Case study : Planting methods and beneficial substrate microbes effect on the growth of vegetated roof plants in Finland

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Abstract

Vegetated roofs, often called “green roofs”, are popular and necessary in urban greening in densely populated areas. Well-functioning vegetated roofs can provide various ecosystem services to urban residents (e.g., stormwater management, air pollution mitigation, and aesthetic value). Plants essentially determine the actualization of the ecosystem services, thus finding effective ways to establish and maintain the roof plants is important. While greenhouse experiments can be better controlled than field experiments, it is critical to test whether results gained in the greenhouse hold in actual roof conditions. Therefore, we investigated the effects of microbial inoculant, plant species, planting method, and their interactions on plant growth and the beneficial microbes in the roof substrate at the initial establishment of vegetated roofs. The selected plants (i.e., *Antennaria dioica*, *Campanula rotundifolia*, *Fragaria vesca*, *Geranium sanguineum*, *Lotus corniculatus*, *Thymus serpyllum*, *Trifolium repens*, and *Viola tricolor*) were established using pre-grown vegetation mats, plug plants, and seeds, each with and without co-inoculation with *Rhizophagus irregularis* and *Bacillus amyloliquefaciens*, two plant growth-promoting microbial species. Eventually, only *F. vesca*, *T. serpyllum*, *T. repens*, and *V. tricolor* were found successfully settled in either of the three planting methods. Dry aboveground plant biomass was measured to assess the effects of co-inoculation on plant growth. *R. irregularis* colonization level and *B. amyloliquefaciens* bacterial density were detected from root and substrate samples, respectively. The results indicated that co-inoculation with *R. irregularis* and *B. amyloliquefaciens* successfully colonized target plant species and significantly increased the initial growth of the vegetated roof plants by 18 to 292%. Additionally, the abundance of *R. irregularis* was affected by plant species (*F. vesca* > *T. serpyllum* > *T. repens*) and planting methods (seed > plug > mat), while the bacterial density of *B. amyloliquefaciens* was higher in *T. repens* roots than the other plant species, and was not affected by planning methods.

The results suggest that co-inoculating *R. irregularis* and *B. amyloliquefaciens* at the installation phase of vegetated roofs could improve microbial settlement and colonization in the substrate, and consequently achieve synergistic effect on plant growth. The study also provides basis and reference for future vegetated roofs research.

Keywords: *Bacillus amyloliquefaciens*, plant growth promoting, planting methods, *Rhizophagus irregularis*, vegetated roof

1. Introduction

Even though urban green spaces essentially contribute towards biodiverse, self-sustaining, climate change-resistant and aesthetic living environments, urban land use often prioritizes other forms of urban development (Arnfield, 2003; Bowler et al., 2010). Where urban space is congested by several interests, vegetated roofs provide opportunities for urban greening (Yang et al., 2008). A vegetated roof, often called a "green roof", is a rooftop of a building where vegetation is grown in substrates. The past few decades have witnessed an expansion of the vegetated roof industry and applications on both public and private buildings, primarily because of the environmental advantages that vegetated roofs provide, e.g., managing stormwater, mitigating air pollution, lessening the urban heat-island effect, and enhancing the aesthetics of the urban setting (DeNardo et al., 2005; Oberndorfer et al., 2007; Yang et al., 2008). Plants are key for providing such ecological advantages, yet it is often challenging to grow plants on rooftops, especially when shallow substrate (<4 cm for moss-sedum roofs and <15 cm for grass-herbaceous plant roofs) is often used due to load capacity restrictions of the building (Forschungsgesellschaft Landschaftsentwicklung Landschaftsbau, 2008). In contrast to the thicker substrate, a shallow substrate on vegetated roofs is usually limited in water availability

during continuously dry and hot seasons and is not resistant to temperature fluctuation due to continuous, direct exposure to sunlight and wind (Lazzarin et al., 2005; Henry and Frascaria-Lacoste, 2012; Klein and Coffman, 2015). The mortality of non-succulents on vegetated roofs is generally high at the initial establishment phase, even with irrigation (Monterusso et al., 2005; Wolf and Lundholm, 2008;). Therefore, studies have been conducted to identify the factors that influence the survival of vegetated roof plants, such as substrate depth (Durhman et al., 2007), hydrogel amendment (Savi et al., 2014), and microbial community (Fulthorpe et al., 2018).

Plant-microbe interaction is an important part of any functional ecosystem, and inoculants of plant growth-promoting microbes (PGPMs) have been proven effective in promoting the growth of agro-economically important crops (Gangwar et al., 2017; Mishra et al., 2017). Recently, more and more researchers have laid their eyes microbial community and PGPM application on vegetated roofs. Rumble (2013) has conducted extensive vegetated roof research on microbial community surveys and manipulation. She reported that microbial community on vegetated roofs is low in abundance, and the microbial community will eventually adapt to arid conditions and exhibited seasonal change. She also found that inoculation did not significantly alter microbial mass in new vegetated roofs, but remediated soil food webs in mature vegetated roofs. Molineux et al. (2014) found that when AMF and compost tea (containing beneficial bacteria) were applied together on vegetated roofs, the microbial biomass was significantly reduced compared to single application of the compost tea, indicating competition between the AMF and beneficial bacteria. These studies suggest that microbial manipulation on vegetated roofs is possible and the outcomes are affected by various factors.

There are four commonly used methods to establish plants on rooftops according to Dvorak (2011), i.e., precultivation, plugs, seed, and cutting. Emilsson and Rolf (2005) reported

that sedum coverage was higher in mat plots than cutting and plug plots, while moss coverage was highest in cutting plots and lowest in mat plots. Monterusso et al., (2005) found no difference in sedum coverage when planted as seeds or plugs on the vegetated roof. Another study reported that adding AMF inoculant directly to plug plants (*Prunella vulgaris*) on vegetated roof resulted in the highest AMF colonization than applying in the surrounding substrate or between plug and substrate (Young et al., 2015).

Despite the fast-growing number of studies on vegetated roofs, there are clear gaps in the knowledge. Firstly, most inoculants applied were unspecified inoculant mixtures, e.g., compost tea (Molineux et al., 2014), with unknown compatibilities among the microbes. As a result, the desired effect might be diminished due to internal competition and suppression. Secondly, the inoculation effect on plant growth was seldomly tested on other than succulent plants on vegetated roofs. Thirdly, the effects of planting method on vegetated roof microbial communities were still not investigated. To fill these gaps, we inoculated newly built vegetated roofs with *Rhizophagus irregularis* (Blaszk, Wubet, Renker & Buscot) and *Bacillus amyloliquefaciens* (Fukumoto) to test their initial impact on some forb species for vegetated roofs and microbial population in substrates. These selected microbes were reported to be compatible with each other (Xie et al., 2018).

R. irregularis and *B. amyloliquefaciens* (Fukumoto) are two acknowledged PGPMs (Idriss et al., 2002; Lenoir et al., 2016). *R. irregularis* is an arbuscular mycorrhizal fungus (AMF) that is symbiotic with the host plant roots. Typical structures of AMF are hyphae, arbuscules, and vesicles. They function as nutrient exchange/storage organs and transportation ducts. *B. amyloliquefaciens* is a Gram-positive, spore-forming bacterium that is attracted by root exudates and resides on the root surface. A layer of *B. amyloliquefaciens* cells on the root surface indicates effective colonization (Chen et al., 2013). Both microbes can promote plant growth by increasing nutrient uptake. They can produce microbial metabolites and enzymes,

100 such as phytase, to hydrolyze the normally indigestible organic phosphorus (P), thereby
101 providing usable inorganic P to host plants (Koide and Kabir, 2000; Idriss et al., 2002). Both
102 microbes can induce systemic resistance against pathogens by producing natural biocontrol
103 chemicals such as antifungal phenolics and lipopeptides (Xavier et al., 2003; Chowdhury et al.,
104 2015). They also improve host-plant resistance to environmental stresses, especially salinity
105 and drought. Such resistance may be related to increased antioxidant activity in host plants and
106 suppressed production of reactive oxygen species, which may damage plant tissues during
107 stress (Pandey and Garg, 2017; Wang et al., 2017).

108 In previous greenhouse experiment using *Antennaria dioica*, *Campanula rotundifolia*,
109 *Fragaria vesca*, *Geranium sanguineum*, *Lotus corniculatus*, *Thymus serpyllum*, *Trifolium*
110 *repens*, and *Viola tricolor* as hosts, all plants, except for *C. rotundifolia*, were co-colonized by
111 *R. irregularis* and *B. amyloliquefaciens* (Xie et al., 2018). *B. amyloliquefaciens* inoculation
112 significantly increased the colonization level of *R. irregularis* in the roots of most of the studied
113 plant species. More importantly, co-inoculation with the two PGPMs increased both plant
114 biomass and photosynthesis compared with single inoculation (Xie et al., 2018). The present
115 study hypothesized that similar results could be obtained from vegetated roofs.

116 The same plant species were used in our present experiment. Firstly, all of them are
117 Finnish native species (http://www.luontoportti.com/suomi/en/). Secondly, other researchers
118 have expressed interest or recommendation in using these plants on vegetated roofs (Latocha
119 and Batorska, 2007; Gabrych et al., 2016), which might be attributed to their stress tolerance
120 (Lewis, 1969; Taschler and Neuner, 2004; Striker et al., 2005; Stevens and Wilson, 2012;
121 Moradi et al., 2014; Kipkeev et al., 2015). Thirdly, they have been proven to form mutualistic
122 interactions with and benefit from the selected PGPMs (Xie et al., 2018). Lastly, the different
123 plant species represent flower, berry, and grass, increasing vegetated roof biodiversity.

In the present study, the substrate on the roofs was inoculated with *R. irregularis* and *B. amyloliquefaciens*. The scientific objectives of this study were 1) to test if the plant growth-promoting effects of the inoculation could be obtained from vegetated roofs, and 2) to test which planting methods, plant species, inoculation treatments (treated and non-treated), and their interactions could maximize the initial plant growth and microbial colonization on the vegetated roofs.

2. Materials and methods

2.1 General layout of the rooftop experiment

The vegetated roofs on a residential building in Jätkäsaari, Helsinki (60.155062, 24.915783) was completed by mid-April 2018. A series of experiments were carried out between April and September 2018 (Table 1). The roofs were selected as the experimental sites because 1) the roofs were initially designed and constructed to meet our experimental requirements, such as the plant species, drainage system, and planting methods; 2) inoculation of PGPMs on newly built vegetated roofs would be more successful than mature ones (Rumble and Gange, 2017); 3) the roofs are private property with limited accessibility, which would limit interference from human activities; and 4) there are two similar roofs at the same elevation so that two independent and repetitive experiments could be conducted simultaneously.

The western and eastern roofs are 32 meters above ground level, and 40 meters apart. Each roof is divided into testbed sections based on their drainage topography, allowing 4 sections on the western roof and 5 on the eastern roof (Fig 1). Each section has a slight slope towards a separate drainage outlet in the middle part the roof. The slopes of the sections are between 1:72 and 1:22 (0.8-2.6°). A walkway divides each roof into two halves. The walkway and the section create 8 plots on the western roof (plots 1-8 in Fig 1) and 9 on eastern roof (9-17

148 in Fig 1). Plots 1-3 and 9-11 were treated with the inoculants while plots 5-7 and 13-15 were
149 controls. Three planting methods, namely pre-grown vegetation mats, plug plants, or direct
150 sowing of seeds on the roof, were used according to Fig 1. Plot 4 and 8 on the western roof and
151 plot 12, 16 and 17 on the eastern roof were not included in our experiment as they are close to
152 shaft structures (2.5 m in height), which would likely block sunlight and rain of these nearby
153 plots, creating extra sources of variation. The walkways performed as barriers to prevent
154 inoculants from spreading from treated to control plots via runoff. Unintentional colonization of
155 control plants might grow better than actual control plants. This would diminish the plant
156 growth-promoting effect produced by microbial inoculation when comparing control and
157 treated plants, leading to a false conclusion.

158 The specifically manufactured substrate used on the vegetated roofs was based on
159 crushed brick (Hyvinkään Tieluiska Oy, Finland). Plant and substrate samples were collected
160 from even and flat areas with an average depth of 11.7 cm according to a separate
161 measurement, and no difference was recorded among all the tested plots according to one-way
162 ANOVA. Substrate depth was thicker (< 20 cm) where a few junipers seedlings (< 30 cm) were
163 planted at the border of the plots (Fig 1). The substrate was a mixture of lightweight expanded
164 clay aggregates (3-8 mm, 70%), bark chips (15%), and compost (15%). The substrate properties
165 were pH 5.5-5.7, organic matter 2.7-5.4%, P 12 mg/kg, K 80-160 mg/kg, and N 4 mg/kg. Using
166 low nutrient substrate was to reduce nutrient leaching and induce plants to proactively reach out
167 to PGPMs for mutualistic symbiosis.

168 The plant species in this experiment were selected based on our previous study, i.e., *A.*
169 *dioica*, *C. rotundifolia*, *F. vesca*, *G. sanguineum*, *L. corniculatus*, *T. serpyllum*, *T. repens*, and
170 *V. tricolor* (Xie et al., 2018). All seeds were purchased from Suomen Niittysiemen (Jyväskylä,
171 Finland). The seedlings of plant plugs and the vegetation mats were produced by Terola Plant
172 Nursery (Tuulos, Finland). For the mat-grown plots, the seed mixture was cultivated in a

greenhouse at a density of 50-150 plants/m^2. For the plug plant plots, the plants were cultivated in a greenhouse before transplanting on the vegetated roofs at a density of 16 seedlings/m^2. For seed plots, the seed mixture used for the vegetation mats was applied at 1 g/m^2 on the vegetated roofs.

Weather condition data, i.e., hourly air temperature and precipitation intensity, were retrieved from the public archive of the Finnish Meteorological Institute. The Kaivopuisto weather station (60.15, 24.96) locates 1.5 km to the east of the experimental site. Irrigation was conducted by professional gardeners who used an automatic portable sprayer to try to water evenly when necessary.

This case study had some unavoidable limitations typical for field experiments on vegetated roofs. Firstly, the growing period for vegetated roof plants was five months (from mid-April to mid-August), and the survey window was only two and a half months (from early June to mid-August). Such a short monitoring period was due to the short growing season in Finland (Ylhäisi et al., 2010). Secondly, to avoid microbial contamination from treated to untreated plots, the treated plots were placed on one side of the walkway, and the untreated plots on the other side. Therefore, the experimental plots could not be completely randomized. Thirdly, the amount of replication was limited, because we had to delineate the plots according to the sections defined by the roof drainage, in order to avoid stormwater from mixing between the sections. However, we do have 2 to 3 within-roof replicates of each factor level: 3 plots with microbes, and 3 without, and 2 with plug plants, 2 with mats and 2 with sown seeds. Lastly, since the vegetated roofs are private property, we could not conduct any destructive samplings, such as collecting the whole roots to evaluate root biomass, as well as a large number of shoot sampling.

2.2 Inoculation and samplings

MYC4000 and Rhizocell are powdery inoculant products purchased from Lallemand Plant Care (Castelmaurou, France), whose nutritional properties were tested in Natural Resources Institution Finland. MYC4000 contains 4000 spores/g of *R. irregularis* strain DAOM 181602, and Rhizocell contains $>10^9$ CFU endospores/g of *B. amyloliquefaciens* strain FZB42. MYC4000 and Rhizocell were simultaneously dissolved in water and evenly applied to the treated plots. The non-treated plots were simply irrigated with the same amount of water. The inoculations were conducted twice to ensure successful inoculation (Table 1). For each inoculation, 0.012 g of MYC4000 and 0.1 g of Rhizocell was applied to 1 m^2 treated plots according to the manufacturer's recommendation. The products were also sent to Natural Resources Institute Finland (Luke) for the nutrient content analyses.

Sampling was conducted in mid-August to evaluate plant growth, *R. irregularis* colonization, and *B. amyloliquefaciens* density, separately. Six replicates of shoots, roots and root-adhering substrates were collected per plant species in each plot. The replicate number was determined according to other studies on vegetated roofs (John et al., 2014; Young et al., 2015; Xie et al., 2018). This resulted in a total of 108 sample sets of roots, shoot and substrates per roof. To avoid destructive sampling, a small amount of fine root and substrate samples were carefully dug up and collected. No uprooting was performed. According to Xie et al. (2018), the collected samples were processed as follows before further treatments: 1) shoot samples were first oven-dried at 70°C for 48 h and then weighed; 2) roots samples were carefully brushed and stored in 70% ethanol at 4°C; 3) root-adhering substrate samples were collected in screw-cap tubes, and each substrate sample was thoroughly mixed and stored at 4°C shortly before DNA extraction.

2.3 Detection of *R. irregularis* in root samples

Detection and quantification of *R. irregularis* were based on root staining and microscopy (Phillips and Hayman, 1970; Vierheilig et al., 2005; Xie et al., 2018). The protocols were adjusted slightly for different plants. In general, roots were first soaked in KOH solution to soften the root cell walls for effective staining and to remove the root pigment. Then the roots were immersed in hydrogen peroxide containing ammonia (H_2O_2+NH_3) to further remove the root pigment. Next, the roots were transferred into the HCl solution to neutralize the remaining KOH. Finally, the roots were stained in hot Trypan Blue solution (lactic acid containing 63 ml/l glycerol, 63 ml/l water, and 0.02% Trypan Blue) before storing in pure glycerol (Table 2). Slides were made by mounting stained roots on microscope slides with the polyvinyl-lacto-glycerol solution (10 ml/l water, 10 ml/l lactic acid, 1 ml/l glycerol, and 1.66 mg/l polyvinyl alcohol). The abundance of hypha, arbuscule, and vesicle of *R. irregularis* was quantified using a modified gridline intersect method (McGonigle et al., 1990; Xie et al., 2018).

2.4 Detection of *B. amyloliquefaciens* in substrate samples

The *B. amyloliquefaciens* was quantified by detecting the amount of the *gyrB* gene in the total substrate DNA. *gyrB* encodes the subunit B protein of DNA gyrase and can be used as a phylogenetic marker (Bavykin et al., 2004). DNA was extracted from the substrate using the PowerSoil DNA extraction kit (MO BIO, Carlsbad, CA, USA). Genomic DNA from Rhizocell product was isolated using the DNeasy Plant Mini kit (QIAGEN, Hilden, Germany). DNA concentrations were measured with a Nanodrop 2000 spectrophotometer (Thermo Fisher, Waltham, MA, USA). PCR using the primer pair BaG3F (5´-GTCGACCACTCTTGACGTTACGGTT-3´) and BaG4R (5´-CGATCACTTCAAGATCGGCCACAG-3´) was conducted to amplify a 94-bp fragment from both substrate and Rhizocell-product DNA. Both PCR products were sent to Macrogen (Seoul, South Korea) for sequencing to verify that the *Bacillus* species in the substrate matched the

Bacillus species from the Rhizocell product. Before quantitative PCR (qPCR), substrate DNA samples were diluted to 5 ng/µl, and the Rhizocell DNA was serially diluted by 10-fold (1:1, 1:10, 1:100, 1:1000, and 1:10000) and used to construct a standard curve and calculate amplification efficiency. Finally, qPCR was carried out as follows: 5 min at 95°C, followed by 45 cycles of 10 s at 95°C, 10 s at 62°C, and 10 s at 72°C, with final incubation for 5 min at 72°C.

The *B. amyloliquefaciens* bacterial densities in substrate samples were calculated based on the standard curve equation (Bavykin et al., 2004; Xie et al., 2018), as follows:

$$Bacillus \text{ density (ng/g substrate)} = {}^{10^{(\mathrm{Ct}-\mathrm{m})/-\mathrm{slope}}}/_{\mathrm{weight}}$$

in which "Ct" denotes the cycle threshold value from the qPCR, "slope" and "m" denote the slope value and intercept value of the standard curve, respectively, "weight" is the weight of the substrate from which the DNA was extracted, and "n" denotes the dilution ratio of each substrate DNA sample.

2.5 Statistical analysis

A. dioica, *C. rotundifolia*, *G. sanguineum*, and *L. corniculatus* were hardly found on the vegetated roofs. As a result, samples of *F. vesca*, *T. serpyllum*, *T. repens*, and *V. tricolor* were collected accordingly (Table 3). For statistical analyses, the datasets were divided into three subgroups: 1) *F. vesca*, *T. serpyllum*, and *T. repens* from the mat and plug planting method; 2) *T. serpyllum* and *T. repens* from the mat, plug and seed planting methods; and 3) *T. serpyllum*, *T. repens*, and *V. tricolor* from the seed planting method (Table 3).

The effects of treatment, host-plant species, planting method, roof location and their interactions on colonization level of *R. irregularis* and density of *B. amyloliquefaciens* were assessed using ANOVA (IBM SPSS Statistics 25, Armonk, NY, USA). The roof location (eastern and western roofs) was not a study subject, but it was tested as a factor to ensure it had

no effect. Mean values for the colonization of *R. irregularis* and density of *B. amyloliquefaciens* were compared using the least significant difference ($LSD_{0.05}$).

3. Results and Discussion

3.1 Weather conditions and nutrient property of inoculants

According to Finnish Meteorological Institution, from April to August 2018, the absolute air temperature ranged between 1 and 30.2°C (Fig 2a). Small rain events occurred in April, followed by a dry period until late-June, after which bigger rain events occurred and lasted until the end of August (Fig 2b). Comparing to other years between 2015 and 2019, monthly mean air temperatures in 2018 were moderate in April and June, and the highest in May, July, and August. Monthly precipitation intensity in 2018 was generally low among other years. The accumulative precipitation in 2018 was the smallest, which was only 48.7% of the year 2016 (Fig 2c).

In general, the weather condition in 2018 was hotter and drier among the recent 5 years. However, low rain intensity was complemented with additional irrigation according to weather conditions.

According to analytical results from Natural Resources Institute Finland, MYC4000 inoculant contains P 0.20 g/kg, K 0.19 g/kg, and N 0.22 g/kg; while Rhizocell inoculant contains P 8.34 g/kg, K 13.7 g/kg, and N 5.55 g/kg. As a result, the inoculation contributed to the P content increase of 0.015mg/kg, the K content increase of 0.022 mg/kg, and the N increase of 0.082 mg/kg in the vegetated roof substrates. Such small increases would unlikely to create differences in the nutrient levels between the control and treated substrates.

3.2 Increased plant biomass following co-inoculation with *R. irregularis* and *B. amyloliquefaciens*

Comparisons between treated and non-treated plants within the same plant species and planting method indicated that four of our tested plant species grew larger in the co-inoculated plots than in the control ones (Fig 3). For the mat and plug planting methods, *T. repens* showed a smaller increase than *F. vesca* and *T. serpyllum* on both roofs. The seed-sown *V. tricolor* showed the highest shoot biomass increase in our experiment: 223.1% on the western roof and 292.0% on the eastern roof. In general, the co-inoculation increased plant biomass between 20 and 300% for the plants species that grew sufficiently to be included in the analysis (Fig 3).

Our findings suggest that the co-inoculation of *B. amyloliquefaciens* and *R. irregularis* can increase plant aboveground biomass on vegetated roofs. However, the improvement of growth in this rooftop experiment was much less than that in the greenhouse experiments (Xie et al., 2018) (Table 4).

It has been reported that the PGPMs in substrate improve plant growth to a higher level in control than in field conditions. Shoot biomass of *Pisum sativum* was found to increase 93.9% when inoculated with *Gloums deserticola* in the sterile substrate in the greenhouse, compared with 27.9% in the field (Fracchia et al., 2000). Another study also recorded higher grain yield in *Triticum aestivum* inoculated with *Pseudomonas putida* 108 in the greenhouse (56%) than in the field (37%) (Zabihi et al., 2011).

A likely reason why plant growth increase was smaller in field conditions is attributed to the more stressful growing conditions on the rooftops. It has been found that under stress, AMF inoculation could increase plant growth compared to non-inoculation by ameliorating the impact of the stress. However, AMF inoculation cannot completely counteract the negative effect of such stress. For instance, leaf number and leaf area of *Vigna unguiculata* in different treatments followed the pattern: AMF+watering = non-AMF+watering > AMF+drought stress

> non-AMF+drought stress (Oyewole et al., 2017). We suggest that AMF promoting effect was restricted due to its investment in resistance under stressed conditions. On the contrary, in stress-free conditions, AMF could invest more its potential in plant growth-promoting.

Furthermore, substrate nutrient in greenhouse substrate was richer: the greenhouse substrate had extremely low available P (2.2 mg/kg) and N (0.4 mg/kg), compared with rooftop substrate (12 mg/kg and 4 mg/kg, respectively). One plant growth-promoting mechanism delivered by *R. irregularis* and *B. amyloliquefaciens* is to improve nutrient availability for host plants (Xie et al., 2018). When the nutrient is abundant in the substrate, plant growth-promotion via improved nutrient availability by PGPM inoculation is curtailed, and vice versa. For instance, high P content in the substrate has been repeatedly reported to reduce AMF colonization level, suggesting that plants favor direct and non-symbiotic P uptake by roots (Balzergue et al., 2013). Consequently, lower AMF colonization may likely deliver less plant growth promotion (Treseder, 2013). Another recent study found out that without fertilizer, plant growth-promoting rhizobacteria *B. subtilis* No.2 increased fruit and plant mass of tomato variety (cv. Moldova) by 20.8% and 21.7%, respectively. When applying humic fertilizer, the increase dropped to 9.7% and 2.7%, respectively (Pishchik et al., 2018). Therefore, we conclude that the nutrient-rich substrate in the present study might have overshadowed the plant growth-promoting effect of PGPM inoculation, which led to a smaller, yet still statistically significant increase in biomass.

Some studies have reported cases about microbial competition in vegetated roof substrates and subsequent less successful inoculation, which was not observed in our study. For instance, Rumble and Gange (2017) hypothesized that the local microbial community might compete with commercial inoculants and limit their success in colonization on matured vegetated roofs. Molineux et al., (2014) reported that AMF inoculant reduced bacterial biomass in the vegetated roof substrate. However, their findings do not contradict ours. Our experiment

was conducted on newly built vegetated roofs where microbial community was not fully established yet, diminishing the possibility of such suppression and competition. Furthermore, the compatibility of *R. irregularis* and *B. amyloliquefaciens* in our study was confirmed in controlled and sterile conditions before the field experiment. Not only could they co-exist in the rhizosphere, but also *B. amyloliquefaciens* was found to promote *R. irregularis* colonization. Whereas in Molineux's study, the species of inoculants were not specified, and their compatibility was unknown.

In conclusion, plant growth improvement via PGPM inoculation on vegetated roofs is an outcome of mutualistic interaction between host plants and PGPMs to support each other to survive under stressful growing conditions. Additionally, the reduced promoting effect on the vegetated roof might be due to distributing PGPMs' ability against stresses and nutrient-rich substrate.

3.3 Plant species and planting method significantly affected *R. irregularis* colonization

Hypha and arbuscule structures were detected in the four plant species, and they were significantly more abundant in treated plants than control ones. However, vesicles resided only in the treated roots of mat-grown *F. vesca* (2% on the western roof, 8.7% on the eastern roof), plug-grown *F. vesca* (3.2% on the western roof and 4.5% on the eastern roof) and seed-grown *V. tricolor* (8.3% on the western roof and 2.3% on the eastern roof). In the previous greenhouse experiment, *F. vesca*, *T. serpyllum*, and *T. repens* had vesicle abundance of 14%, 8%, and 21.3% respectively, while *V. tricolor* exhibited a low vesicle abundance of 1.3%. In general, arbuscules occurred less frequently than hyphae but more frequently than vesicles. This is in line with the progression of AMF development (Strack et al., 2003). Additionally, roof location did not exhibit significant effect on *R. irregularis* colonization (data not shown).

Analysis of the first group (*F. vesca*, *T. serpyllum*, and *T. repens* from the mat and plug methods) revealed that the plant species, planting method, and microbial inoculation, with three-way interactions, had a significant effect on the abundance of hypha and arbuscules (Table 5). *F. vesca* was the most colonized host plant by *R. irregularis*, followed by *T. serpyllum*, and *T. repens* was the least colonized. Additionally, *R. irregularis* presented smaller abundance in plug-grown plants than mat-grown plants (Fig 4a & 4b).

Similarly, for the second group (*T. serpyllum* and *T. repens* from the mat, plug, and seed planting methods), the plant species, planting method and microbial inoculation, again with three-way interactions, significantly affected the abundance of hyphae and arbuscules (Table 5). *T. serpyllum* was colonized by *R. irregularis* to a greater extent than *T. repens* in all the three planting methods. Seed-grown plants were the most colonized, whereas the mat-grown plants were the least (Fig 4c & 4d).

Results from the analysis of the third group (*T. serpyllum*, *T. repens*, and *V. tricolor* from the seed planting method) revealed that hyphae and arbuscules occurred significantly less in *T. repens* than *V. tricolor* and *T. serpyllum* (Table 5). The difference between *V. tricolor* and *T. serpyllum* was not statistically significant (Fig 4e & 4f).

The abundance of *R. irregularis* in host roots follows the order *F. vesca* > *T. serpyllum* > *T. repens*, which is consistent with our previous greenhouse experiment (Xie et al., 2018). Studies have shown that a given AMF might colonize a wide range of plant species, but the AMF abundance varies (Sanders, 2003). For one thing, lacking plant specificity ensures successful colonization, which allows AMF to create a continuous fungal web for nutrient flow among adjacent plants (Sanders, 2003). For another, different plant properties might lead to different AMF colonization, such as root exudates and morphology. Root exudates from different plant species could induce different responses of AMF, leading to various AMF colonization levels (Bever et al., 1996; Legay et al., 2016; Popescu, 2016). Plants with fine

roots (thin-walled cells) are more easily penetrated and colonized by AMF (Wilcox, 1983), and taproot plants were hypothesized to be more dependent on mycorrhiza than plants with the fibrous root system (Yang et al., 2015).

As regards the planting method, seed-grown plants had the greatest *R. irregularis* colonization, followed by plug-grown plants, and mat-grown plants had the poorest colonization. Considering that the vegetation mats and plug plants grew ahead of seed-grown plants before *R. irregularis* inoculation, the differences in colonization levels among the planting methods might depend on the host plant developmental stage at the time of AMF inoculation. Two mechanisms might explain such dependency. Firstly, AMF is mainly attracted to host plants by their root exudates, and the exudates change with the plant development stage (Buee et al., 2000; Buée et al., 2009; Micallef et al., 2009). Secondly, young seedlings with thin-walled root cells and cortex can be easily penetrated by AMF hyphae. This is why younger terminal roots are usually more colonized than older and mature roots, even in the same plant (Wilcox, 1983). Therefore, we suggest an a posteriori hypothesis that when host plants grow to maturity, the change of AMF-related root exudates and thickening of root cells and cortex cause low AMF abundance.

Additionally, the significant difference between mat-grown and plug-grown plants in *R. irregularis* colonization might be attributed to competition for *R. irregularis* spores. In this experiment, plug plants were more sparsely arranged than the mat-grown plants (50-150 per m^2 in mat planting vs 16 per m^2 in plug planting). Therefore, the competition for *R. irregularis* colonization was less intense, and there was more space for plug plants to grow and thus become colonized. Koide and Dickie (2002) also concluded that lower AMF colonization in a densely populated plant community is likely attributed to the competition for AMF fungal spores.

R. irregularis colonization was minimal in the control plants, and neither plant species nor planting method significantly affected AMF abundance (Table 6). Such naturally occurred AMF in vegetated roof substrates were reported ranging between 0 to 90% with different geographical locations, host species, and mycorrhizal species (John et al., 2014; Young et al., 2015; Rumble and Gange, 2017). However, when the occurrence of native AMF is extremely low (<4%), such as in our controlled plants, plant growth would not be significantly affected (Young et al., 2015).

To sum up, the results suggest that the abundance of *R. irregularis* was affected by plant species, planting method, and their interactions.

3.4 The density of *B. amyloliquefaciens* in the substrate was affected by plant species but not by planting method

B. amyloliquefaciens was detected in all the inoculated plots, but not in the controlled ones. DNA sequencing confirmed that the *B. amyloliquefaciens* in the roof substrate and Rhizocell product were the same species. Based on ANOVA, the planting methods, as well as roof location (data not shown), had no significant effect on the density of *B. amyloliquefaciens*. For plant species, the *B. amyloliquefaciens* density in the rhizosphere of *T. repens* was greater than other plant species (Table 7).

Why was *B. amyloliquefaciens* bacterial density not affected by planting methods? In a greenhouse experiment lasting 40 days, *T. aestivum* was inoculated with *Azospirillum brasilense* (a nitrogen-fixing bacterium) either once on day 8 (single inoculation) or 4 times on day 8, day 16, day 24, and day 32 (successive inoculation). Eventually, by day 40, the bacterial density of *A. brasilense* did not differ between single and successive inoculation (Bashan, 1986). Through this example, we could suggest two reasons why the planting method could influence *R. irregularis* colonization level, but not *B. amyloliquefaciens* density. Firstly, *B.*

amyloliquefaciens resides outside roots, which means no root penetrating is required. In that sense, root cell wall thickness, determined by plant age and species, would not influence the growth of *B. amyloliquefaciens*. Secondly, host plants need to compete with adjacent plants in attracting AMF spores, which may become a limiting resource in dense plant communities. On the contrary, *B. amyloliquefaciens* can simply reproduce in the rhizosphere, as long as the host plants provide root exudates. In this study, by the time of inoculation, mat and plug plants had formed leaves that might prevent the inoculant solution from reaching the substrate and roots, while seed plots were still plantless. Given that the mat plants were denser than the plug plants, it assumed that the amount of *B. amyloliquefaciens* endospore followed the pattern: seed > plug > mat. However, *B. amyloliquefaciens* propagated to the same density level in different planting methods, as long as the host plant could provide nutrients through root exudates.

Why did plant species affect *B. amyloliquefaciens* density? So far, no similar studies investigated the dependency of *Bacillus* density in the substrate on host plant species, but others confirmed that different plant species can maintain substrate bacteria at various density levels. Bergsma-Vlami et al. (2005) concluded that lily (cv. Vivaldi) supported *Pseudomonas* spp. (a PGPM) at a significantly lower level than wheat (cv. Bussard), sugar beet (cv. Auris), and potato (cv. Bintje). Such microbial composition and density were determined by plant traits, including root biomass, root surface, root porosity and root exudates (terHorst and Zee, 2016).

Moreover, in the field, *B. amyloliquefaciens* density was higher in the rhizosphere of *T. repens*. While in the greenhouse, plant species did not affect *B. amyloliquefaciens* density in the rhizosphere (Xie et al., 2018). The inconsistent results might be owing to different growing conditions between the two studies. The plants in the greenhouse were cultivated in sterile substrate and favorable conditions, while the plants on the roofs grew in adverse conditions. For one thing, stresses might stimulate plants to proactively attract PGPMs via specific root exudates. For instance, it was found that flood, drought and nutrient stresses would induce

plants to release exudate containing malic acid (Keeley, 1978; Henry et al., 2007), which directly stimulates *Bacillus* growth (Rudrappa et al., 2008; Chen et al., 2012). For another, plants can regulate substrate microbial community and their functions also through exudates in response to specific changes and stressors, which can influence *B. amyloliquefaciens* density indirectly via competition, antagonism or synergy (Pantastico-Caldas et al., 1992; Young et al., 1995). In sterile substrate, plants could not exert such influence on *B. amyloliquefaciens* density. In conclusion, stressed conditions on roofs might induce *T. repens* to release specific exudates to directly attract and support *B. amyloliquefaciens*, or to reshape substrate microbial community to indirectly affect *B. amyloliquefaciens* density. While greenhouse conditions could not initiate such a process.

3.5 Methodological limitation in our study and their consequences

Located in the boreal region, Helsinki experiences relatively short growing seasons and long winters. This allowed us a relatively short sampling period, yet critical for the establishment of plants. Without continuous monitoring the growth of microbes and plants in the following years, the long-term impact of inoculation on vegetated roofs cannot be confirmed.

Compared to larger field studies, our vegetated roofs were small in size with the designed water flow direction. Therefore, complete randomization was not possible. However, if one of the experimental roofs had an unexpected disturbance that remained unobserved from us, it was unlikely that the results would have been similar between the two roofs. We suggested that similar results on the impact of microbial inoculants will be reported if complete randomization can be arranged on a vegetated roof with larger areas.

Since the complete root system of each plant was not collected due to restrictions, root biomass, and root:shoot ratio were not assessed. These are important plant growth indices missing from our study (Lloret et al., 1999). We can only speculate that both indices would be

494 increased via microbial inoculation, and such speculation should be verified from another
495 vegetated roof where destructive measurements are allowed.
496

497 4. Conclusions

498 This study confirmed that commercial *R. irregularis* and *B. amyloliquefaciens* products can
499 successfully colonize a number of plants on vegetated roofs. And more importantly, the co-
500 inoculation of the microbes can promote plant shoot biomass between 20 and 300%. Young and
501 colleagues' (2015) reported that successful colonization increased leaf P content, but no
502 changes in leaf N content or plant biomass, when inoculating *P. vulgaris* with AMF inoculum
503 mixture. A possible explanation would be devoid of mycorrhizal helper bacteria (Garbaye,
504 1994; Xie et al., 2018) or such PGPM/plant combination that did not contribute to visible plant
505 growth (Sanders, 2003). Young used a commercial inoculant containing different unspecified
506 AMF species. Therefore, we could not make a nuanced comparison between their and our AMF
507 species in plant growth-promoting. In conclusion, the exhibited promoting effects depend on
508 the combination of plant and PMPG species. We suggest further tests of PGPMs and plant
509 species on vegetated roofs.

510 We found that *R. irregularis* colonization level depends on the plant species and follows
511 the pattern *F. vesca* > *T. serpyllum* > *T. repens*, which is consistent with our previous study in
512 greenhouse conditions (Xie et al., 2018). Furthermore, plug plants were more colonized than
513 mat-grown plants, but less than seed-grown plants, which was likely the outcome of different
514 plant development stage and competition for AMF endospores.

515 *B. amyloliquefaciens* density was affected by plant species but not by planting method.
516 *T. repens* supported *B. amyloliquefaciens* at a higher density than *T. serpyllum* and *F. vesca* in
517 the mat and plug planting methods, and also higher than *T. serpyllum* and *V. tricolor* in seed
518 planting method.

519 The low abundance of *R. irregularis* and non-existence of *B. amyloliquefaciens* in
520 control plots suggests that the experimental design using a walkway and drainage system
521 effectively limited contamination from treated to non-treated plots.
522 *A. dioica*, *C. rotundifolia*, *G. sanguineum*, and *L. corniculatus* hardly survived on the
523 vegetated roofs, as they might not be suitable plants for vegetated roofs in Southern Finland
524 (Latocha and Batorska, 2007; Gabrych et al., 2016). For instance, *A. dioica* is a Finnish red list
525 species affected by environmental changes, and it is poor in competing with neighboring plants
526 (Vega-Frutis et al., 2014; Varga et al., 2017).
527 According to our findings, we suggest constructing vegetated roofs that utilize the same
528 planting regime in a similar climate as ours by 1) co-inoculating *R. irregularis* and *B.*
529 *amyloliquefaciens* to harvest synergistic effects on plant growth; 2) co-inoculating *R.*
530 *irregularis* and *B. amyloliquefaciens* on vegetated roofs at the installation phase to reduce the
531 competition from the established native microbial community, achieving a higher colonization
532 of *R. irregularis* and *B. amyloliquefaciens*; and 3) using the mat and plug plants to achieve
533 instant greening, but also seeds to maintain high AMF compatibility in substrates. Thus, by
534 improving plant growth and tolerance, vegetated roofs would deliver better eco-services to
535 manage stormwater, increase urban biodiversity, mitigate air pollution and heat island effect,
536 and reducing nutrient leaching caused by fertilization.
537 For future studies, we suggest testing these methods on different substrates, such as
538 recycled materials which are lower in carbon footprint, and across different climate conditions
539 to verify its functionality in broader geographic conditions.
540 In conclusion, this case study laid a scientific basis for further vegetated roof
541 experiments that involve the PGPM application. After confirming the initial establishment of
542 plants and inoculants, the next step for this research is to monitor the microbial and plant
543 growth on the roofs for the coming years to assess the long-term effect of PGPM inoculation.

Also, we can investigate the effect of inoculating plug- and mat- grown plants in the nursery before installing on vegetated roofs, while saving efforts of on-site inoculation (Young et al., 2015). Ideally, PGPM inoculation could become a cost-effective solution to support vegetated roofs in providing strengthened ecosystem services to citizens.

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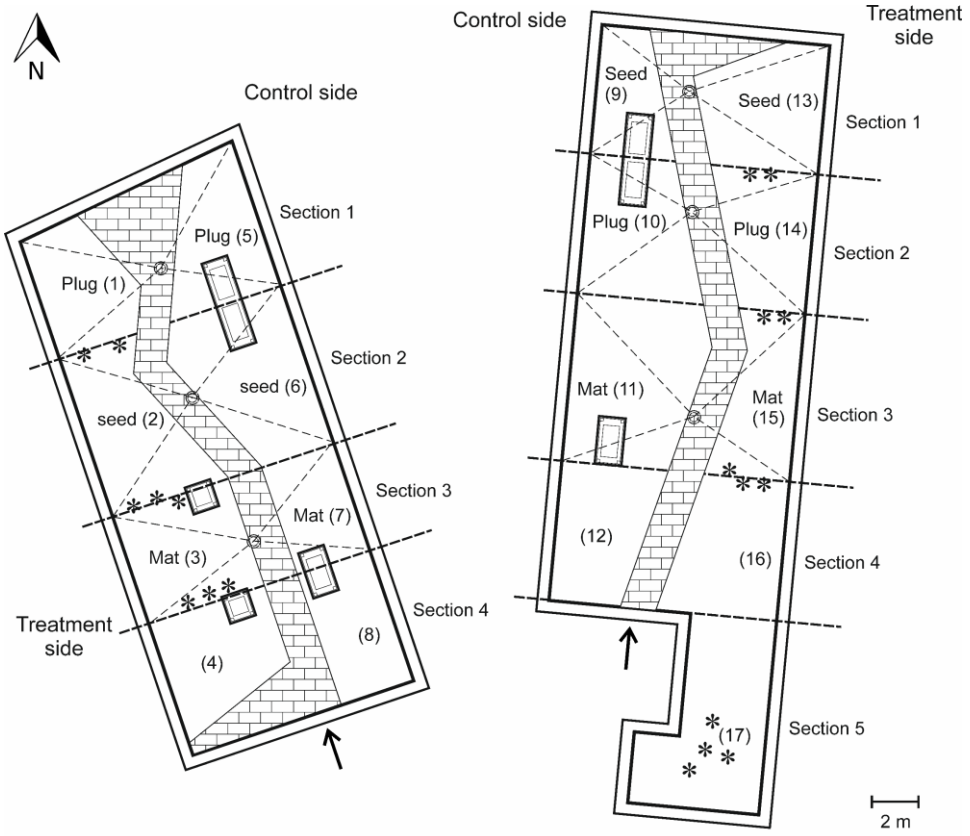
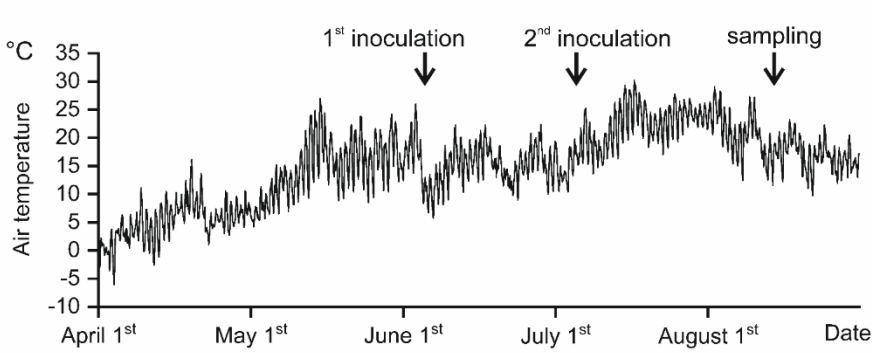


Fig 1. The layout of the vegetated roofs. Planting methods are indicated as "Plug", "Mat", and "Seed". For the western roof, the left half was the treatment side, whereas, for the eastern roof, the right half was the treatment side. Rectangle stands for the ventilation outlet and "*" stands for each juniper seedling (< 30 cm) on the vegetated roofs. Arrows indicate the entrances. Each number in the figure represents an individual experimental plot.

a



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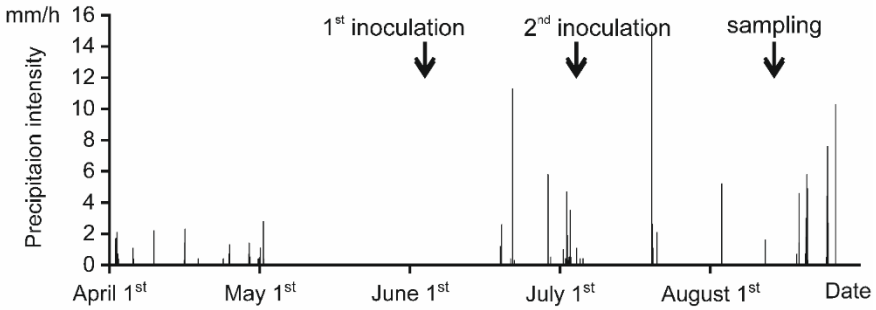
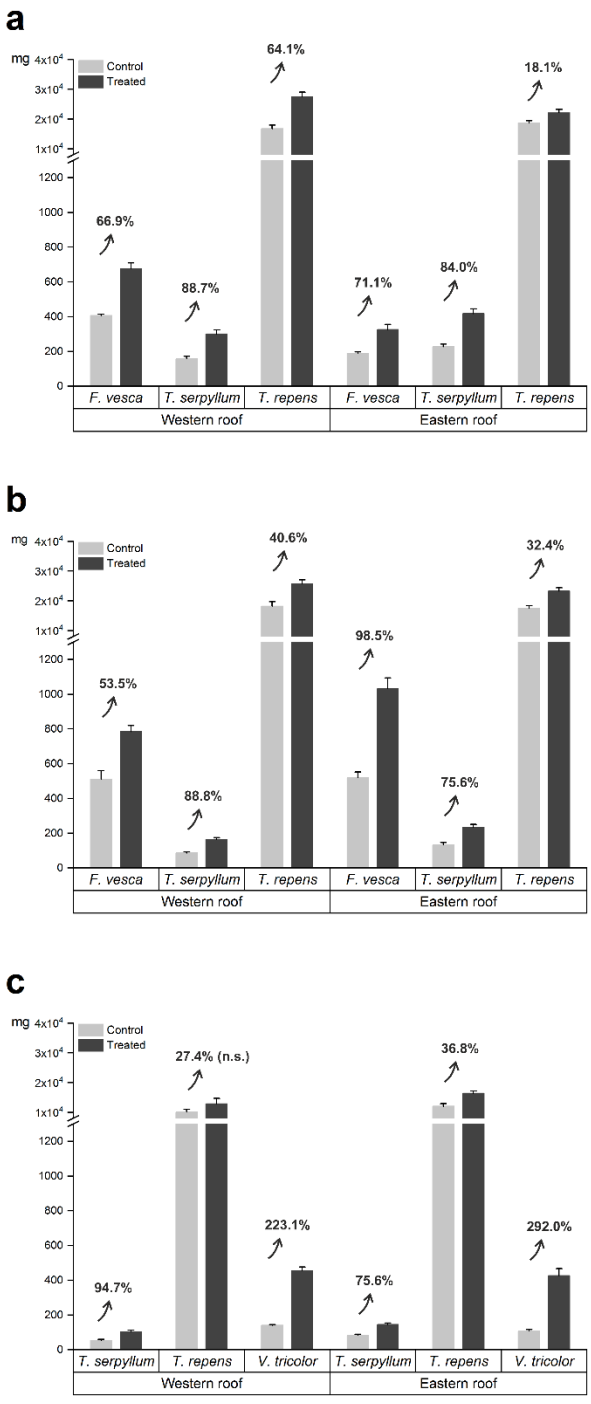


c

	Monthly mean air temperature (°C)					Monthly accumulative rain intensity (mm)				
Month	2018	2019	2017	2016	2015	2018	2019	2017	2016	2015
April	5.0	6.7	2.8	4.8	5.3	29.8	7.9	35.5	67.3	54.7
May	14.6	10.3	9.5	13.9	9.2	8.9	48	10.5	7.1	39.8
June	15.3	17.2	13.6	15.2	13.3	25.1	9.1	98.9	159.1	78
July	21.1	17.5	16.0	17.8	16.4	37.6	63.3	28.1	42.1	69.9
August	18.6	17.3	16.2	16.4	17.4	64	93	112	63.7	40.0
Sum	/	/	/	/	/	165.4	221.3	285.0	339.3	282.4

Fig 2. Air temperature and precipitation intensity data from Finnish Meteorological institute. Data of hourly air temperature (a) and hourly precipitation intensity (b) between April and August in 2018. The arrows pointed out the time points of the tasks. Comparison of monthly average air temperature and monthly accumulative precipitation among the years between 2015 and 2019 (c).



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774 **Fig 3. Comparisons of dry aboveground biomass of inoculated (treated) and**
775 **non-inoculated (control) plants for each species and planting method: mat**
776 **(a), plug (b) and seed (c).** Bars (n=6, mean ± SE) represent the absolute value of dry
777 aboveground biomass for treated (light gray) and non-treated (dark gray) plants. The increase
778 percentage numbers are shown between inoculated and control plants (n.s.: no significance).

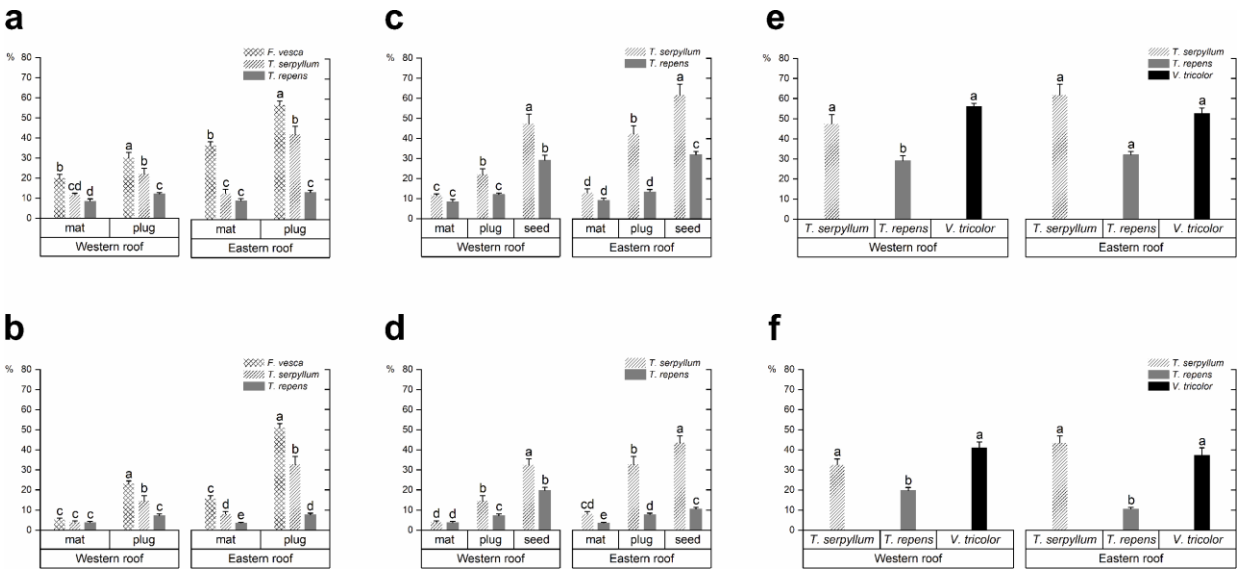

Fig 4. The abundance of hyphae and arbuscules of *R. irregularis* in inoculated plant roots. Bars (n=6, mean ± SE) represent the occurrence percentages of hypha (a, c, and e) and arbuscules (b, d, and f). Graphs a and b present data for the first group (*F. vesca*, *T. serpyllum*, and *T. repens* for the mat and plug planting methods). Graphs c and d present data for the second group (*T. serpyllum* and *T. repens* for the mat, plug and seed planting methods). Graphs c and d present data for the third group (*T. serpyllum*, *T. repens*, and *V. tricolor* for the seed planting method). The AMF abundance on western and eastern roofs is presented and compared separately. Different lowercase letters indicate statistically significant differences ($LSD_{0.05}$).

799 **Table 1. Timetable presenting the experimental procedure in this study.**

Tasks	**Time**	**Aims**
Vegetated roof construction completion	04.2018	
1st inoculation	05.06.2018	Ensuring successful inoculation of the target PGPMs
2nd inoculation	05.07.2018	
Shoot, root and substrate sampling	14.08.2018	Sample collection
Shoot desiccation	15-17.08.2018	Measuring shoot dry weight
Substrate DNA extraction and qPCR	15.08-01.11.2018	Detecting *B. amyloliquefaciens* content in substrate samples
Root staining and microscopic quantification	02.11-06.12.2018	Detecting *R. irregularis* abundance in root samples

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Table 2. Detailed staining protocol for plant species.

	Staining solutions			
Plant species	**KOH**	**H_2O_2+NH_3[1]**	**HCl[2]**	**Trypan Blue[3]**
A. dioica	48 h in 2.5% KOH at RT[4]	30 min at RT[4]	90 min at RT[4]	90 min at 90˚C
C. rotundifolia	60 min in 2.5% KOH at 80˚C	40 min at RT[4]	30 min at RT[4]	60 min at 80˚C
F. vesca	48 h in 1.25% KOH at RT[4]	None	60 min at RT[4]	60 min at 80˚C
G. sanguineum	30 min in 2.5% KOH at 121˚C	None	30 min at RT[4]	75 min at 90˚C
L. corniculatus	60 min in 2.5% KOH at 80˚C	None	30 min at RT[4]	45 min at 95˚C
T. repens	60 min in 2.5% KOH at 80˚C	None	30 min at RT[4]	90 min at 90˚C
T. serpyllum	20 min in 2.5% KOH at 90˚C	None	60 min at RT[4]	90 min at 80˚C
V. tricolor	60 min in 2.5% KOH at 80˚C	None	30 min at RT[4]	75 min at 95˚C

[1]1.5% hydrogen peroxide containing 5 ml/l ammonia;
[2]1% Hydrochloric acid;
[3]Lactic acid containing 63 ml/l glycerol, 63 ml/l water, and 0.02% Trypan Blue;
[4]Room temperature.

Table 3. Shoot, root and substrate samples from different plant species and planting methods from two roofs (western and eastern) and two treatments (control and inoculation). The checkmarks indicate the samples collected, and the hyphens indicate no sampling. Each checkmark represents 6 individual plants from which 6 sample sets were collected, respectively. One sample set included shoot, fine root, and root-adhering substrate samples. The analysis groups are denoted by rectangles of different colors. The first group encompasses *F. vesca*, *T. serpyllum*, and *T. repens* from the mat and plug planting method; The second group encompasses *T. serpyllum* and *T. repens* from the mat, plug and seed planting methods; The third group encompasses *T. serpyllum*, *T. repens*, and *V. tricolor* from the seed planting method.

Plant species	**Mat**				**Plug**				**Seed**			
	Western roof		Eastern roof		Western roof		Eastern roof		Western roof		Eastern roof	
	Con.	Tre.	Con.	Tre.	Con.	Tre.	Con.	Tre.	Con.	Tre.	Con.	Tre.
F. vesca	√	√	√	√	√	√	√	√	–	–	–	–
T. serpyllum	√	√	√	√	√	√	√	√	√	√	√	√
T. repens	√	√	√	√	√	√	√	√	√	√	√	√
V. tricolor	–	–	–	–	–	–	–	–	√	√	√	√

Con.: control; Tre.: treatment.

Table 4. Increase in dry aboveground biomass of co-inoculated plants compared with non-inoculated control plants in the previous study (greenhouse conditions) and present study (field conditions).

	Biomass increase (%)							
	Greenhouse		Field (mat)		Field (plug)		Field (seed)	
Tested plants	Exp. 1	Exp. 2	W roof	E roof	W roof	E roof	W roof	E roof
F. vesca	648	578	66.9	72.1	53.5	98.5	/	/
T. repens	717	2447	64.1	18.1	40.6	32.4	27.4 (n.s.)	36.8
T. serpyllum	1883	388	88.7	84.0	88.8	75.6	94.7	75.6
V. tricolor	579	712	/	/	/	/	223	292

Exp. 1 and Exp. 2: the first and second greenhouse experiments in Xie et al. (2018);
W roof and E roof: western roof and eastern roof;
n.s.: no significance.

Table 5. Effect of plant species, planting method, inoculation treatment, and their interactions on the abundance of hyphae and arbuscules of *R. irregularis* in roots of co-inoculated plants (n=6). P, M, and T refer to plant species, planting methods, and treatments, respectively.

	1st group						**2nd group**					
Source	Hypha			Arbuscule			Hypha			Arbuscule		
	df	F	Sig	df	F	Sig	df	F	Sig	df	F	Sig
P	2	100.579	<.001	2	108.974	<.001	1	92.146	<.001	1	129.147	<.001
M	1	75.611	<.001	1	257.129	<.001	2	129.607	<.001	2	113.207	<.001
T	1	859.399	<.001	1	738.917	<.001	1	771.068	<.001	1	583.457	<.001
P×M	2	9.199	<.001	2	42.490	<.001	2	13.236	<.001	2	24.668	<.001
P×T	2	93.835	<.001	2	108.024	<.001	1	88.985	<.001	1	132.303	<.001
M×T	1	68.996	<.001	1	246.671	<.001	2	113.556	<.001	2	104.047	<.001
P×M×T	2	10.470	<.001	2	43.766	<.001	2	12.761	<.001	2	25.735	<.001
	3rd group											
Source	Hypha			Arbuscule								
	df	F	Sig	df	F	Sig						
P	2	33.045	<.001	2	48.841	<.001						
T	1	925.831	<.001	1	681.376	<.001						
P×T	2	29.141	<.001	2	47.789	<.001						

Table 6. Effect of plant species, planting method, and their interactions on the abundance of hypha and arbuscule of *R. irregularis* in the roots of the control plants (n=6). P and M refer to plant species and planting methods, respectively.

1st group						
Source	Hypha			Arbuscule		
	df	F	Sig	df	F	Sig
P	2	.559	.575	2	.251	.779
M	1	.402	.529	1	.431	.514
P×M	2	.269	.765	2	.682	.510
2nd group						
Source	Hypha			Arbuscule		
	df	F	Sig	df	F	Sig
P	1	.098	.755	1	.091	.764
M	2	2.012	.143	1	1.045	.358
P×M	2	.498	.610	1	.578	.564
3rd group						
Source	Hypha			Arbuscule		
	df	F	Sig	df	F	Sig
P	2	.657	.526	2	.054	.948

Table 7. Effect of different plant species, planting method, and their interactions on the density of *B. amyloliquefaciens* in the substrates of co-inoculated plants (n=6). P and M refer to plant species and planting methods, respectively.

Source	**1st group**		
	df	F	Sig
P	2	10.781	<.001
M	1	1.462	.231
P×M	2	1.714	.189
Source	**2nd group**		
	df	F	Sig
P	1	30.694	<.001
M	1	1.258	.292
P×M	2	2.946	.060
Source	**3rd group**		
	df	F	Sig
P	2	7.969	<.001