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Influence of *Trichoderma asperellum* and *Bacillus subtilis* as biocontrol and plant growth promoting agents on soil microbiota

Maris Senkovs*, Vizma Nikolajeva, Galina Makarenkova and Zaiga Petrina

Abstract

Purpose: The manipulation of soil microbiota can involve changes in microbial diversity and microbial activities, and it is carried out for practical purposes. The microbial diversity can be modified by the inoculation of beneficial microorganisms into soil or by agricultural management practices. This study provides information on the influence of introducing beneficial soil microorganisms on soil indigenous microbiota. The aim of this study was to determine how biocontrol and plant growth promoting agents *Trichoderma asperellum* and *Bacillus subtilis* and their consortium affect indigenous soil microbiota without placing emphasis on the plant as a determinant of change.

Methods: Experimental soil samples were treated with *B. subtilis* and *T. asperellum* and their consortium. The shift of number of bacterial and fungal CFUs in soil was determined. Biolog EcoPlate assay demonstrated the metabolic activity of microorganisms in soil. The concentration of *Trichoderma* genus, *Firmicutes, Gammaproteobacteria* and *Acidobacteria* DNA in soil samples was determined by molecular methods. A correlation analysis was performed between microbiological and molecular data.

Results: Soil treatment with *T. asperellum*, *B. subtilis* and the plant pathogenic fungus *Botrytis cinerea* changed the number of CFUs and amplified DNA fragments of certain taxa. The study showed that added microorganisms did not significantly affect the metabolic diversity of the community and Shannon-Wiener biodiversity index but change the utilization of carbohydrates, complex carbon compounds and organic phosphorus compounds.

Conclusion: Introduced biocontrol and plant growth promoting agents *T. asperellum* and *B. subtilis* survive in soil during a 60-day experiment and influence composition and functionality of indigenous populations. Whereas *B. subtilis* and *T. asperellum* and their consortium are inhibitors of the pathogenic fungus *B. cinerea*, this could further positively affect specific crops.

Keywords: *Bacillus subtilis*, *Trichoderma*, Biolog EcoPlate, Microbial consortium, qPCR, Soil

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Introduction

Microbiological research is increasingly focusing on the uniqueness of a particular soil microbiota, where the interaction of different groups of microorganisms plays an important role. Positive effects may be achieved through both natural microbial associations and laboratory-based consortia that promote the development of plant-beneficial microorganisms in soil and are capable of suppressing certain plant infectious agents and the diseases they cause. In order to judge the effectiveness of this type of consortium, multiple studies, both culture-dependent and molecular, are needed to determine if and how the soil microbiota has changed (Glick 2012; Song et al. 2016).

Soil is a natural, non-consolidated material of minerals and organic matter that provides living organisms with resources for survival (Compant et al. 2005). Soil can be considered as one of the most complex habitats that maintain biodiversity (Briones 2014). A soil habitat can be defined as a set of living organisms coexisting in it, including plants, animals and microorganisms and the abiotic environment around it. Geological and climatic factors, as well as the presence of certain flora, are decisive for the establishment of the soil habitat. One gram of soil can contain a kilometre of fungal hyphae and about 10^9 eubacteria and archaea cells belonging to tens of thousands of species, most of which are not cultivated under laboratory conditions. Soil areas located closer to the surface of the earth contain more organic and other available nutrients, resulting in higher biodiversity, while nutrient deficiencies and microbiological poverty can be observed in the deeper soil layers (Birkeland 1999). Concentration of microorganisms is significantly higher in the root area called rhizosphere, where the amount of organic matter is sufficient (Lynch and Whipps 1990). Soil constantly interacts with living organisms (biosphere) with rocks and minerals (geosphere), water (hydrosphere), atmosphere or organic matter (detritosphere). Soil research is fundamental to understanding the dynamics of geochemical, biochemical and biophysical interactions on the Earth's surface, especially in the context of global climate change (Voroney 2007).

For practical purposes, various manipulations of the soil microbiota are possible, involving changes in microbiological diversity and activity. Microbiological diversity can be altered by inoculating soil-friendly microorganisms (*Bacillus subtilis*, *Trichoderma* spp. etc.) and inhibiting the development of possible plant pathogenic organisms (*Botrytis cinerea* etc.) (Nannipieri et al. 2003). Plant growth-promoting bacteria are able to supply the plant with the necessary elements (fix nitrogen, synthesize plant hormones, dissolve minerals) and compete with the pathogenic microbiota for occupying a specific ecological niche or substrate availability, producing inhibitory allelochemicals or promoting systemic plant-host resistance to pathogens (Compant et al. 2005). The interaction between bacteria and microscopic fungi, which can form certain physical associations depending on the type of molecular communication established, plays an important role in ensuring soil health. These bacterial-fungal interactions often result in changes in pathogenicity or affect one or more types of organization suitable for the use of nutrients in soil (Frey-Klett et al. 2011). There is also widespread attention given to the poorly cultivated groups of *Proteobacteria* and *Acidobacteria*, which physiology and ecology are poorly studied, but they are widely represented in soil and occupy a certain place in ecological processes (Jones et al. 2009). Group members have a variety of morphological, biochemical and physiological characteristics that make their community important (Spain et al. 2009). In order to characterize the soil microbiota as successfully as possible, it is best to use both culture-based and DNA-based culture-independent methods (Stefani et al. 2015).

Microorganisms of the soil microbiota can be classified into physiological groups involved in various biological processes: ammonifiers, nitrogen binders, nitrate reducers, bacteria and fungi degrading cellulose and other important plant components. The microbiota is responsible for a number of processes that occur in soil, from the mobilization of chemical elements to their accumulation (Pajares and Bohannan, 2016). Microorganisms can both stimulate plant growth and suppress certain plant infectious agents that lead to disease and are transmitted through the soil (Glick 2012). It is difficult to accurately characterize the soil microbiota due to the high phenotypic and genetic variability and the heterogeneity of the soil itself (Sayer et al. 2017).

Information on the ability of groups of microorganisms to influence and modify the total soil microbiota excluding plant interactions is still poor; therefore, the aim of this work was to investigate whether and how the fungus *Trichoderma asperellum* and the bacterium *Bacillus subtilis* widely used as biocontrol and plant growth promoting agents and, respectively, their consortium influence the indigenous microbiota of the soil and related biochemical processes. In addition, some soil samples were also treated with the pathogenic fungus *Botrytis cinerea*, which causes grey mould in many hosts of different plant species.

Materials and methods

Experimental soil samples and microorganisms

For the study, six soil samples were placed in three replicates in Sterivent 107 × 94 × 96 mm plant tissue culture containers (Duchefa Biochemie, Netherlands). In each container, 150 g of Suliflor premium peat moss based

substrate (humidity 60%, pH 5.5–6.5, organic matter > 80%, Ca 1.2%, Mg 0.06%, raw materials: mineral fertilizer liming material, wetting agent; Sulinkiai, Lithuania) was placed and mixed with 50 ml of the suspension of microorganisms except for the control sample which was mixed with 50 ml of sterile water. The following microorganisms and consortia were used for soil inoculation: (1) *Bacillus subtilis* MSCL 897, (2) *Botrytis cinerea* MSCL 433, (3) *Trichoderma asperellum* MSCL 309, (4) *B. subtilis* and *T. asperellum* and (5) *B. subtilis*, *T. asperellum* and *B. cinerea*. The initial concentration of microorganisms in the suspension was 10^8 colony-forming units (CFUs)/ml. The pH of the soil was determined using a pH metre AD1405 (Adrona, Latvia). Soil humidity was determined with moisture analyser Axis AGS120/T250 (Sp. z o.o, Poland) by heating 3 g of soil at 75 °C for 20 min.

Microbiological analyses

At the beginning (0 day) and 30 and 60 days after treatment, the number of CFUs of cultivated bacteria and fungi (CFUs g^{-1} of wet soil) was estimated by plating of soil samples on Malt Extract Agar (ME, Biolife, Italy) and R2A (Sifin, Germany) media in duplicate at various dilutions. The samples were stored at 23–24 °C for 60 days. Petri plates were incubated at 21 °C for 7 days. The temperature was controlled using a room thermometer (Alerton, USA). Predominant fungal genera were identified using macroscopic and microscopic appearance (Sayer et al. 2017).

Biolog EcoPlate method

One hundred microlitres of soil suspension was transferred to a well of a Biolog EcoPlate™ 96-well microplate (Biolog, USA) and incubated at 21 °C. Absorbance readings were taken after 48 h, and the ability of various samples to utilize the relevant carbon compounds was determined (Jia et al. 2013; Xu et al. 2015). Magellan for F50 computer program (Tecan, Switzerland) was used to process the microplate reader data. From the data obtained, average metabolic response (AMR) and community metabolic diversity (CMD) were calculated according to Laboratory for Microbial Ecology (Mo Bio Laboratories, USA). CMD was calculated by summing the number of positive responses (purple-coloured wells) observed following incubation. AMR = $\Sigma(OD_{well} - OD_{neg})/6$, where AMR is the average metabolic response, OD_{well} is the carbon source optical density, OD_{neg} is the optical density of the control and 6 is the six groups of carbon compounds. Soil microbial biodiversity (Shannon-Wiener index) was calculated from Biolog EcoPlate results (Song et al. 2016).

Total soil DNA extraction and qPCR

0.25 g of soil was removed from each soil sample for DNA extraction using the PowerSoil™ DNA isolation kit according to the manufacturer's protocol (Mo Bio Laboratories, USA). *Pantoea agglomerans* MSCL 652, *Bacillus subtilis* MSCL 897 and *Trichoderma asperellum* MSCL 309 DNA 1–10^4 pg/μl were used to obtain standard melting curves. qPCR mixture in volume of 25 μl contained Maxima SYBR Green/ROX qPCR Master Mix (Thermo Fisher Scientific) with primer 0.2 μM and 1 μl (5–10 ng) of DNA. Samples were prepared in triplicate. A separate qPCR plate was used for day 0, day 30 and day 60 samples. qPCR programs for the quantification of different groups of microorganisms: initial denaturation 15 min at 95 °C, denaturation 30 s at 95 °C, annealing 30 s at 60 °C (for *Firmicutes* 30 s at 50 °C) and extension 30 s at 72 °C. The denaturation, annealing and extension steps were repeated 40 times (for *Trichoderma* 37 times). For each group of microorganisms, individual qPCR primers (Sigma-Aldrich, USA) were selected (Table 1). 7300 RT-PCR equipment (Applied Biosystems, USA) was used for qPCR reaction. To confirm the specificity of amplified PCR products, all qPCR reactions were followed by melting curve analysis and agarose gel electrophoresis. The obtained data were analysed with the 7300 System SDS program.

Statistical analysis and processing of data

The statistical calculator available on Socscistatistics.com, 2019 web resource, was used to calculate the Pearson correlation and its reliability, as well as to determine the chi-square criterion. The Pearson coefficient and significance of correlation were reported ($P \leq 0.05$). The correlation strength values used were as follows (absolute value of r): 0.00–0.19—very weak, 0.20–0.39—weak, 0.40–0.59—moderate, 0.60–0.79—strong and 0.80–1.0—very strong correlation (Evans 1996).

Physical indicators of soil

In all experimental soil samples except for the control, the percentages of moisture increased from 60 to 65% to 67–71% of the total weight after 60 days ($P < 0.05$). Comparing the pH values at the beginning and end of the experiment, as well as between different treated samples, it can be concluded that they did not differ significantly and fluctuated between pH 6.5 and 7.0 ($P > 0.05$).

Results

Number of cultivated microorganisms

The control soil contained an average 0.28 ± 0.15 × 10^9 fungal CFUs and 12.65 ± 1.79 × 10^9 bacterial CFUs per gram without significant changes during the experiment. The soil contained *B. subtilis* 1.63 ± 0.42 × 10^9 CFUs/g and *Actinobacteria* 1.63 ± 0.23 × 10^9 CFUs/g as assessed

Table 1 Primers used qPCR

Specificity	Primer	Sequence	Reference
Gammaproteobacteria	Gam877F	5′-GCTAACGCATTAAGTRYCCCG-3′	Yang et al. 2015
	Gam1066R	5′-GCCATGCRGCACCTGTCT-3′	
Firmicutes	Lgc353F	5′-GCAGTAGGGAATCTTCCG-3′	Fierer et al. 2005
	Eub518R	5′-ATTACCGCGGCTGCTGG-3′	
Acidobacteria	Acid31	5′-GATCCTGGCTCAGAATC-3′	Fierer et al. 2005
	Eub518R	5′-ATTACCGCGGCTGCTGG-3′	
Trichoderma	uTr	5′-AAGTTCAGCGGGTATTCCT-3′	Fierer et al. 2005
	uTf	5′- AACGTTACCAAACTGTTG-3′	

by bacterial colony morphology. After addition of microorganisms, in soil samples of the 0 days, an increase in the number of fungal CFUs could be observed compared to the control without introduced microorganisms except where *B. cinerea* had been introduced (Fig. 1a). The number of CFUs differed significantly ($P < 0.05$) 30 days later in the sample containing only *T. asperellum* and after 60 days in the sample containing the *B. subtilis* + *T .asperellum* + *B. cinerea* consortium. The number of fungal CFUs in all soil samples decreased significantly ($P < 0.05$) over 60 days except in the control (Fig. 1a). After microscopic examination, it was found that fungi belonging to genera *Geomyces*, *Penicillium* and *Mucor* were predominant in the control. *Mucor* and *Verticillium* were predominant in soil treated with *B. cinerea*. *Trichoderma* was predominant in all samples where *Trichoderma* was introduced alone or in consortium. *B. cinerea* colonies were not detected in any of the samples.

The total bacterial CFUs did not increase significantly in any of the samples compared to the control, but they decreased ($P < 0.05$) in the samples with *B. cinerea*, *T. asperellum* and three microorganisms together within 60 days (Fig. 1b), whereas the amount of *Actinobacteria* decreased significantly in all samples except treated with *B. cinerea* (Fig. 1d).

After 60 days, the number of *B. subtilis* CFUs in the *B. cinerea* and *B. subtilis* + *T. asperellum* + *B. cinerea* samples was significantly reduced compared to the 0 days in the controls. At days 0, 30, and 60, the number of CFUs in the *B. subtilis*, *B. subtilis* + *T. asperellum* and *B.*

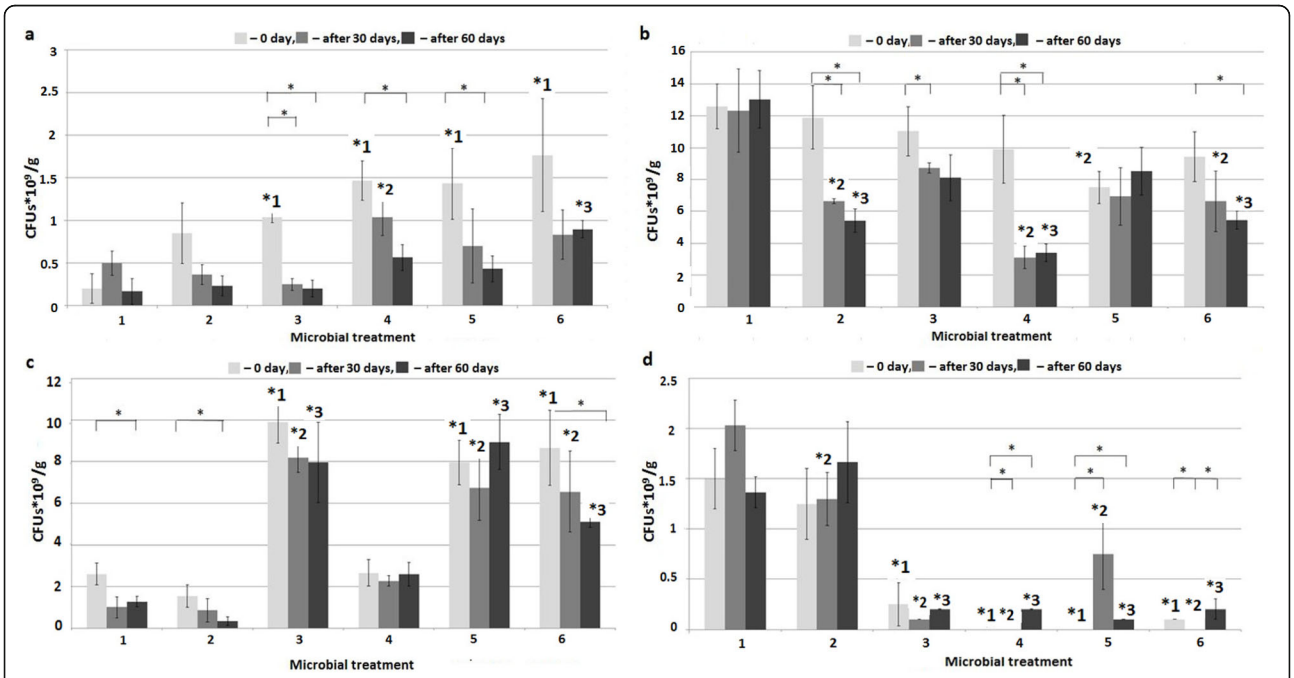

Fig. 1 Number of CFUs of fungi (**a**), bacteria (**b**), *B. subtilis* (**c**) and *Actinobacteria* (**d**) in control and in soil samples treated with different microorganisms and their consortia over different time periods. *—significant ($P < 0.05$) difference between samples at the same treatments at different times; $*^{123}$—significant difference from control at the same time; standard deviation is obtained from three replicate

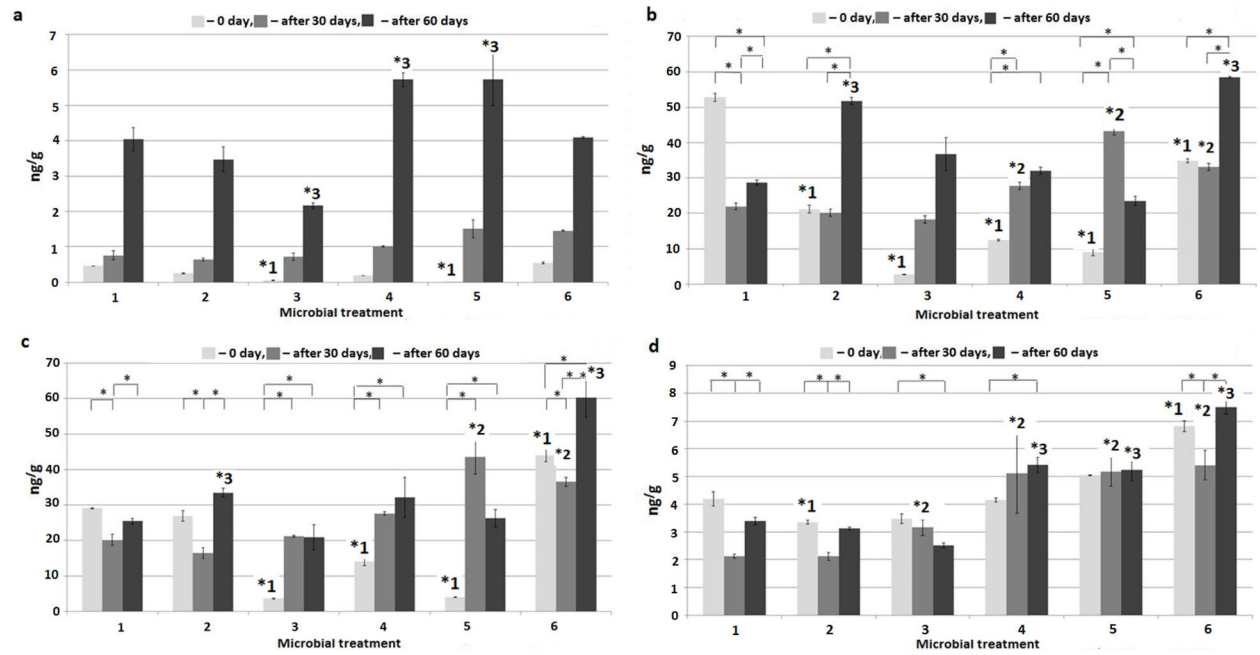

Fig. 2 Concentrations of amplified products of *Gammaproteobacteria* (**a**), *Acidobacteria* (**b**), *Firmicutes* (**c**) and *Trichoderma* (**d**) in treated soil samples at different time periods. *—significant ($P < 0.05$) difference between samples at the same treatments; $*^{123}$—significant difference from control at the same time; standard deviation is obtained from three replicates

subtilis + *T. asperellum* + *B. cinerea* samples was significantly higher than in corresponding controls ($P < 0.05$; Fig. 2a).

qPCR results for microorganism amplicon concentrations

The concentration of *Gammaproteobacteria* DNA amplicons between the differently treated soil samples differed significantly at all times ($P < 0.05$). After 30 and 60 days, *Gammaproteobacteria* DNA amplicon concentrations increased in all samples regardless of treatment (Fig. 2a). All samples showed increase of *Acidobacteria* DNA amplicons after 60 days ($P < 0.05$) except for the control and the sample containing *B. subtilis* + *T. asperellum* (Fig. 2b). Samples in which *B. subtilis* or the consortium were introduced did not show a significant increase in *Firmicutes* DNA amplicon concentration after a certain number of days except for the sample in which three microorganisms were introduced (Fig. 2c). Significant increases in *Trichoderma* amplified DNA amplicons over time could only be detected in a sample treated with the consortium of three microorganisms (Fig. 2d).

Biolog EcoPlate results

Decreases in the AMR of amines and organophosphorus compounds were observed in all differently treated soil samples after 60 days. Decreases in the AMR of amino acids and carboxylic acids could be seen in all samples except controls. In contrast, an increase in the AMR of carbohydrates and complex carbon sources were observed in all samples except the one in which *B. cinerea* was introduced. Total AMR values increased only in the control sample, unchanged in the sample containing the consortium of three microorganisms, but decreased in all other samples after 60 days (Fig. 3a).

The chi-square statistical criterion showed that the control CMD values (Fig. 3b) did not differ significantly from other samples at corresponding days ($P > 0.05$), whereas the AMR values at day 60 differed significantly between the control and *B. cinerea* samples ($P = 0.0026$) as well as between the control and the sample in which *B. subtilis* + *T. asperellum* were introduced ($P = 0.0160$).

It was estimated that the Shannon-Wiener diversity index decreased during incubation in all differently treated samples, from 1.73 to 1.58 in control, from 1.72 to 1.54 in *B. cinerea* sample, from 1.75 to 1.50 in *B. subtilis*, from 1.73 to 1.45 in *T. asperellum*, from 1.76 to 1.56 in *B. subtilis* + *T. asperellum* and from 1.75 to 1.63 in *B. subtilis* + *T. asperellum* + *B. cinerea* sample. Comparison of the Shannon-Wiener diversity index at day 0 and day 60 showed that the greatest diversity was in samples containing a consortium of all three microorganisms.

Results of the correlation analysis

Simple linear correlation analysis (Pearson correlation) was performed to test the correlation between data on CFUs numbers and the concentration of amplified DNA fragments in treated soil samples (Fig. 4a–d). The correlation between different groups of microorganisms was

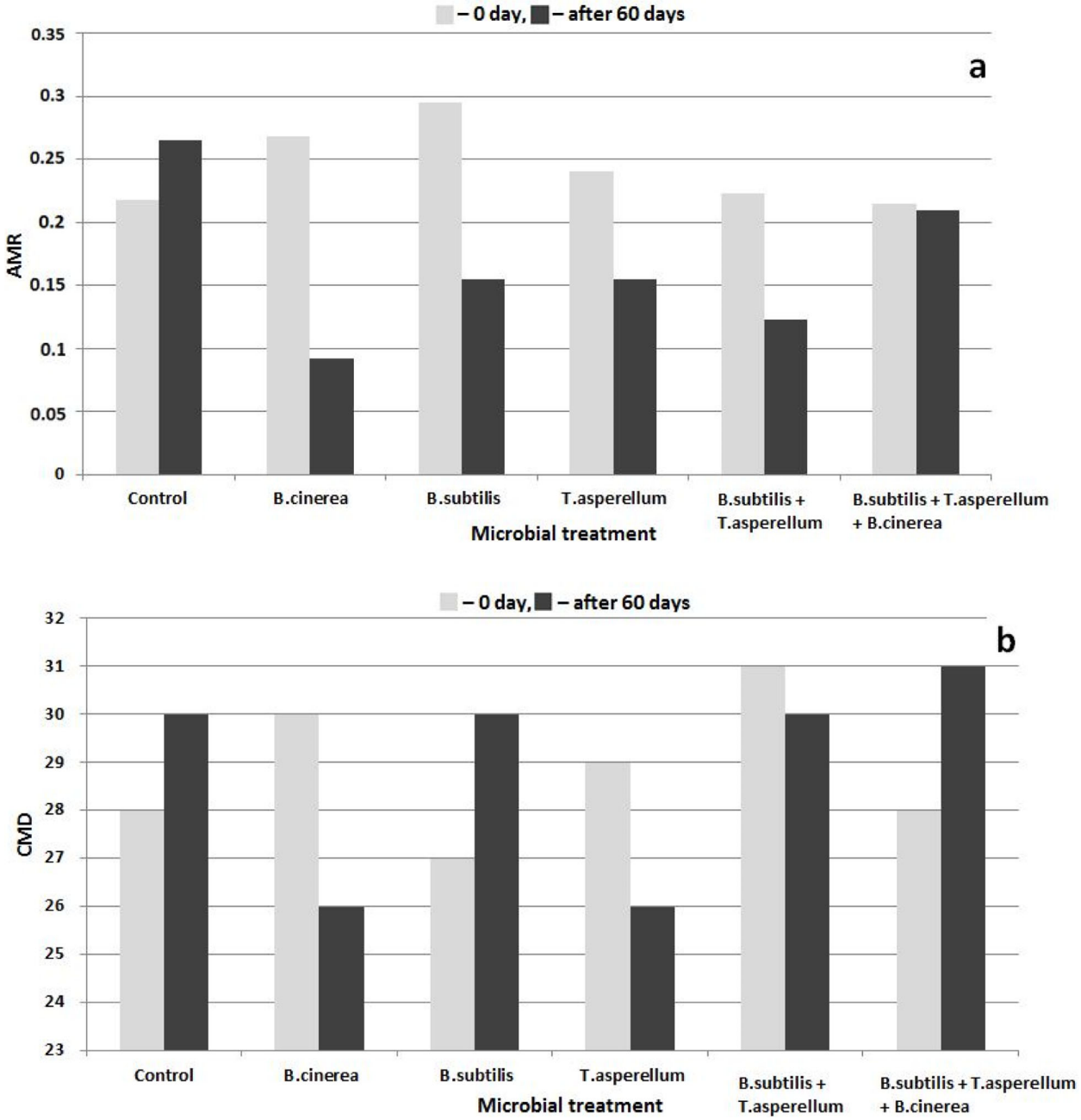

Fig. 3 a Average metabolic response (AMR) and **b** community metabolic diversity (CMD) in differently treated soil samples at different times. *—significant ($P < 0.05$) difference from control at the same time; *—significant ($P < 0.05$) difference from control at the same time

also tested separately on the basis of the concentration data of the amplified DNA fragments (Fig. 4 e and f). At 0 days, a non-significant correlation was found between the microorganism concentrations in all samples obtained by the various methods, except for *Gammaproteobacteria* and *Acidobacteria* (Fig. 4e). The Pearson correlation analysis indicated a very strong correlation ($r \geq 0.8$, $P \leq 0.05$) between the concentration of the amplified DNA fragments of *Trichoderma* and fungal CFUs/g (Fig. 4a). A significant very strong correlation was found between *Gammaproteobacteria* and *Acidobacteria* and between *Trichoderma* and *Firmicutes* amplified DNA fragment concentrations ($r \geq 0.8$, $P \leq 0.05$) (Fig. 4e and f).

Discussion

The study investigated the effect of recognized beneficial soil rhizosphere microorganisms *T. asperellum* and *B. subtilis*, as well as grey mould causing *B. cinerea,* and

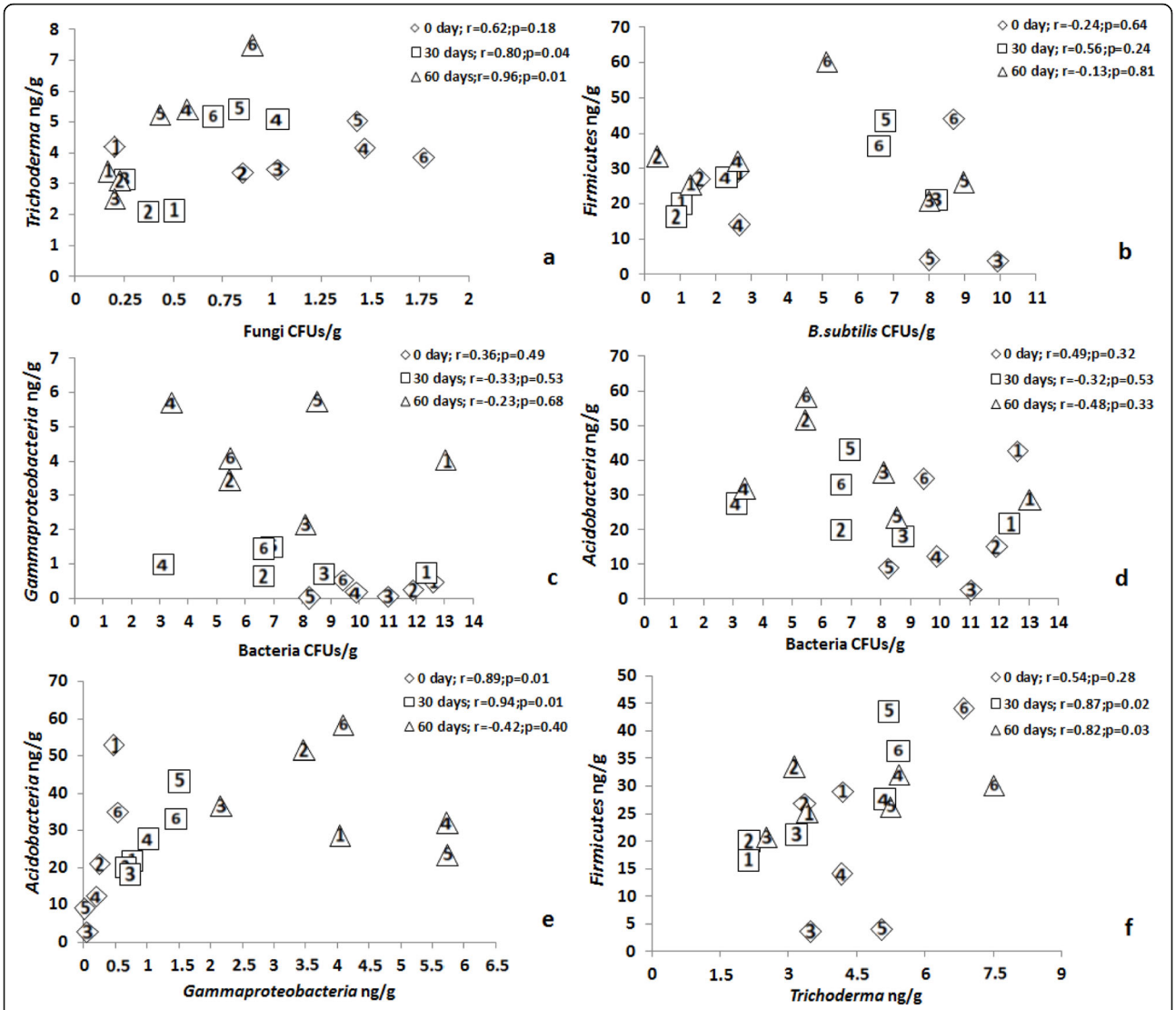

Fig. 4 Pearson correlation analysis between microbiological and molecular analysis data. Treatment of experimental soil samples: (1) control, (2) *Botrytis cinerea*, (3) *Bacillus subtilis*, (4) *Trichoderma asperellum*, (5) *B. subtilis* + *T .asperellum* and (6) *B. subtilis* + *T. asperellum* + *B. cinerea*. Pearson correlation coefficient and the significance of correlation are indicated. Correlation between fungal CFUs/g and amplicons of *Trichoderma* ng/g (**a**), between *B. subtilis* CFUs/g and *Firmicutes* ng/g (**b**), between bacterial CFUs/g and *Gammaproteobacteria* amplicons ng/g (**c**), between bacterial CFUs/g F and *Acidobacteria* amplicons ng/g (**d**), between *Acidobacteria* and *Gammaproteobacteria* amplicons ng/g (**e**) and between *Trichoderma* and *Firmicutes* amplicons ng/g (**f**)

their combinations on indigenous soil bacteria and fungi and related biochemical processes.

Soil physical data showed an increase in the total moisture content of the samples after treatment with microorganisms, which may be related to aerobic respiration: water and energy produced from substrates and oxygen and carbon dioxide released (Griffin 1996). The pH values found in experimental soil samples (pH 6.5–7.0) were suitable for optimal growth of microorganisms (Fierer et al. 2005).

With the exception of pH and soil moisture, the concentration of available nutrients in the soil also is an important factor that can directly affect the total number of bacteria and fungi. The introduction of additional microorganisms or consortia actively breeds and intensifies competition for nutrient availability. In the long run, this may result in a decrease in the total number of one or another group of microorganisms (Fitzsimons and Miller 2011). This could be confirmed by our results, where the number of fungal CFUs in all treated soil samples decreased significantly ($P < 0.05$) during incubation, except control (Fig. 1a). In contrast, McKenney et al. (2013) stated that *B. subtilis* endospores and their multilayer envelope provide long-term survival of bacteria under

adverse environmental conditions, such as competition with other microorganisms. This was not confirmed by part of our results, i.e. when number of *B. subtilis* CFUs decreased over a period of 60 days in samples with multispecies consortium *B. subtilis* + *T. asperellum* + *B. cinerea* (Fig. 1b).

Antibiotics, secondary metabolites, or degrading enzymes produced by *Actinobacteria* may actively act on certain soil microorganisms, including phytopathogens by inhibiting their growth. The ability of *Actinobacteria* to produce siderophores is effective, for example, in fighting the plant pathogenic *Fusarium oxysporum* (Getha et al. 2005; Barka et al. 2015). Previously, an increase in *Actinobacteria* and *Alphaproteobacteria* was observed in soil carrying pathogenic bacterium *Ralstonia solanacearum* (Wang et al. 2017). A similar situation could be observed with the introduction of *B. cinerea* into the soil, but the amount of *Actinobacteria* did not change significantly (Fig. 1d).

Quantitative PCR is now widely used in studies of microbial ecology to determine the number of genes and/or amplicons in specific samples (Smith and Osborn, 2009). Quantitative PCR is a sensitive method with a high degree of reproducibility; however, several disadvantages can also be observed, for example, the numerical values of the detected microorganisms may not reflect their objective number in different soil habitats. However, qPCR provides a reproducible measurement system standard for estimating the relative amount of bacteria and fungi in samples (Fierer et al. 2005). If we are talking about culture-dependent methods, it is important to mention that CFU is a very limited method too for a sample such as soil because this method selects a very small portion of the soil microbes to grow in the selective media. These methods are complementary but barely will give the same results in a complex environmental matrix such as soil (Cangelosi and Meschke 2014).

The above deficiencies may explain—at least partially—the results of the correlation analyses between different methods and groups of microorganisms. No significant correlation was found between the amplicon concentrations of *Firmicutes* and CFUs of *B. subtilis*, between the *Gammaproteobacteria* and total CFUs of bacteria and between the *Acidobacteria* and total CFUs of bacteria (Fig. 4b–d).

Soil taxa of *Proteobacteria*, *Actinobacteria* and *Acidobacteria* are the most abundant, according to the presence of the 16S rRNA genes, in taiga, scrub steppe and tropical forest ecosystems (Delgado-Baquerizo et al. 2018). Our results showed a significant effect of the soil treatment on the amount of specific microbial amplicons. Thus, according to qPCR data, the number of *Acidobacteria* decreased initially (day 0), but then approached the baseline ($P < 0.05$, Fig. 2b). The number of *Gammaproteobacteria* amplicons increased in all groups. This issue should be explored in future experiments. It is expected that the amount of *Trichoderma* increased in all samples with introduced *Trichoderma*, alone or in consortium (Fig. 2d). The number of *Trichoderma* amplicons correlated with the number of *Firmicutes* including *B. subtilis* amplicons, though in some cases (samples with added *B. subtilis* alone or *T. asperellum* alone), it did not match the amount of *B. subtilis* CFUs (Fig. 1c). Further studies will need to investigate the factors that led to a drastic reduction in *Actinobacteria* cultivability immediately after the introduction of the microorganisms in the soil, with no subsequent improvement (Fig. 1d). A relatively high concentration of *Acidobacteria* DNA amplicons was found in all samples (16.4–40.6 ng g^{-1}, Fig. 2b). These bacteria are ubiquitous and abundant members of soil bacterial communities but are difficult to culture or un-culturable at present (Jones et al. 2009); therefore, the observed non-correlation with bacterial CFUs was expected.

The biochemical characterization of soil microbial communities confirmed the ability of soil microorganisms to metabolise all substrates offered in Biolog EcoPlate. CMD values fluctuated insignificantly but, despite the addition of microorganisms, AMP values decreased in *B. cinerea* samples and samples with *B. subtilis* + *T. asperellum* (Fig. 3). AMR also decreased during incubation in all samples with added microorganisms. It is consistent with the Shannon-Wiener diversity index which also tended to decrease. However, the addition of microorganisms other than *B. cinerea* has been found to increase the ability of soil microbial populations to utilize carbohydrates and complex carbon compounds. The method does not distinguish between who does it, added microorganisms or indigenous soil populations stimulated by interspecies interactions (Javorekova et al. 2015). .

Smalla et al. (1998) believe that the Biolog EcoPlate method can determine only the ability of the most competitive soil microorganisms to utilize a particular carbon substrate without determining the potential of entire soil populations. In several studies, Biolog EcoPlate assay has been used in greenhouse or field experiments with plants. For example, the functional diversity of microorganisms in soil grown on common maize (*Zea mays*) was determined using Biolog EcoPlate and biochemical methods. A positive correlation can be observed between the biodegradation rates of the soils analysed and the utilization of the major carbon compounds except the amino acid group. Productivity of conventional maize correlated with soil biological activity (Galazka et al. 2017).

From this study, it can be concluded that the introduced agents of biocontrol and plant growth *T.*

asperellum and *B. subtilis* survive in the soil during a 60-day experiment and affect the composition and functionality of the indigenous populations. It is known that the functioning of microbiological consortia can also be influenced by the physiological properties of certain plants, such as the excretion of roots or their structure. From the point of view of plant protection, it is important to consider *B. subtilis* and *T. asperellum* and their consortium as an inhibitor of phytopathogenic fungus *B. cinerea*, which negatively affects to the cultivation of specific crops.

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Authors' contributions
MS: writing—final draft, data analysis. VN: conceptualization, data analysis, review and editing. GM: methodology. ZP: writing—review. All authors read and approved the final manuscript.

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Availability of data and materials
All data generated or analysed during this study are included in this article.

Declarations

Ethics approval and consent to participate
Not applicable.

Consent for publication
Not applicable.

Competing interests
The authors declare that they have no competing interests.

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