roofs' entirety. Substrate type is particularly important (Molineux et al., 2009; Graceson et al., 2014b; Bates et al., 2015; Molineux et al., 2015; Eksi and Rowe, 2016), as it is the main green roof component that will support the vegetation. Previous studies suggest that engineered substrates may be biologically limited but that microbial inoculants could be used to enhance the functioning belowground (Molineux et al., 2014; Ondoño et al., 2014; Young et al., 2015). Thus a physically engineered substrate, that has considered biological functionality, will underpin the success of a specified planting scheme.

Soil microbial communities at ground level have been well studied in many habitats. These microscopic organisms, including bacteria and fungi, are vital for colonisation of a substrate by plants (Lavelle et al., 2006). They offer favourable conditions for plants to extract limited nutrients, either by breaking down and recycling dead and decaying matter, or by providing access to nutrient pools that can be unexploitable (Smith and Read, 2010). One group in particular, the arbuscular mycorrhizal fungi (AMF), facilitate this via hyphal networks in plant root cells (Van der Heijden et al., 1998) and in doing so also increase root hair surface area allowing access to water films on soil particles in times of extreme drought stress (Allen, 2009). AMF comprise of about 150 known fungal species and are said to be associated with around 80% of all plant species root systems (Hodge, 2000).

The microbial ecology of green roof habitats is beginning to receive attention McGuire et al., 2013, Rumble and Gange, 2013, John et al., 2014, Buffam and Mitchell, 2015, however little of this research links the effects of microbial communities to plant growth on green roofs (Young et al., 2015) or their effects on substrate nutrient levels. Green roofs can be extreme environments for many plant species; thus microbial groups such as AMF could potentially provide vegetation with a better chance of survival at roof level (Molineux et al., 2014). This in turn would help maintain ecosystem services, like building cooling, evapotranspiration and reduction in the urban heat island effect (Oberndorfer et al., 2007; Lundholm et al., 2010); as well as increased storm water retention (Connop et al., 2016), carbon sequestration (Parras-Alcántara et al., 2015) and urban soil security (Anaya-Romero et al., 2015).

The aim of the research was to determine how substrate type and depth effected plant species richness and plant 'health' determined by performance measurements such as heights, leaf numbers, root and shoot biomass. It also explores the additions of microbial inoculants to green roof substrates and the effect this had, not only on the microbial communities themselves (as described in Molineux et al., 2014), but also on the substrate nutrients and bait plant leaf nutrients. The main research questions regarding the addition of microbial inoculations to various substrate types and depths (described in Methods section) were, did they (i) produce larger plants (heights, leaf numbers, root and shoot biomass), (ii) increase root colonisation by beneficial arbuscular mycorrhizal fungi, (iii) effect leaf nutrient levels, (iv) increase species diversity and (v) increase available soil nutrients?

2. Methods

2.1. Field site

To study the effects of substrate type and depth, an existing experimental set-up on the gift shop at London Zoo (Regents Park, London) was utilised and microbial inoculation treatments were applied. The experimental green roof is approximately 180 m^2 and split into 2 m × 2 m plots which contain various substrates at five different depths (further details in Kadas, 2007). Molineux et al. (2014) fully describes the additions of the microbial treatments, but in short: two substrate types (brick-based and concrete-based) at two of the depths (5.5 cm and 8 cm) were chosen for the investigation, each replicated 3 times. Substrate properties data can be found in Appendix I. The existing plots were further divided into quarters, which were then used for the microbial manipulation experiments. The inoculations were applied three times over the summer of 2007. The treatments were a commercial arbuscular mycorrhizal fungal mix (hereafter referred to as 'Fungi'), a live compost tea containing bacteria and fungi (Tea), a combination of both treatments (Fungi + Tea), and finally control plots where no inoculants were added (Control). Information on product content is available at: http://www.symbio.co.uk/horticulture_datasheets.aspx.

2.2. Bait plants

Before microbial manipulation could begin, bait plants – to be used as indicators for any changes in plant growth due to the addition of microorganisms – were planted into the experimental plots. The bait plant species chosen was *Plantago lanceolata*; as a perennial it retains some leaves over winter and re-sprouts each spring from the rootstock, making the recording of growth from one year to the next possible. It is strongly mycorrhizal and is often used as a model plant in field studies (e.g. Walter et al., 2016). By growing the *P. lanceolata* in pumice, in a controlled temperature room, the bait plant roots remained mycorrhizal-free until added to the green roof plots. Colonisation of the roots could then be analysed in the different treatments, by removing one bait plant from each treatment plot annually. This also allowed for the collection of dry shoot and root biomass data whilst leaving the established green roof *P. lanceolata* population undisturbed by the experiment. Four bait plants of *P. lanceolata* were planted into each of the designated experimental plots in May 2007, after three months of growth in a control temperature room at Royal Holloway University. This was to ensure that at least two plants would survive for removal after treatments were applied. Plants were selected for similarity in size in order for height comparisons to be made, and to reduce plant phenotypic variability.

2.2.1. Plant heights & leaf numbers

Plant heights and leaf numbers for the bait plants of *P. lanceolata* were recorded in November 2007, following treatments and November 2008, a year after treatments were first applied. Means taken from three replicates were used to determine any differences between the underlying substrate types (including depth) and the microbial treatments.

All samples were taken in November, so that seasonal variation in microbial biomass (Blume et al., 2002) was reduced as much as possible, many studies have also shown microbial biomass is increased under cool and wet conditions, thus November represented an ideal soil sampling time (Van Gestel et al., 1992; Arnold et al., 1999; Papatheodorou et al., 2004). November also represented the end of the growing season on our zoo green roof and therefore the plants were at their largest before the frost began to restrict their growth.