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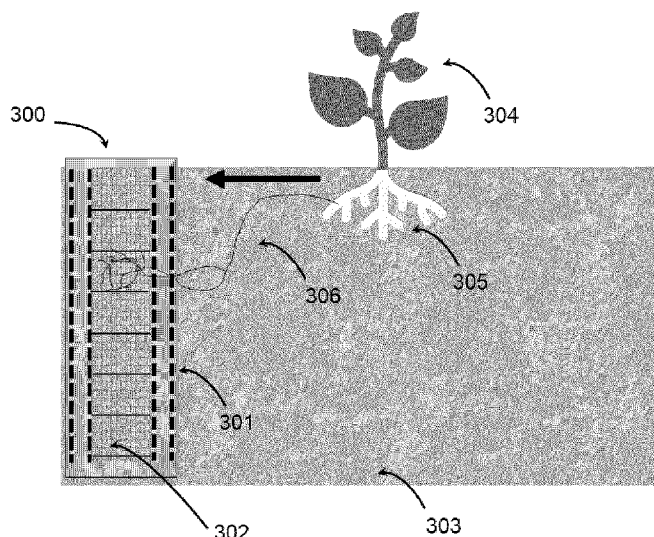


Fig. 3

(57) Abstract: The present invention relates to a lab-on-a-chip device with modular layout for determining a plurality of biological, molecular, chemical or physical parameters associated with soil, in particular soil health and quality. Also disclosed is method for using the device.



DEVICE FOR MEASURING SOIL PARAMETERS

TECHNICAL FIELD

[001] The present disclosure relates to a device and a method for measuring one or more biological, molecular, chemical or physical parameters associated with soil. This can be useful for the efficient and reliable on-the-spot assessment and long-time monitoring of soil health and quality.

BACKGROUND

[002] Plants are capable of hosting a diverse array of microorganisms, collectively referred to as the plant microbiome, in different areas of the plant such as the rhizosphere (the soil surrounding plant roots), endosphere (the internal tissues of the plant), and phyllosphere (the leaves, stem, and flowers). These microbiomes can establish long-term interactions with the host plant, which can have varying effects on crop performance and microbe-mediated biogeochemical processes, ranging from positive to neutral or negative impacts. The microorganisms that form a symbiotic relationship with the host plant and contribute to its health, function, and evolution are referred to as microsymbionts.

[003] As an example of such plant-microbe interactions, so-called arbuscular mycorrhizal associations are mutually beneficial relationships between the roots of most plant species and fungi present in the soil. These mutualistic symbiotic relationships involve the exchange of nutrients between the two organisms, with the fungi receiving carbohydrates from the plant and the plant receiving essential nutrients such as phosphorus and nitrogen from the fungi.

[004] The presence of mycorrhizal associations in soil is an indicator of overall soil health quality for several reasons. First, the fungi involved in these associations help to improve soil structure by producing a network of fungal threads that bind soil particles together, improving soil stability and reducing erosion. Additionally, mycorrhizal associations can increase the ability of plants to take up nutrients from the soil, which can lead to increased plant growth and productivity.

[005] Furthermore, mycorrhizal associations can help to improve soil health by promoting the growth of beneficial soil microorganisms such as bacteria and other fungi. This, in turn, can lead to the formation of soil aggregates that improve soil structure, water-holding capacity, and nutrient availability.

[006] Overall, the presence and abundance of plant-microbe interactions, such as mycorrhizal associations, in soil can be seen as an indicator of the health and fertility of the soil. The absence of such associations can suggest soil degradation or disturbances that have disrupted the natural functioning of the soil ecosystem. Hence, monitoring the presence, quantity and quality of plant-microbe interactions can be a useful tool in assessing overall soil health quality.

[007] It would be advantageous if such plant-microbe interactions could be reliably, economically and efficiently measured in a variety of locations.

[008] Devices and methods for studying plant-microbe interactions are known in the art. For example, Mafla-Endara et al., *"Microfluidic chips provide visual access to in situ soil ecology"*, COMMUNICATIONS BIOLOGY, vol. 4, Article number 889, 20 July 2021, pages 1-12, describes microfabricated, transparent 'soil chips' that can be buried in the field, where soil microbes and minerals can enter the device. Mafla-Endara et al. particularly focus on assessing the influence of pore space medium (air, water, nutrients) and pore space geometry on nutrient cycling, feedback between microbes and soil physical processes, inter-kingdom interactions, and biodiversity-function relationships. Only external visual inspection and optical microscopy (inverted microscope, bright-field imaging, real-time video) and Raman scattering microspectroscopy of the 'soil chips', without any detectors being present in the device per se, is taught.

[009] Ozoe et al., *"Early characterization method of plant root adaptability to soil environments"*, PROCEEDINGS OF THE 2015 28TH IEEE INTERNATIONAL CONFERENCE ON MICRO ELECTRO MECHANICAL SYSTEMS (MEMS), 18 January 2015, pages 702-705, describe a device developed for quantitatively studying physical (as opposed to chemical and biological) mechanisms of plant root growth in soil environments. The device incorporates a force displacement sensor for registering microforces associated with response of growing roots to physical barriers (e.g. stones, leaves).

[010] Borer et al., *"Spatial organization of bacterial populations in response to oxygen and carbon counter-gradients in pore networks"*, NATURE COMMUNICATIONS, vol. 9, Article number 769, 22 February 2018, pages 1-11 describes a study of the response of heterogeneous bacterial soil populations (i.e., differing in metabolic capabilities) to oxygen and carbon counter-gradients in pore networks. The study was conducted using micrometric pore networks etched in glass. The device does not contain separate chambers for colonisation, growth etc. of microbial species and merely describes an "oxygen optode" to assess oxygen levels at the centre of the pore network and peripheral ports.

[011] Richter et al., *"Fungi-on-a-Chip: microfluidic platforms for single-cell studies on fungi"*, FEMS MICROBIOLOGY REVIEWS, vol. 46, 24 August 2022, pages 1-29, review "fungi-on-a-chip" microfluidic devices and mentions several microscopic techniques (bright-field, high-resolution, fluorescence). The devices are for laboratory research rather than for in-field deployment in the soil.

[012] Stanley et al., *"Soil-on-a-Chip: microfluidic platforms for environmental organismal studies"*, LAB ON A CHIP, vol. 16, 27 November 2015, pages 228-241, provides a general review of so-called soil-on-chip devices.

[013] Sylvain et al. *"The EcoChip: A Wireless Multi-Sensor Platform for Comprehensive Environmental Monitoring"*, IEEE TRANSACTIONS ON BIOMEDICAL CIRCUITS AND SYSTEMS, vol. 12, 1 December 2018, pages 1289-1300, describes the "EcoChip", which is aimed at the culturing of microorganisms and real-time monitoring, in the field, of specifically bacterial growth. The system comprises two distinct parts, an embedded electronic board which includes different sensor interface circuits, and a layered structure including a microfabricated gold-plated electrode array as well as a 96-well plate.

[014] There is a need for improved devices and methods for assessing soil health parameters.

SUMMARY OF THE INVENTION

[015] Thus, it is an object of the invention to be able to measure one or more biological, molecular, chemical or physical parameters associated with soil, in particular to soil health and quality in a variety of locations, such as a farmer's field, in an easy, reproducible and inexpensive manner. More specifically, it is an object to provide field-deployable, low-cost means for rapid, preferably real-time, monitoring of biological, molecular, chemical or physical parameters associated with soil, particularly with mycorrhizal associations.

[016] This objective is achieved with the device and associated methods according to the present invention. Accordingly, in first aspect there is provided a lab-on-a-chip device with modular layout for determining a plurality of biological, molecular, chemical or physical parameters associated with soil, the device comprising

an inner space confined by an exterior surface, wherein the exterior surface is configured to be at least partially and sustainably contacted with soil, and wherein the exterior surface comprises one or more inlet channels configured to provide any of fluid communication, organismal mobility, and colonization between the soil and the inner space,

wherein the inner space comprises one or a plurality of discrete modules embedded in an inner matrix material,

wherein each module comprises a self-contained chamber, the chamber being configured to provide any of fluid communication, organismal mobility, and colonization with an inlet channel and/or one or more other chambers,

wherein one or more chambers are configured to attract, collect, grow and/or to become colonized with a microorganism present in the soil; and

wherein one or more chambers are configured to provide data in relation to one or more preselected biological, chemical, molecular, or physical parameters associated with the soil.

[017] This device allows monitoring the presence, abundance and nature of interactions, such as mycorrhizal associations, between soil-dwelling microorganisms and their environment, thus providing a useful indicator of overall soil health quality. For example, soils with a healthy microbial, particularly mycorrhizal, population are likely to have good nutrient cycling and retention, as well as good soil structure and water-holding capacity. Conversely, soils with poor microbial populations may be an indicator of reduced plant growth and yields, and may be more prone to erosion and nutrient leaching.

[018] The lab-on-chip device as disclosed herein comprises one or a plurality of discrete modules. In one embodiment, the device contains one discrete module. Preferably, the device contains a plurality of discrete modules. The modular layout of this device design provides a high degree of flexibility and

adaptability. By utilizing separate modules that can be easily assembled, disassembled, and replaced, the design enables customization and modularity, allowing the addition or removal of features, thus making the device more versatile and adaptable to various use cases and environments. As a result, this design is ideal for a wide range of applications and users. Additionally, the modular design facilitates easy upgrades and repairs in the event of module wear or failure. Advantageously, the modular design of the device may promote sustainability by reducing material waste and extending the lifespan of the device.

[019] The modular lab-on-a-chip design facilitates simultaneous testing of multiple biological, physical, chemical and molecular parameters related to soil-dwelling organisms in a single device. The organisms may be loaded directly onto the device or attracted to the device from the soil by means of, for example, nutrients, carbon or nitrogen gradients, or chemical attractants.

[020] The device can be implemented as a portable, field-deployable unit that allows farmers, to quickly and easily obtain on-the-spot information on local soil health parameters, and to use these data, if desired, to make any adjustments to sowing, planting, irrigation, fertilisation, pest control etc. strategies. The device may also be implemented into one or more pots or other units that allows users, such as agricultural scientists, to quickly and easily obtain on-the-spot information and/or collect data over a certain time period.

[021] Accordingly, in a second aspect of the present invention, there is provided a method for determining one or more biological, chemical, molecular, or physical parameters associated with soil, the method comprising:

providing the lab-on-chip-device as disclosed herein,
deploying the lab-on-a-chip device at least partly in the soil,
optionally removing the lab-on-a-chip device from the soil after a predetermined time period,
and
obtaining data in relation to a plurality of preselected biological, chemical, or physical parameters associated with the soil during the predetermined time period, and
optionally further processing the obtained data.

[022] The one or more preselected biological, molecular, chemical, or physical parameters may be selected from spore germination, colonization events, presence and/or concentration of one or more microorganisms; degree of soil biodiversity; plant-microbe interactions; root exudation(s); degree of response to pesticides, xenobiotics, heavy metals, or other inorganic species; response to one or more chemical attractants or repellents; degradation or migration of chemicals, such as the degradation of lignin, cellulose, or other plant materials indicative of carbon cycling, the solubilization of phosphorus or other elements from minerals, the mineralization of nitrogen (from e.g. polymers, proteins, or other organic sources), and the degradation of microplastics and other contaminants.

[023] In one embodiment of the second aspect, the collected data comprise data in relation to arbuscular mycorrhiza fungi (AMF) in the soil.

BRIEF DESCRIPTION OF THE DRAWINGS

[024] Fig. 1 displays a schematic drawing of modular lab-on-a-chip device comprising six discrete modules.

[025] Fig. 2 displays a modular lab-on-a-chip device comprising three discrete modules.

[026] Fig. 3 displays a modular lab-on-a-chip device comprising seven discrete modules with selective barriers for colonisation of AMF.

[027] Fig. 4 displays a modular lab-on-a-chip device comprising seven discrete modules for spore germination and colonization of chambers

[028] Fig. 5 displays a modular lab-on-a-chip device comprising fourteen discrete modules comprising different fungal lineages for active ingredient (AI) testing.

[029] Fig. 6 displays a schematic overview of how data from the device of the invention may be collected to identify the degradation, solubilization, mobilization of compounds or the deposition or growth of microorganisms.

[030] Fig. 7 displays the results of a soil parameter test carried out using the device according to an embodiment of the invention.

[031] Fig. 8 displays an embodiment of a device or a device module for use in a device according to the invention.

[032] Fig. 9 displays an embodiment of a device, or a device module for use in a device according to the invention.

[033] Fig. 10 illustrates the arrangement of a plurality of devices or device modules according to the present disclosure in an array.

[034] Fig. 11 displays the results of electrical conductivity measurements in a device module according to the invention.

[035] Fig. 12 displays the results of electrical conductivity measurements in a device module according to the invention.

DETAILED DESCRIPTION

[036] The invention primarily relates to lab-on-a-chip device for measuring a plurality of biological, chemical or physical parameters associated with soil health. As used herein, the term soil health refers to refers to the overall condition of the soil as a living ecosystem that supports plant growth, biodiversity, and environmental sustainability. Good soil health is characterized by a balanced mix of physical, chemical, and biological properties, including good structure, adequate nutrients, and a diverse population of microorganisms. A healthy soil is able to maintain its fertility, resist erosion, and support

healthy plant growth, while also providing essential ecosystem services such as carbon sequestration, water filtration, and nutrient cycling.

[037] As used herein, a lab-on-a-chip device refers to a miniaturized system that is capable of integrating multiple laboratory functions, such as collecting sample material, providing media, attractants, repellents and/or nutrients, growing of microbial species, mixing of compounds, separation of compounds, and a variety of biological, chemical or physical analysis tools, onto a single chip-like device.

[038] As will be explained in more detail below, the lab-on-a-chip devices according to the present invention are not limited to sub-centimetre devices but also encompass devices with chambers having dimensions in the order of several square centimetres. Accordingly, many embodiments of the present invention do not require complex miniaturisation and tedious integration of sensors, as many types of sensors (such as, for example, electrodes for determining electrical conductivity) are already routinely available at micrometre to nanometre scales. Also, populating the measurement chamber does not necessarily require complicated device designs, as migration of micro-organisms can be controlled naturally and/or by adding (gradients of) nutrients or attractants (or conversely, repellents).

[039] The established knowledge in the field of lab-on-a-chip devices, as well as the proven advantages of such devices, make them ideally suited for the applications as described herein. For example, small working volumes are possible due to the high surface-to-volume ratio of microfluidic channels, which allows for efficient mixing and interaction of species and agents. Additionally, the use of controlled flow paths enables precise control over the location and timing of delivery and removal of such species and agents. Physical heterogeneity, such as the introduction of barriers or varying surface properties, can be achieved through microfabrication techniques such as soft lithography. This allows for the creation of complex microenvironments that can mimic the natural physical heterogeneity of biological systems.

[040] Physical and chemical gradients can be generated using lab-on-a-chip devices through the controlled provision of different media, attractants, repellents and/or nutrients. This allows for the precise mimicking or manipulation of environmental factors and the creation of well-defined gradients.

[041] Patchiness, or the creation of localized areas with different environmental conditions, can be achieved through the use of distinct types of media, attractants, repellents and/or nutrients in specific regions or chambers of the device, and/or the use of size-selective barriers and root-guidance systems. Guided root growth can be achieved through the use of microfabricated channels with precise geometries that mimic natural root pathways. These channels can be used to guide the growth of roots in a specific direction or to study the effects of different environmental factors on root growth.

[042] In other embodiments, the device may be provided with a gauze or mesh having a mesh size sufficiently small to prevent plant roots from entering the one or more of the chambers. Herein, the mesh or gauze forms a selective barrier for entrance of pre-selected species, allowing, for example, the activities of selected species, such as mycorrhizal fungi or other symbiotic fungi, to be monitored. The

mesh or gauze may be provided, for example, at an external surface of the device, such as between the soil and an inlet channel, or within an inlet channel. The mesh size chosen for providing a size-selective barrier for penetration by plant roots, whilst allowing entrance of species under investigation such as symbiotic fungi, depends on various conditions, including the plant species, but is generally smaller than 100 microns, preferably smaller than 75 microns, preferably smaller than 50 microns, most preferably smaller than 35 microns. In some embodiments, a mesh size of 1 micron or below may be used to also exclude both plant roots and fungal species.

[043] The lab-on-a-chip device according to the present invention typically comprises a plurality of discrete modules, wherein each module comprises a self-contained chamber adapted to collect data in relation to one or more pre-selected soil-related biological, chemical, molecular, or physical parameters. In one embodiment, the device comprises at least two modules. Typically, the device comprises at least three, four, five, six, seven, eight, nine, ten, eleven, twelve, thirteen, fourteen, fifteen, sixteen, seventeen, eighteen, nineteen, or twenty discrete modules. In one embodiment, the device comprises five, six, seven, eight, or nine discrete modules.

[044] The chambers of each module can have different specific functions. For example, they can be used as reservoirs for population of the device and distribution to other chambers, for germination or colonisation of microorganisms, for isolating or separating microorganisms, for analytical purposes such as quantification of particular events, to prepare for visual (e.g. with microscope) inspection, for accommodating specific sensors, and combinations thereof. Thus, each chamber comprised in the multi-module device may be adapted to perform one or more functions and/or actions selected from attracting, selecting, repelling, deterring, incapacitating, growing, transforming, colonizing, dispersing, separating, isolating, counting, characterizing, and visualizing of soil microorganisms and their interactions. In order to realise such functions or actions, each chamber comprises one or more components capable of eliciting such functions and/or actions, including but not limited to distinctive nutritional content, selective chemistries, attractants, microorganisms, and sensors. Depending on the particular chamber component or combination of components, each chamber may be sampled for specific metabolomic, DNA, eDNA, RNA or other molecular, elemental, or chemical analysis, or for the collection of microorganisms for subsequent isolation or characterization.

[045] In alternative embodiments, the present invention provides a device for determining one or more biological, molecular, chemical or physical parameters associated with soil, the device comprising

an inner space confined by an exterior surface, wherein the exterior surface is configured to be at least partially and sustainably contacted with soil, and wherein the exterior surface comprises one or more inlet channels configured to provide any of fluid communication, organismal mobility, and colonization between the soil and the inner space,

wherein the inner space comprises one or more discrete modules embedded in an inner matrix material,

wherein each module comprises a self-contained chamber, the chamber being configured to provide any of fluid communication, organismal mobility, and colonization with an inlet channel and/or one or more other chambers,

wherein one or more chambers are configured to attract, collect, grow and/or become colonized by a microorganism present in the soil; and

wherein one or more chambers are configured to provide data in relation to one or more preselected biological, chemical, molecular, or physical parameters associated with the soil.

[046] In a preferred embodiment of this device, the inner space comprises a single discrete module, wherein the module comprises a self-contained chamber, wherein the chamber is configured to provide any of fluid communication, organismal mobility, and colonization with an inlet channel,

wherein the chamber is configured to attract, collect, grow and/or become colonized by a microorganism present in the soil; and

wherein the chamber is configured to provide data in relation to one or more preselected biological, chemical, molecular, or physical parameters associated with the soil.

[047] Preferably, in this embodiment the module is capable of monitoring electrical conductivity of a soil solution containing soil-dwelling microorganism, such as symbiotic fungi. Thus, in an embodiment the chamber of the module comprises an electrical conductivity sensor.

[048] Preferably, in this embodiment plant roots are prevented from entering the module chamber by means of a size-selective barrier as described herein.

[049] As used herein, "microorganisms" are understood to include bacteria, fungi, protozoa, arthropods, nematodes, and algae. Particularly interesting microorganisms for studying the interactions as described herein are mycorrhizal fungi and other symbiotic fungi, free-living and pathogenic nematodes, phosphorous-solubilizing and nitrogen-mineralizing bacteria, and lignocellulolytic microorganisms.

[050] In one embodiment, the device comprises at least one colonization chamber. Suitably, the colonization chamber comprises nutrients, auxotrophies (i.e., metabolites required for an organism to grow, but which it cannot synthesize itself) and/or other compounds suitable for colonization of pre-selected microorganisms such as amino acids, vitamins, fatty acids, lipids carbohydrates, proteins and lignocellulosic biomass.

[051] Typically, the chamber of at least one discrete module comprises one or more sensors. Preferably, the one or more sensors are nanosensors, microsensors or colorimetric sensors. Preferably, the one or more sensors comprise at least one microsensor, at least one colorimetric sensor, or any combination thereof. Other examples of suitable sensors for implementation into the chamber of at least one discrete module include optical, electrical, electrochemical, thermal, magnetic, mechanical, gas or volatile, and biological sensors, and combinations thereof.

[052] Alternatively or additionally, any chamber may be equipped with one or more components or compounds that allow assessment, qualitatively or quantitatively, or a combination thereof, by an external analytical tool or device, such as visual inspection, optical microscopy (optionally using a portable microscope), fluorescence spectroscopy, electrical conductivity measurements, electrical impedance measurements, temperature measurements, antigen testing (e.g. glomalin), antibodies testing, enzymatic testing, gas detection, water flow measurements, vibration measurements, humidity measurements, pressure measurements, photoresistance measurements, ultrasonic testing, and acoustic measurements. Examples of optical or visual measurements include the amount of light let through by the degradation of lignocellulosic material or the solubilization/mineralization of inorganic or organic phosphate or nitrogen containing compounds. Examples of acoustic measurements include the sound of chewing of plant roots by parasitic nematodes and the sound of enhanced water flow due to hyphal structure.

[053] In an exemplary embodiment, the chamber of at least one discrete module of the device of the invention comprises an electrical conductivity sensor. Electrical conductivity may be used as a qualitative or quantitative indicator of the presence of nutrients and fertility of soil. More specifically, the absorption of nutrients and breakdown of organic matter by symbiotic fungi, and the subsequent transport and release of ionic nutrient species, such as phosphate, nitrate, potassium, and magnesium ions, into the surrounding soil solution by these fungi increases the total ionic concentration of the solution. As electrical conductivity is directly proportional to the concentration of ionic species in the solution, electrical conductivity sensing is an effective means for assessing the activity and effectiveness of symbiotic fungi in nutrient uptake and transfer to the plant. In an embodiment, symbiotic fungi, such as arbuscular mycorrhizal fungi (AMF) or other mycorrhizal species, are allowed to access nutrients present in the soil in a selective manner, such as by providing a small-pore, size-selective mesh which plant roots cannot pass, and subsequently release those nutrients within the chamber, resulting in an increase in electrical conductivity measured within the chamber.

[054] These measurements and tests, and associated sensors, devices and compounds allow assessing the relevant processes indicative of key biological parameters, such as nutrient bioavailability and biogeochemical cycling related to carbon, nitrogen, phosphorus and other elements, water usage and transport, the presence or absence of particular microorganisms contributing to these processes, or the presence or absence of pathogens and plant parasites.

[055] In one embodiment, the lab-on-a-chip device according to the present invention is configured to be buried in the soil to a specified depth or a range of specified depths, allowing the sensors to collect data at the desired depth or, for example a series of specific depths. Accordingly, preferably, the exterior surface of the device is made of a material, or combinations of materials, that can withstand the effects of burial in the ground, such as, for example, glass, fibreglass, stone, ceramic, plastic, rubber, corrugated metal, geotextiles, and various composite materials. Combinations of such materials are also possible.

[056] Advantageously, one or more modules of the device are configured for populating the device with one or more microorganisms. Population can be effected without discriminating between different species of microorganisms present in the soil, or the device may be equipped with means to populate the device with preselected species. These species can be an array of indicator microorganisms such as mycorrhizal fungi, phosphorus-solubilizing or nitrogen-mineralizing bacteria, or pathogenic or parasitic fungi and nematodes respectively.

[057] In order to differentiate between microorganisms present in the soil, at least one chamber of the device may suitably comprise one or more chemical signals to attract or repel pre-selected microorganisms. Examples of such chemical signals include, but are not limited to volatile organic compounds (VOCs), phytohormones and other signaling molecules, which are used as attractants to signal the presence of nutrients or as repellents to signal the presence of toxins or other harmful conditions.

[058] One or more chambers of the device may suitably be provided with means to selectively control microorganisms, such as inhibiting growth, preventing reproduction, or killing. In one embodiment of the present invention, the chamber of at least one discrete module comprises one or more pesticides adapted to select against one or more microorganisms. In the device according to the invention, such pesticides can be applied for selecting against the presence of unwanted microorganisms in the device, or for investigating the effect of certain pesticides or combinations thereof on soil quality and health.

[059] As used herein, the term "pesticide," means and includes an active material or substance that kills, regulates, or otherwise adversely affects the growth of pests (e.g., insects, mites, ticks, nematodes, bacteria, fungi, diseases, and plants).

[060] The pesticides may comprise fungicides, herbicides, insecticides, nematocides, molluscicides, acaricides, slimicides, algicides, viricides, rodenticides, bactericides, chemosterilants, anthropodocides, anthelmintics, and antibiotics.

[061] As used herein, the term "insecticide," means and includes an active material that kills, regulates, or otherwise adversely affects the growth of insects.

[062] As used herein, the term "fungicide," means and includes an active material or substance that kills, controls, or otherwise adversely affects the growth of fungi or fungal spores.

[063] As used herein, the term "herbicide," means and includes an active material that kills, controls, or otherwise adversely affects the growth of plants.

[064] Examples of suitable pesticides which may be selected include those identified in "The Pesticide Manual" (The Pesticide Manual - A World Compendium; Thirteenth Edition; Editor: C. D. S. Tomlin; The British Crop Protection Council). In one embodiment, the pesticide is a pesticide yet to be developed or tested, wherein the device as described herein can be applied to obtain specific information on the effect of this new pesticide or group of pesticides on one or more aspects of soil health.

[065] One or more chambers of the device may suitably be provided with means to selectively attract and support growth of one or more microorganisms, such as a carbon gradient, a nitrogen gradient or a nutrient composition.

[066] In one embodiment, the chamber of at least one discrete module comprises a carbon gradient adapted to attract and support growth of one or more, preferably pre-selected, microorganisms. For example, in order to attract and support growth of aerobic bacteria, a spatial glucose gradient may be used, which serves to attract such aerobic bacteria along a pathway of increasing glucose concentration. Other examples of suitable carbon gradients for attracting and supporting growth of aerobic bacteria include fructose, lactose, ethanol and specific amino acids and fatty acids. Other examples of suitable carbon gradients for attracting and supporting growth of methanogenic bacteria include specific amino acids and fatty acids.

[067] In one embodiment, the chamber of at least one discrete module comprises one or more compound gradients such as cellulose which select for organisms having a lignocellulolytic function, such as cellulase, lignin peroxidase, xylanase, cellobiohydrolase, endoglucanase, β -glucosidase, and laccase.

[068] In one embodiment, the chamber of at least one discrete module comprises a nutrient composition adapted to attract and support growth of one or more, preferably pre-selected, microorganisms. Suitable nutrient compositions may contain any one of carbon sources, nitrogen sources, phosphorous sources, sulphur sources, trace elements, vitamins and water, and combinations thereof. The nutrient composition may be provided in solid form, in liquid form, or in the form of a solution, emulsion, suspension, sludge or biomass. The solid nutrient composition may be provided, for example, in powder, pill, or pellet form.

[069] Examples of suitable carbon sources include sugars, amino acids, and fatty acids. Examples of suitable nitrogen sources include ammonium, nitrate, amino acids, proteins and other inorganic and organic nitrogen sources. Examples of suitable phosphorus sources include phosphate ions, inorganic phosphate minerals, and organic phosphorus compounds. Examples of suitable sulphur sources include sulphate ions and organic sulphur compounds. Examples of trace elements required by microorganisms for various metabolic processes include metals such as iron, copper, and zinc. Examples of vitamins required by microorganisms for various metabolic processes include vitamin B12 and folic acid.

[070] The device as described herein is particularly suitable to study the presence, abundance and nature of interactions between soil-dwelling microorganisms and their environment. Accordingly, the chamber of at least one discrete module comprises one or more microorganisms, the one or more microorganism preferably comprising spores, resting cells, resting spores, or another resting stage of a microorganism.

[071] Each chamber comprised in the plurality of discrete modules in the device may have shapes and dimensions that are tailored to the specific function of the module and the chamber. Suitable shapes

are square, rectangular, oval, circular, etc. Typically, at least one of the dimensions of the chamber, for example the diameter of a circular chamber, is in the range of 100-10000 microns, preferably in the range of 500 and 5000 microns, more preferably in the range of 100 and 500 microns.

[072] The chamber matrix material is typically designed to form a selective medium and/or indicator of organismal activity. For example, if the inner matrix comprises National Botanical Research Institute's phosphate growth (NBRIP) medium, organisms capable of solubilizing phosphorus are selected. Furthermore, by visually inspecting the chamber, the activity of phosphorus solubilization can be assessed, as the medium starts off cloudy and becomes clear as phosphorus is solubilized by microorganisms.

[073] In one embodiment of the lab-on-a-chip device according to the present invention, the perimeter of at least one discrete chamber comprises a semi-permeable or permeable seal. Typically, the material of this seal surrounding the chamber is different from the inner matrix material, wherein the seal suitably forms a physical selective barrier between the chamber and the soil environment. Preferably, the permeability of this seal is tuneable. For example, by increasing the concentration (e.g., weight % of agar), diffusion through the permeable seal will be reduced. The incorporation of other materials such as cellulose into the permeable seal may further adjust the characteristics of this seal, making it more or less permeable, and making it more or less accessible to penetration by a microorganism. Selective barriers other than (semi-)permeable seals may also be incorporated.

[074] Typically, the chamber of at least one discrete module comprises an impermeable, partially permeable or permeable matrix material, wherein the matrix material has a level of permeability that is tailored relative to the preselected biological, chemical, molecular or physical parameter. For example, a chamber may comprise a porous, non-porous, solid or gel-like matrix material, optionally comprising nutrients, fertilizers, chemical signals (such as attractants or repellents), and/or pesticides as described herein. In one embodiment, the chamber of at least one discrete module comprises a nitrogen, phosphorus, and potassium (NPK) composition in order to serve as a fertilizer chamber for microorganisms. In an exemplary embodiment, the chamber of at least one discrete module comprises nitrogen, phosphorus, and potassium (NPK) embedded in a polymer matrix, wherein the polymer matrix is only accessible to arbuscular mycorrhizal fungi (AMF).

[075] Due to the modular design of the device of the present invention, data can be captured about many different taxonomic (bacterial, fungal, nematode, protist, etc.) or soil functions (e.g. phosphorus solubilization, nitrification, cellulose degradation) and/or about the presence and/or concentrations of indicator molecules or metabolites (e.g., CO₂, ethylene, environmental DNA) using a single device.

[076] In one embodiment, the device comprises modules for measuring at least two indicators selected from bacterial, fungal, nematode, protist, metabolomic, molecular, and functional indicators.

[077] In another embodiment, the device comprises modules for measuring at least three, four, five, six, seven, eight, nine, ten, eleven, twelve, thirteen, fourteen, fifteen, sixteen, seventeen, eighteen,

nineteen, or twenty indicators selected from bacterial, fungal, nematode, protist, metabolomic, molecular, and functional indicators.

[078] In one embodiment, the device comprises one or more gas collection chambers. The gas collection chambers may be inflatable. The, optionally inflatable, gas collection chambers may be connected to one or more chambers in order to collect gas. The production of gas can either be measured based on inflation, collected for subsequent analysis, or through on-line measurement. Inflation may be triggered by a sensor, such as a pressure sensor or an acoustic sensor, thereby triggering an active sampling. Alternatively, passive sampling may be performed using resins or other absorbent materials from which volatiles to be sampled may be extracted. As an example, volatile ammonium measurements may be performed as an indicator of soil nutrient status. Other gaseous nitrogen or carbon compounds may be measured as indicators of greenhouse emissions. As another example, real-time sampling of volatiles such as ethylene and/or carbon dioxide may be performed as a measure of various stress events such as drought, waterlogging and anaerobic conditions.

[079] Typically, the device has a length along a longitudinal axis (y) that extends between a top edge and a bottom edge of the device and a width along a transverse axis (x) that extends between a first side edge and a second side edge of the device. In one embodiment, the device has at least one plane that is essentially rectangular in shape, wherein a length along a longitudinal axis (y) exceeds a width along a transverse axis (x).

[080] Typically, the longitudinal axis (y) has a length in the range of 10-1000 mm, preferably in the range of 20-500 mm, more preferably in the range of 20-200 mm.

[081] Typically, the transverse axis (x) has a length in the range of 5-200 mm, preferably in the range of 10-100 mm, more preferably in the range of 15-50 mm.

[082] Preferably, the device has a virtually two-dimensional shape. That is, the device has a substantially planar or fully planar, preferably rectangular, shape, wherein the plane in which the multiple modules are fitted has dimensions in length and width that are substantially larger than the thickness of this plane. Typically, the device has a thickness (d), formed by the inner matrix and exterior surface materials, between 1 and 20 mm, preferably between 2 and 15 mm, more preferably between 5 and 10 mm. The thickness of the inner matrix may be between 20-2000 microns, preferably between 50 and 1000 microns, more preferably between 100 and 500 microns.

[083] In one embodiment, the device has a length between 1 and 5 cm, a width between 0.5 and 2 cm and a thickness between 5 and 10 mm. typically, the ratio between the thickness of the plane and the smallest dimension of its length and width is at most 1:5, 1:10, 1:15, or 1:20.

[084] In one embodiment, the exterior surface of the device is at least partially transparent, as this allows for visual inspection and analysis by, for example, optical or fluorescence spectroscopy. preferably, the exterior surface is substantially or fully transparent. In one embodiment, the exterior surface of the device is made of glass, such as soda-lime-silicate glass or borosilicate glass. In one

embodiment, the exterior surface of the device is made of a transparent plastic material, such as polymethyl methacrylate (PMMA).

[085] In a preferred embodiment, the device is substantially or fully planar as well as substantially or fully transparent.

[086] Such a virtually two-dimensional, preferably substantially or fully planar and transparent device may be manufactured, for example, by fitting between two flat plates, such as glass slides, the desired chambers in a suitable matrix material, applying desired fluid connections between the chambers, and applying appropriate impermeable and permeable seals between the planes. Components of the chambers may suitably be 3D-printed or placed with micro-pipetting, or a combination thereof. As an example of impermeable seals, the device may be provided with a paraffin wax seal at a plurality of its edges, for example the top edge, bottom edge and one side edge, in order to prevent interaction with the exterior of the device, such as the soil, and to establish a single plane of interaction between the inner space of the device and the one or more inlet channels of the device.

[087] The one or more inlet channels of the device are in fluid communication with at least one of the chambers of a module of the device. As used herein, in addition to the flow of fluids such as liquids and gasses, the term “in fluid communication” encompasses organismal mobility and/or colonization between the one or more inlet channels and at least one chamber of the device.

[088] In one exemplary embodiment, the device has one or more inlet channels in at least one of the first side edge and the second side edge of the device.

[089] In another exemplary embodiment, the device has one or more inlet channels in at least one of the planes defined by transverse axis (x) and longitudinal axis (y), for example in a “perfboard”- type configuration.

[090] In one exemplary embodiment, the one or more inlet channels are configured to form or comprise a selective barrier for entrance of pre-selected species. Such a selective barrier can be formed by a variety of means known to skilled person, and including but not limited to size exclusion, shape exclusion, nutrient content, and auxotrophies, and combinations thereof.

[091] In one embodiment, the one or more inlet channels comprise a permeable or semi-permeable seal. Examples of such a seal include aqueous agar gels, such as 0.5, 1 or 1.2 % water agar, which may have differential and decreasing permeability with increased agar concentrations. In this manner only organisms capable of growth through the membrane may enter a particular channel. Optionally, the membrane seal is further imbued with a selective pesticide, and/or other attractive repellent compounds. In this way, only organisms with the ability to tolerate the selective pesticide or repellent may grow through this membrane. Furthermore, by tuning the permeability of the membrane, the rate at which compounds are released into the soil may be controlled (e.g. slow or quick release). Additionally, the permeability of the membrane may impact gas transfer into and out of the chamber, which may be used to modify the internal environmental by selectively trapping gasses in the chamber or excluding gasses from permeating into the chamber.

[092] In some embodiments, the one or more inlet channels are provided with root guidance means. Such means for guiding roots may involve microfabricated channels with precise geometries that mimic natural root pathways. These channels can be used to guide the growth of roots in a specific direction or to study the effects of different environmental factors on root growth.

[093] The inner matrix of the device may be made of a material that is selective for a pre-selected biological function, such as biofilm formation, cellulose degradation, or phosphorus solubilization. Examples of suitable materials for use as the inner matrix include, but are not limited to an aqueous agar gel, for example National Botanical Research Institute's phosphate growth medium (NBRIP) or Pikovskaya medium (PVK) which both may be used to select for and screen for phosphorus solubilizing organisms.

[094] In other embodiments, the inner matrix of the device is made of a chemically and biologically inert material. Examples of such materials include glass, silicone, carbon fibre, polyethylene, and ceramics, and combinations thereof.

[095] An advantage of the device described herein is that it enables simultaneous assessment of multiple parameters related to soil-dwelling organisms, the device serving as a portable tool for farmers and agronomists to obtain real-time soil health information and, if desired, allowing them to make necessary adjustments to agricultural practices. Accordingly, in a second aspect the invention relates to a method for determining one or more biological, chemical, molecular, or physical parameters associated with soil, the method comprising:

- providing the lab-on-chip-device as described herein,
- deploying the lab-on-a-chip device at least partly in the soil,
- optionally removing the lab-on-a-chip device from the soil after a predetermined time period,
- obtaining data in relation to a plurality of preselected biological, chemical, or physical parameters associated with the soil during the predetermined time period, and
- optionally further processing the collected data.

[096] For each module of the device of the invention, the data in relation to one or more preselected biological, chemical, molecular, or physical parameters associated with the soil may be collected at a single time-point, as in an end-point analysis, or the data may be collected many data points over time to create a time series, advantageously permitting enabling data analysis, fitting time-resolved data to models, interpolation, extrapolation, forecasting etc.

[097] The one or more preselected biological, molecular, chemical, or physical parameters may be selected from spore germination, colonization events, presence and/or concentration of one or more microorganisms; degree of soil biodiversity; plant-microbe interactions; root exudation(s); degree of response to pesticides, xenobiotics, heavy metals, or other inorganic species; response to one or more chemical attractants or repellents.

[098] Preferably, at least one module of the device comprises a sensor configured to collect in situ, preferably digital, data. Preferably the digital data are collected using one or more electronic sensors

configured to collect data selected from optical (absorbance, reflectance, fluorescence, luminescence, refractive index, light scattering), mechanical (e.g. stress, strain, force, pressure), electrical (e.g. impedance and conductivity) chemical (gas and volatile), magnetic (magnetic field, flux, permeability), colorimetric data, image or video data, temperature, and humidity data, or any combination thereof, such as the combination of sensing electrodes pre-coated with antibodies.

[099] In one embodiment, the collected data is qualitative. Examples of qualitative soil data include, but are not limited to, soil biodiversity or the abundance of particular organisms including pathogenic microorganisms, symbiotic fungi such as mycorrhiza, indicator molecules such as volatiles or gases, nitrogen-fixing organisms, phosphorus-solubilizing organisms, cellulolytic microorganisms. Additional examples of qualitative soil data are the function of such organisms such as their phosphorus solubilizing ability, soil odour or sound or other indicator of particular microorganisms or their function.

[100] In one embodiment, the collected data is quantitative. Examples of quantitative soil data include, but are not limited to, levels of particular organisms including pathogenic microorganisms, symbiotic fungi such as mycorrhiza, nitrogen fixing organisms, phosphorus solubilizing organisms, cellulolytic microorganisms and concentrations of indicator molecules such as volatiles or gasses.

[101] In one embodiment, the collected data is a combination of qualitative and quantitative data.

[102] In one embodiment, the collected data comprise data in relation to arbuscular mycorrhiza fungi (AMF) in the soil. Advantageously, the device is used for dynamic monitoring of AMF population over a time period ranging from a few weeks up to few months in connection with a particular field crop cycle.

[103] Generally, depending on the nature of the parameter(s) to be assessed, the data may be collected only once, intermittently or continuously. The compounds or components present in the modules of the device, such as for example chromophores and/or sensors of the device may be configured for single use, or may be left, comprised in the device, in the soil indefinitely and any period in between, thus enabling the provision of information at any potential time-scale.

[104] The one or more sensors comprised in the lab-on-a-chip device of the invention may be connected to a data logger that records and stores the collected data over time, allowing for the analysis of trends and patterns in soil conditions. The collected data may be transmitted wirelessly to a, preferably external, central database for storage and analysis, allowing for remote monitoring and analysis of soil conditions over an extended period. Alternatively or additionally, the sensors or the device as a whole may be equipped with RFID technology to facilitate read-out of data and/or remote data collection and analysis.

[105] The data may be simultaneously collected for a plurality of devices distributed across a land area, such as a field for growing crops or testing pesticides, thereby allowing detailed mapping of one or more biological, chemical, or physical parameters associated with the soil of the land area. For example, different sensors may be used to collect data for various parameters as described herein. The sensors can be connected to a central data collection system through wireless communication, cellular networks, or the Internet of Things (IoT) technology. This allows the data to be transmitted in real-time

to a central database, where it can be processed, analysed, and displayed on a map. Such maps can be created using, for example, geographic information system (GIS) software, which allows for the visualization of the collected data. The map can be used to identify areas with high or low levels of the parameter being measured, and to identify trends and patterns across the land area.

[106] In one embodiment, the device comprises a power source, such as a battery or solar panel, to operate the one or more sensors and/or the data logger.

[107] The collected and optionally analysed and/or mapped data can be used for a wide variety of actions, processes and strategies with regard to the investigated area, including sowing, planting, irrigation, fertilising, pest management, crop rotation, companion planting, intercropping, mulching, irrigation, pruning, and harvesting.

[108] The data can also be used to efficiently and systematically compare the effects of, for instance, a series of different pesticides, combinations of pesticides, and/or different concentrations of a pesticide under otherwise identical soil conditions.

Detailed description of the drawings

[109] Referring to the figures, Figure 1 displays a schematic drawing of a modular lab-on-a-chip device 100 containing six discrete modules 101, 102, 103, 103, 104, 105, and 106 embedded in a matrix 170. The device is designed to facilitate various functions related to microorganism analysis and characterization. The device features entrance channels 110, 120, 130, 140, 150, and 160 that allow the entry of microorganisms while excluding plant roots. The entrance channels may additionally incorporate obstacles or physically or chemically selective elements to enhance the survival and entry of specific microorganisms while selectively filtering out others. The device is equipped with multiple chambers 111, 121, 131, 141, 151, and 161 which may have distinct functionalities. Each chamber may be configured varying nutritional content, selective chemistries, attractants, specific microorganism(s), and sensors/nanosensors (such as colorimetric, electric, visual, or other types). These chambers 111, 121, 131, 141, 151, and 161 serve as controlled environments for analysing and monitoring the behaviour of microorganisms. Each of chambers 111, 121, 131, 141, 151, and 161 can be sampled for various purposes, including metabolomic analysis, DNA/eDNA/RNA analysis, or other molecular, elemental, or chemical analyses. Furthermore, the chambers may allow for the collection of microorganisms for subsequent isolation and/or characterization.

Figure 2 displays an operational modular lab-on-a-chip device 200, which comprises three distinct modules integrated into an inner matrix 203. The inner matrix 201 is composed of 0.5 wt% water-agar, sandwiched between two glass slides. The matrix is sealed on three sides by an impermeable paraffin wax seal 202 and on one side by a permeable seal 203 made of 1.2 wt% water-agar. The permeable seal 201 acts as an entrance channel for AMF chamber 204, where spores are introduced to initiate germination and populate the device. The AMF chamber 204 is interconnected with fertiliser chambers 205 and 206. Both fertiliser chambers 205 and 206 contain NPK (nitrogen, phosphorus, and potassium)

within a polymer matrix, with an approximate thickness of 100 microns. These fertiliser chambers are exclusively accessible to the AMF chamber, ensuring targeted nutrient supply.

Figure 3 displays a schematic drawing of a modular lab-on-a-chip device 300 designed to provide a visual indication of AMF activity in the soil, particularly of hyphal development. The device is deployed in the soil 303 supporting a plant 304 with plant roots 305. The device contains a size selection barrier 301 providing access of AMF hyphae 306 to an array 302 of seven AMF colonization chambers. Further selective barriers, including size exclusion, nutrient content and auxotrophies may be included for colonization of AMF. The visual indication provided by the device offers valuable insights into the effectiveness and extent of AMF colonization, while the multiple-chamber design allows quantification of colonization events. For example, the device may be used for screening of active ingredients, wherein different active ingredients are placed in the chambers and wherein subsequently the colonization of this chamber or the germination of spores is qualitatively or quantitatively assessed. Various sensors may be integrated into the device, optionally equipped with RFID technology to permit data transfer, remote monitoring and (e.g. artificial intelligence-based) screening.

Figure 4 displays a schematic drawing of two modular lab-on-a-chip devices 400 and 401 designed to provide a visual indication of AMF activity in the soil, particularly of hyphal development. The devices are deployed in the soil 403 supporting a plant 404 with plant roots 405. The layout of devices 400 and 401 may be similar identical to device 300 described above. The chambers of device 400 are additionally pre-loaded with spores 402, such as AMF spores. Accordingly, device 400 enables the acquisition of quantitative information on both colonization of the chambers and spore germination 406.

Figure 5 presents a schematic illustration demonstrating the operation of the modular lab-on-a-chip device according to the invention. The figure showcases the following key steps: (A) Loading of Spores: Each module of the device is loaded with spores derived from distinct fungal lineages, highlighting the versatility of the device to accommodate multiple fungal species. (B) Deployment in the Soil: The loaded device is deployed within the soil, strategically positioned to facilitate interactions between the fungal spores and the soil environment. (C) Contact with a pesticide or other active ingredient (AI): The device comes into contact with a pesticide or other active ingredient, representing an experimental scenario where the effect of a specific pesticide on arbuscular mycorrhizal associations is being investigated. (D) Subsequent Analysis: Following the pesticide exposure, a subsequent analysis is conducted. This analysis provides valuable information regarding the impact of the particular pesticide/AI on the establishment and functionality of arbuscular mycorrhizal associations, and other species, allowing for a comprehensive assessment of the pesticide/AI's effect on these important symbiotic interactions.

Figure 6 schematically illustrates an example of how data from the device according to the invention may be collected to identify the (A) degradation, solubilization, mobilization of compounds or (B) the deposition or growth of microorganisms. These data can then be collected by using microscopy, sensors, electrical or light measurements, DNA or other biological materials and converted into a quantitative reading, such as in a (C) end-point analysis, or by collecting a series of data points over time to create (D) time-resolved graphical representations. Due to the modular design of the device,

data can be captured about many different taxonomic (bacterial, fungal, nematode, protist etc.) or soil functions (e.g. phosphorus solubilization, nitrification, cellulose degradation) or on the presence of indicator molecules or metabolites (e.g. carbon dioxide, ethylene, environmental DNA) in a structured manner. (E) For example, information regarding (I) nematode indicators, (II) bacterial indicators, (III) fungal indicators, (IV) metabolic and molecular indicators, (V) protist indicators and (VI) functional indicators can be captured using a single device according to the invention.

Figure 8 provides a schematic representation of an embodiment of the invention, wherein electrical conductivity (EC) monitoring is used as an indicator of the amount of nutrients and fertility of a soil specimen, wherein low EC concentrations may be indicative of low nutrient concentrations whereas high EC may be indicative of high nutrient concentrations. The embodiment represented in Fig. 8 is a root-free environmental compartment device 800, based on a 9 cm petri dish having a bottom 801 and a top 802 provided with a 6 cm diameter nylon 32-micron mesh interface 803 between the device and the soil. The device contains slow-release nutritional compounds that are not available to plants due to the small-pore mesh interface that cannot be penetrated by plant roots. The nutrients may be accessed and released by symbiotic fungi such as Arbuscular Mycorrhizal Fungi (AMF) or other mycorrhizal species. The compartment device 800 further contains an electrical conductivity sensor 804. The device may be placed in soil (either in greenhouse, lab, or in the field), whereby the ability of AMF, other mycorrhizal fungi, or other organisms to liberate nutrients is measured as an increase in the electrical conductivity within the compartment. The liberated nutrients may then be either transported out of the compartment by the fungi or through passive diffusion in water.

Figure 9 provides a schematic representation of another embodiment of the invention wherein electrical conductivity (EC) monitoring is used as an indicator of the amount of nutrients and fertility of a soil specimen. The embodiment represented in Fig. 9 is a root-free environmental compartment device 900, prepared from three laser-cut polyacrylate plastic pieces of 100 mm height, forming the left and right wall (5 mm depth, 4 mm thickness), bottom (30 x 5 mm, 4 mm thickness), back wall (1 mm thickness) and façade frame (30 mm, 1 mm thickness) to form an elongated rectangular compartment 901. A mesh 902 with 32-micron pores (Sefar Petex®, Polyethylene terephthalate monofilament open mesh fabric) assembled in the façade frame leaves a single open interface between the device and the soil. The compartment device 900 further contains an electrical conductivity sensor 903. The compartment may be filled with a standard medium of quartz sand 904, and a solid slow-release fertilizer 905, e.g. comprising N, P and K. The compartment may also be filled with chemistries that act to either repel or kill certain lineages of microorganisms 906, such as pesticides or signalling molecules. Alternatively, or additionally, the compartment may be filled with chemistries used to lure and favour certain microorganisms, such as hormones, signalling molecules and/or carbon substrates 907.

Figure 10 illustrates the arrangement of a plurality of devices or device modules according to the present disclosure in an array in the field for the purpose of intra-field comparison of selected soil health parameters. The devices or device modules may be equipped with means for the wireless transmission of data obtained in each device or device module, which allows for continuous or intermittent, real-time

or historic monitoring of soil health. The devices or device modules may be equipped with an electrical conductivity sensor as described herein to monitor changes in electrical conductivity within the devices or device modules originating from symbiotic fungi such as Arbuscular Mycorrhizal Fungi (AMF) or other mycorrhizal species accessing and releasing nutrients into the device compartment. In general, the array arrangement with continuous or intermittent collection of data permits the identification of areas with differential mycorrhizal functioning indicated through changes in electrical conductivity and/or other parameters indicative of soil health. In addition to the use of these data for identifying areas within a field with differential mycorrhizal functioning, these data may be used for comparing fields or farms at a global level to identify fields or farms with higher or lower functioning.

EXAMPLE

Example 1 – assessing effect of fungicide treatment of soil on fungal colonization

[110] A device with 12 chambers was placed in two separate soils: (a) soil treated with fungicidal compound mancozeb (Dithane® Neotec®; Corteva), and (b) untreated soil. After 2 weeks, the device was removed from the soil and the abundance of total fungal hyphae colonizing the device chambers was determined using optical microscopy. Figure 7 compares the percentage of chambers having become colonized with fungal hyphae in untreated soil and soil treated with mancozeb. In the untreated control soil, fungal hyphae had abundantly colonized all chambers, averaging 84 % colonization (standard deviation 19%). The fungicide-treated controls averaged 31% colonization (standard deviation 10%). Thus, the device of the invention demonstrated a significant effect of fungicide treatment of soil on fungal colonization.

Example 2 – electrical conductivity sensing of AMF activity in soil

Soybean plants were grown in 7-liter pots containing a sandy soil mixture composing 9 parts of river sand and 1 part soil that was sterilized by autoclaving. In each pot, a device as shown in Fig. 8 was buried in the sandy soil mixture such that only the top surface was above the level of the sandy soil mixture. Within each compartment device, the same sandy soil mixture was used, and further 1 gram of Osmocote Exact Mini (Osmocotec Exact Mini - Umhüllte Dauerdünger; ICL Deutschland), a slow release fertilizer including N (15%), P (3.9%), K (9.1%) as well as Mg (1.2%), B (0.023%), Cu (0.071%), Fe (0.46%), Mn (0.064%), Mo (0.023%), Zn (0.027%), all percentages based on total weight of the fertilizer. One pot was inoculated with a commercial blend of mycorrhiza (Symbiom) including *Rhizoglyphus irregulare*, *Funneliformis mosseae*, *Claroideoglomus claroideum*, *Septoglomus deserticola*, *Funneliformis geosporus*, and *Funneliformis caledonius* as AMF. The plants were then grown for 70 days and electrical conductivity was measured within the compartments (Flower Care™ Smart Monitor).

Figure 11A displays the differential electrical conductivity measured in compartment devices in which AMF is present (“+ AMF”) versus those in which they are absent (“- AMF”). The cumulative sum of the electrical conductivity (shown in the inset) was statistically significantly larger, both during the first (0-35 days) and the second (35-70 days) growth phases (p values <0.01). Figure 11B displays the daily (diurnal) fluctuation (Δ) of electrical conductivity, calculated as the maximum value minus the minimum value measured for the day. As can be seen in Figure 11B, the diurnal change is significantly higher when AMF is present (“+ AMF”).

Figure 12A compares the average of the electrical conductivity as displayed in Figure 11A in the presence (“+ AMF”) and absence (“- AMF”) of AMF, during the first (0-35 days; solid fill) and second (35-70 days; shaded) growth phases. Figure 12B compares the average of the daily (diurnal) fluctuation (Δ) of electrical conductivity as displayed in Figure 11B in the presence (“+ AMF”) and absence (“- AMF”) of AMF, during the first (0-35 days; solid fill) and second (35-70 days; shaded) growth phases. These data demonstrate that a device comprising a module capable of measuring electrical conductivity within the module chamber according to the present disclosure may be utilized as an indicator of the amount of nutrients and fertility of a soil, wherein high electrical conductivity may be indicative of high nutrient concentrations, and vice versa.

CLAIMS

1. A lab-on-a-chip device with modular layout for determining a plurality of biological, molecular, chemical or physical parameters associated with soil, the device comprising

an inner space confined by an exterior surface, wherein the exterior surface is configured to be at least partially and sustainably contacted with soil, and wherein the exterior surface comprises one or more inlet channels configured to provide any of fluid communication, organismal mobility, and colonization between the soil and the inner space,

wherein the inner space comprises one or a plurality of discrete modules embedded in an inner matrix material,

wherein each module comprises a self-contained chamber, the chamber being configured to provide any of fluid communication, organismal mobility, and colonization with an inlet channel and/or one or more other chambers,

wherein one or more chambers are configured to attract, collect, grow and/or become colonized by a microorganism present in the soil; and

wherein one or more chambers are configured to provide data in relation to one or more preselected biological, chemical, molecular, or physical parameters associated with the soil.

2. The lab-on-a-chip device of claim 1, wherein a chamber of at least one discrete module comprises an electrical conductivity sensor.

3. The lab-on-a-chip device of claim 1 or claim 2, wherein the chamber of at least one discrete module comprises one or more chemical agents, the one or more chemical agents preferably comprising at least one chemical agent adapted to attract one or more microorganisms, the at least one chemical agent adapted to repel one or more microorganisms, or any combination thereof.

4. The lab-on-a-chip device of any one of the preceding claims, wherein the chamber of at least one discrete module comprises one or more pesticides preferably adapted to select against one or more microorganisms.

5. The lab-on-a-chip device of any one of the preceding claims, wherein the chamber of at least one discrete module comprises a carbon gradient preferably adapted to attract and support the growth of one or more microorganisms.

6. The lab-on-a-chip device of any one of the preceding claims, wherein the chamber of at least one discrete module comprises a nutrient composition preferably adapted to attract and support the growth of one or more microorganisms.
7. The lab-on-a-chip device of any one of the preceding claims, wherein the chamber of at least one discrete module comprises one or more microorganisms, the one or more microorganism preferably comprising spores, cysts, resting cells, resting spores, or another resting stage of a microorganism.
8. The lab-on-a-chip device of any one of the preceding claims, further comprising one or more gas collection chambers, each gas collection chamber arranged in fluid communication with the chamber of one or more of the plurality of discrete modules to allow transfer of gas therebetween.
9. The lab-on-a-chip device of any one of claims 1-8, wherein the device is provided with a gauze or mesh having a mesh size sufficiently small to prevent plant roots from entering the one or more of the chambers.
10. The lab-on-a-chip device of any one of claims 1-8, further comprising a root guidance system, the root guidance system preferably comprising a plurality of channels in one of a top edge, a bottom edge, a first side edge, and a second side edge of the device.
11. The lab-on-a-chip device of any one of the preceding claims, wherein the one or more preselected biological, molecular, chemical, or physical parameters are selected from spore germination, colonization events, presence and/or concentration of one or more microorganisms; degree of soil biodiversity; plant-microbe interactions; root exudation(s); degree of response to pesticides, xenobiotics, heavy metals, or other inorganic species; response to one or more chemical attractants or repellents; degradation or migration of chemicals, such as the degradation of lignin, cellulose, or other plant materials indicative of carbon cycling, the solubilization of phosphorus or other elements from minerals, the mineralization of nitrogen from polymers, and the degradation of microplastics and other contaminants.
12. A method for determining one or more biological, chemical, molecular, or physical parameters associated with soil, the method comprising:
- providing the lab-on-chip-device of any one of claims 1 to 11,
- deploying the lab-on-a-chip device at least partly in the soil,

optionally removing the lab-on-a-chip device from the soil after a predetermined time period,
and

obtaining data in relation to a plurality of preselected biological, chemical, or physical
parameters associated with the soil during the predetermined time period, and

optionally further processing the obtained data.

13. The method of claim 12, wherein the collected data are regularly or continuously transmitted to
an external storage facility for further analysis.

14. The method of claim 12 or 13, in which data are simultaneously collected for a plurality of
devices distributed across a land area, thereby mapping one or more biological, chemical, or physical
parameters associated with the soil of the land area.

15. The method of any one of claims 12 to 14, wherein the collected data comprise data in relation
to arbuscular mycorrhiza fungi (AMF) in the soil.

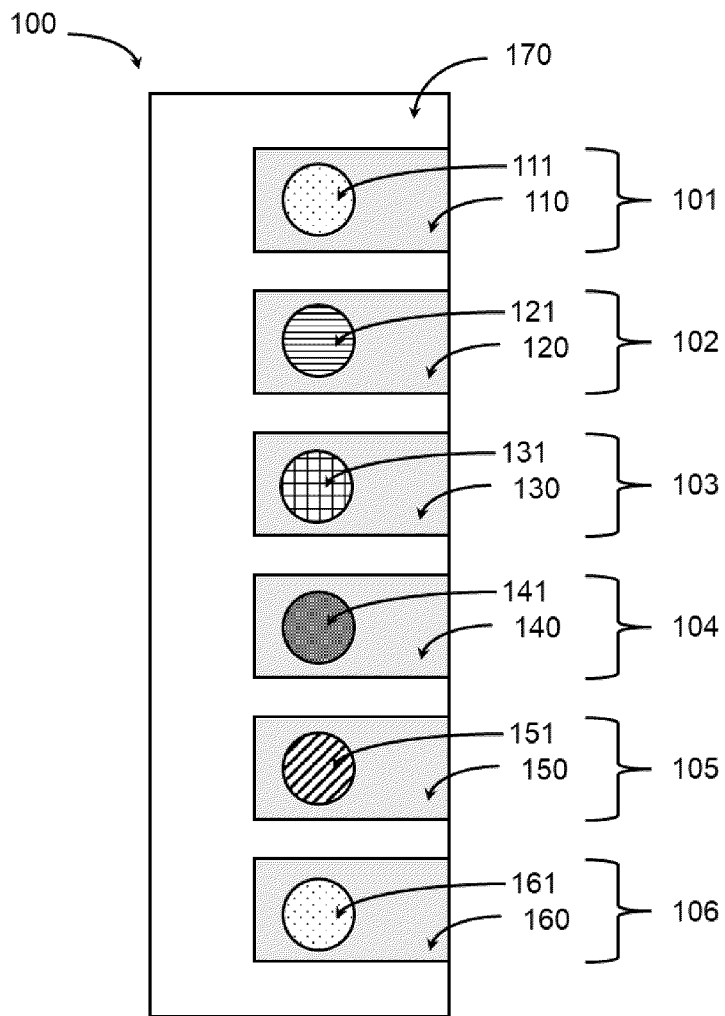


Fig. 1

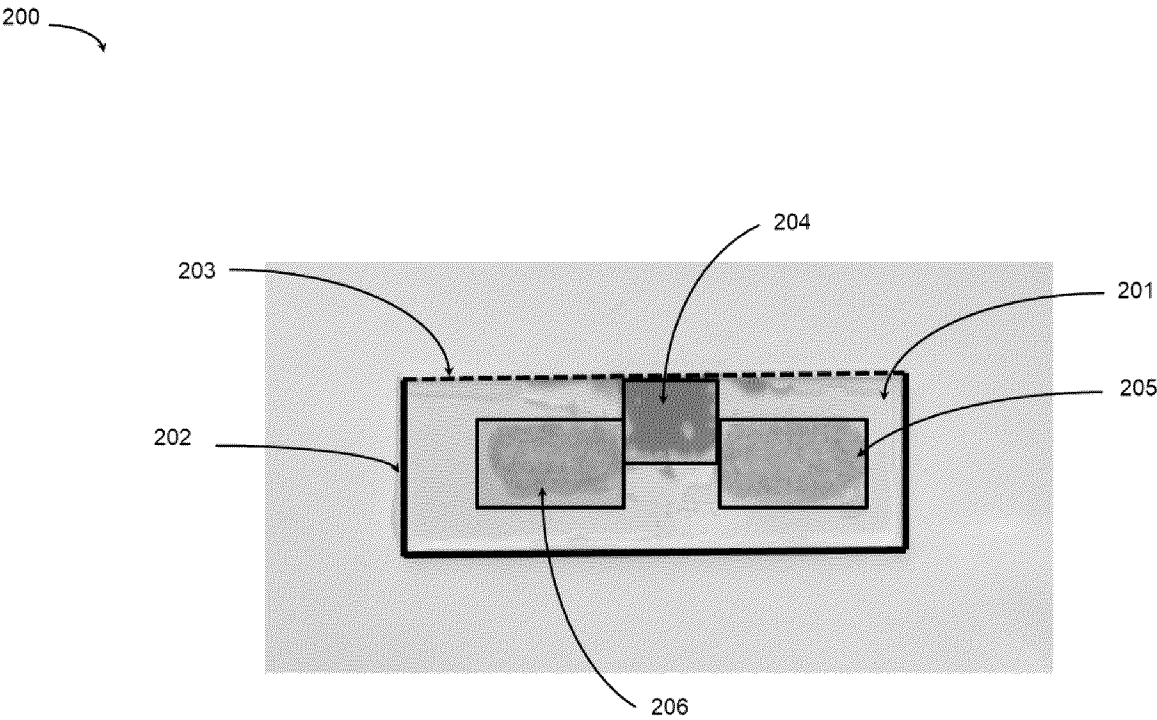


Fig. 2

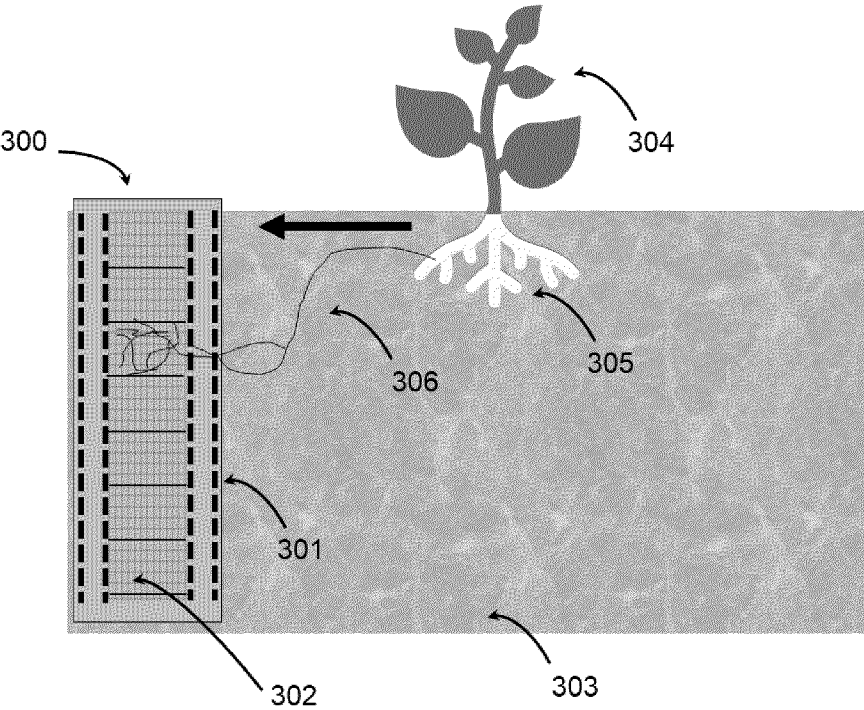


Fig. 3

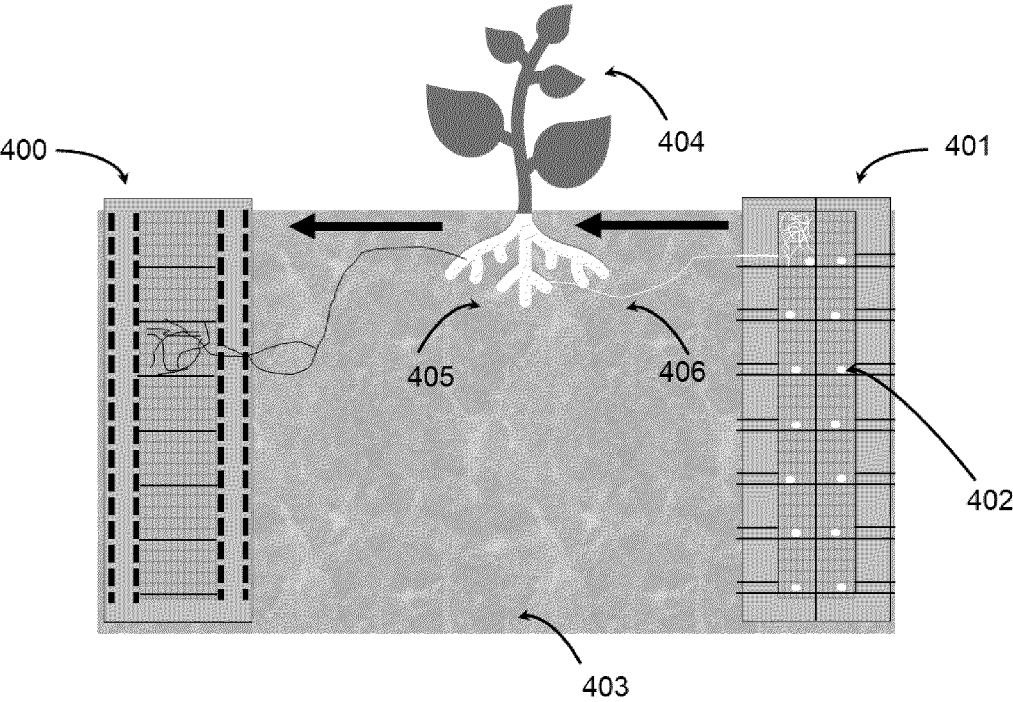


Fig. 4

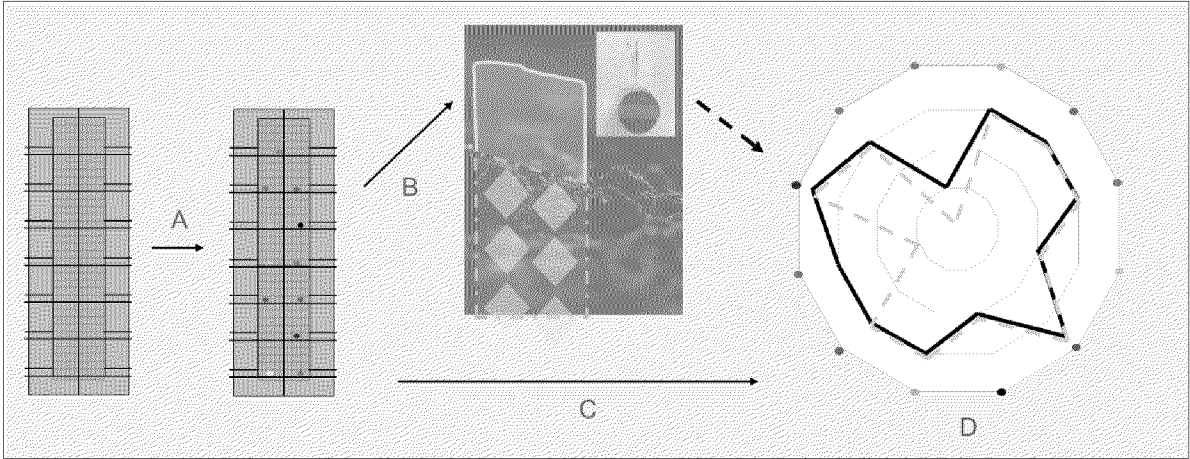


Fig. 5

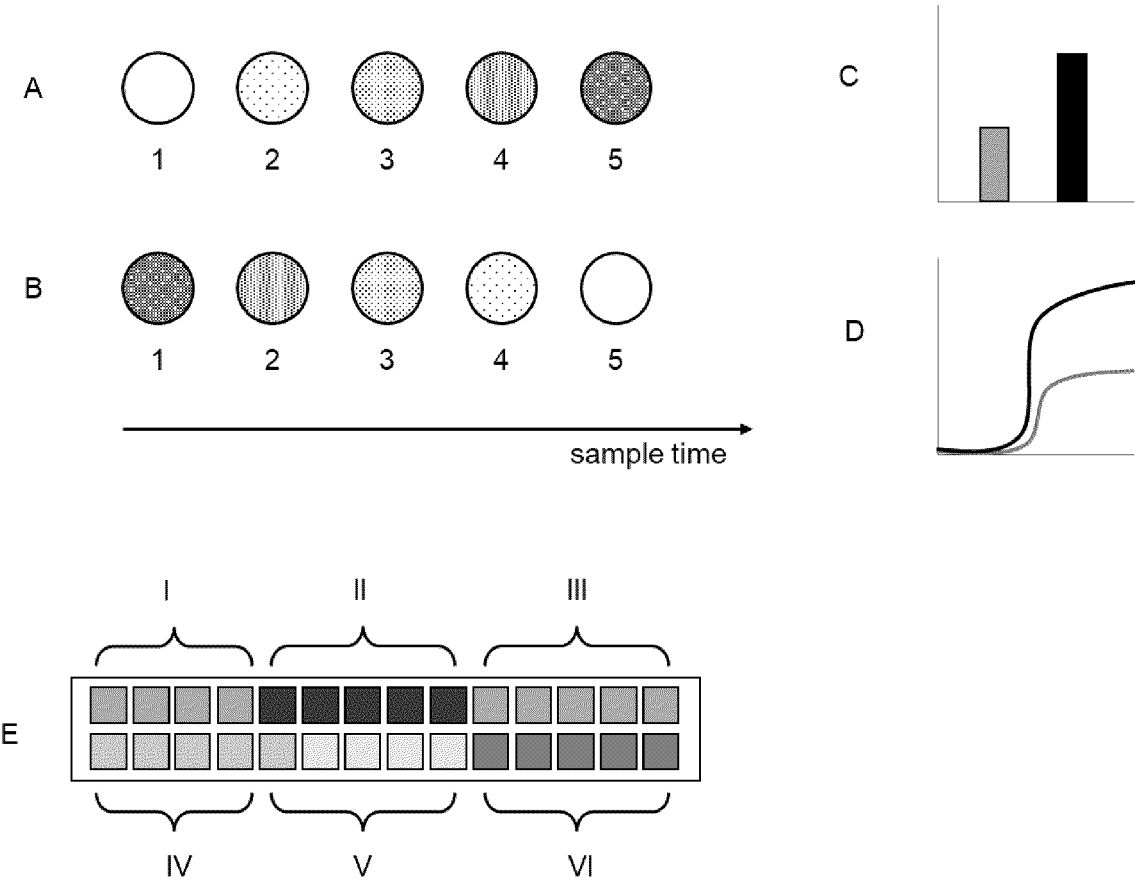


Fig. 6

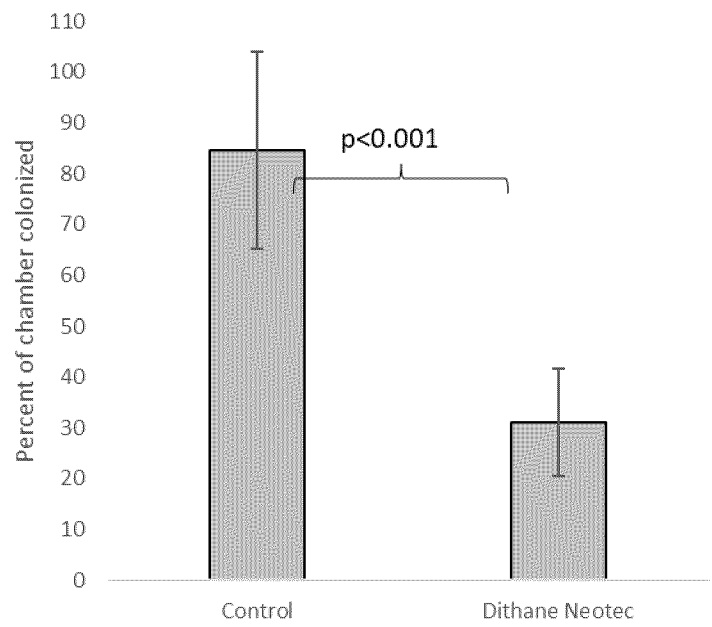


Fig. 7

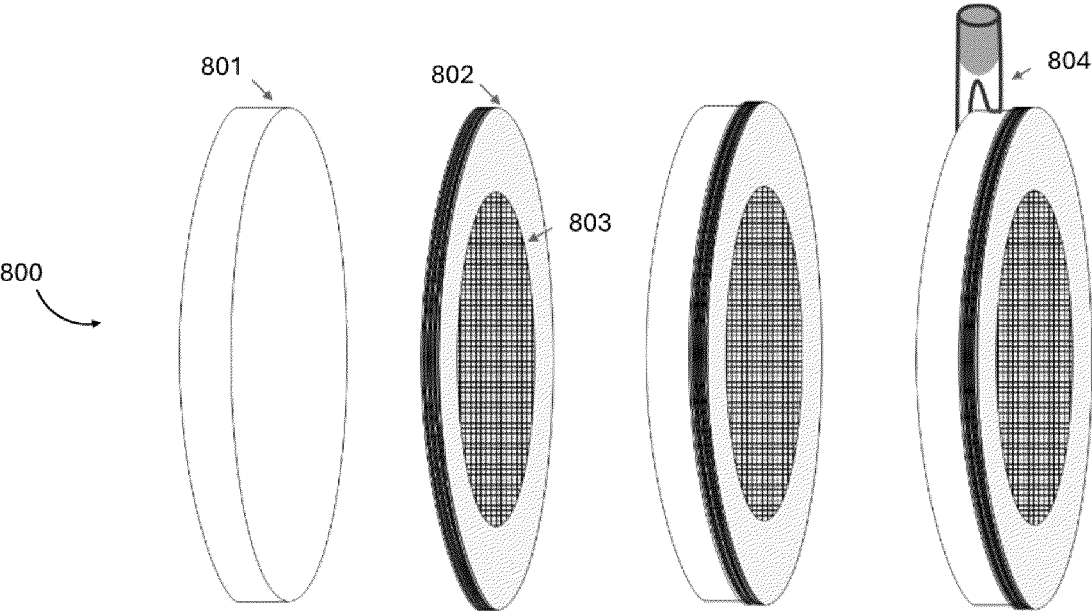


Fig. 8

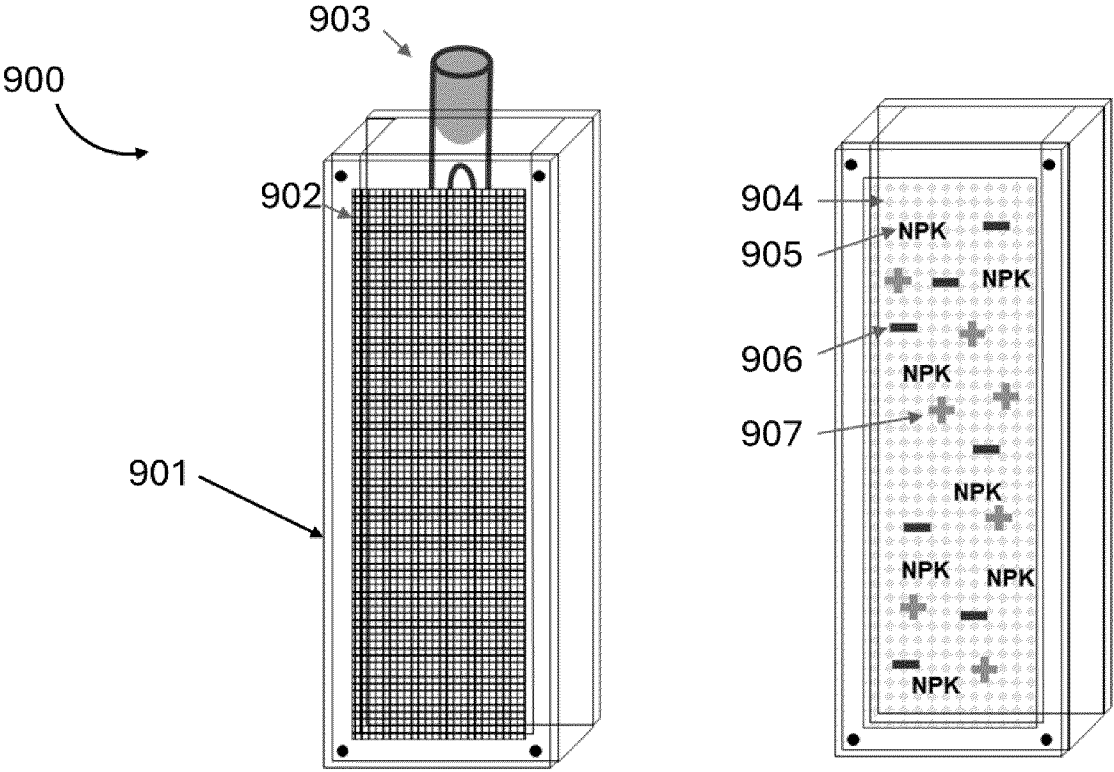


Fig. 9

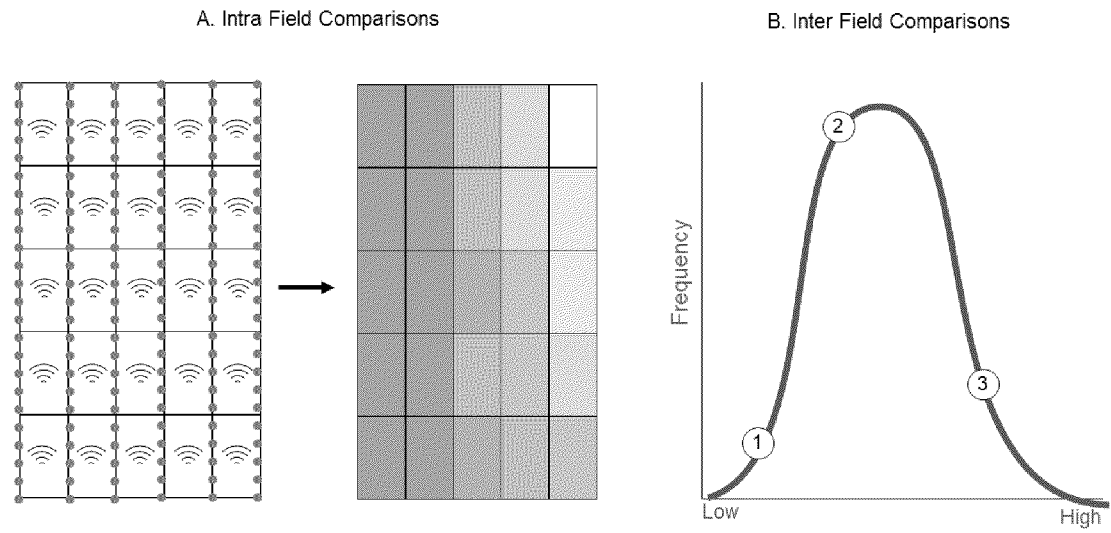
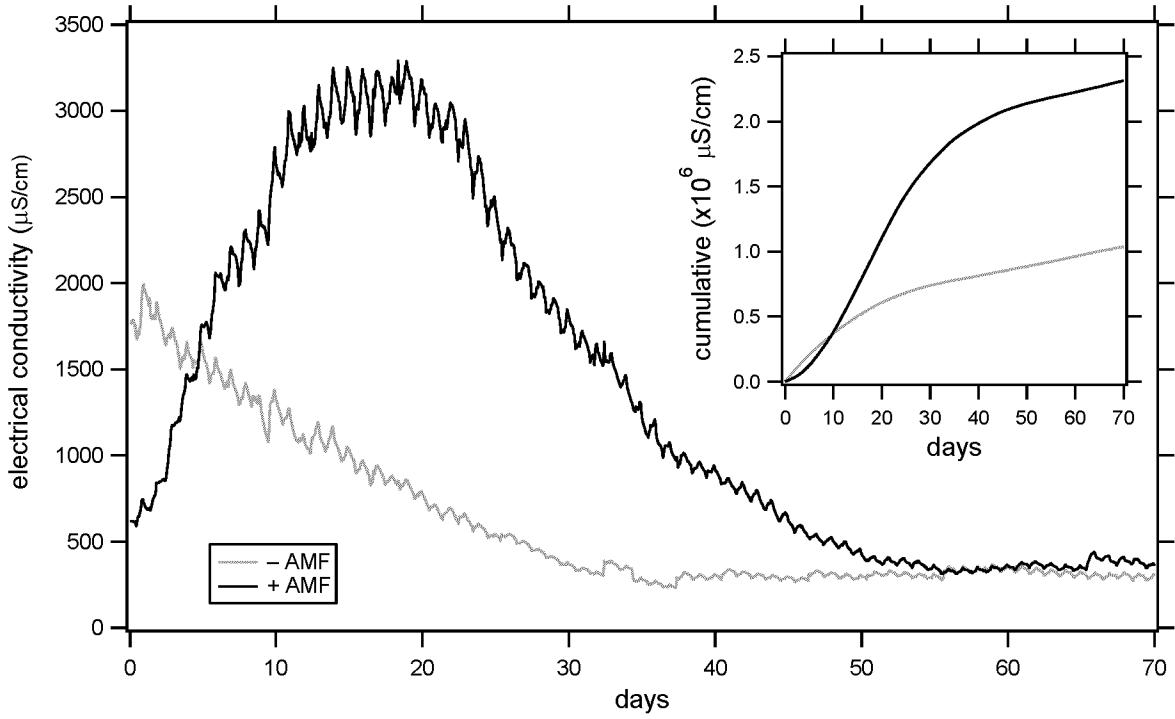


Fig. 10

A



B

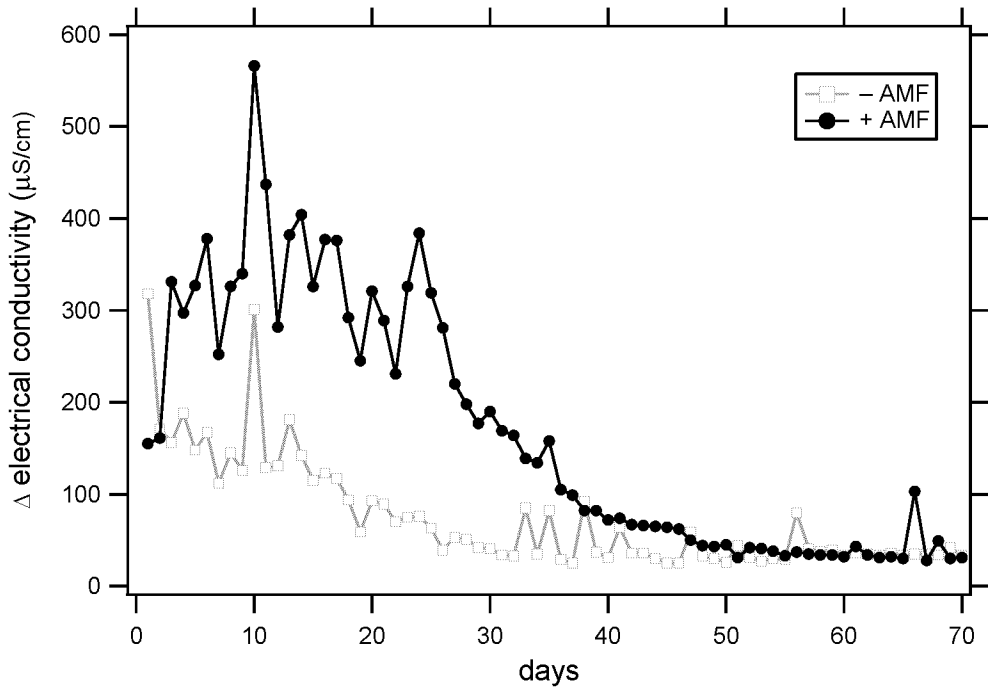
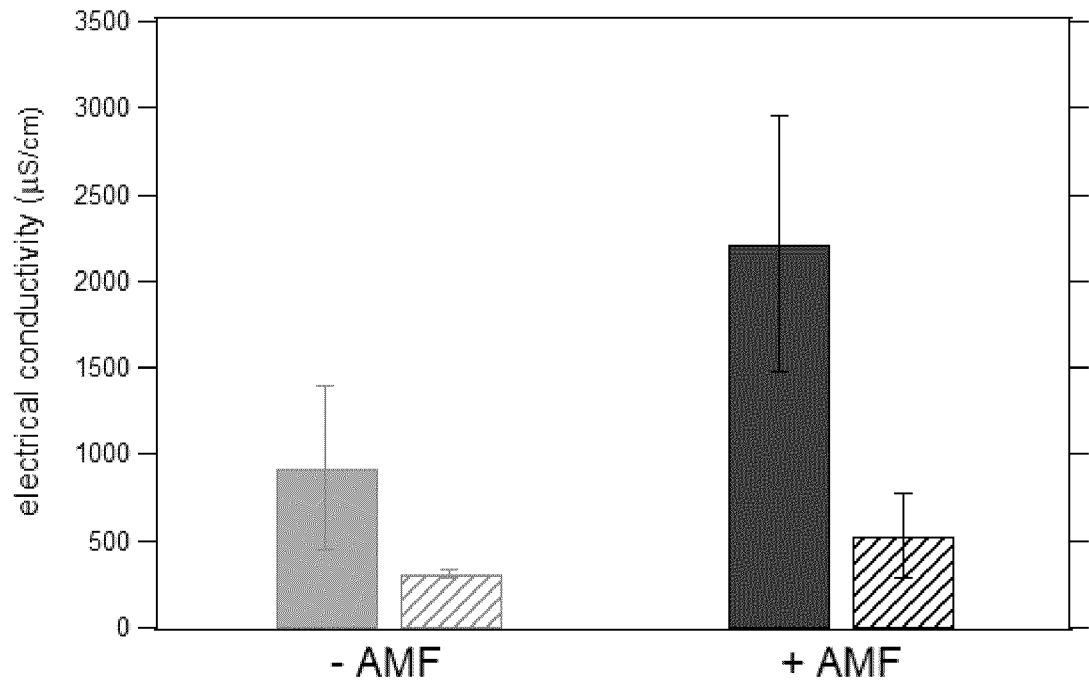


Fig. 11

A



B

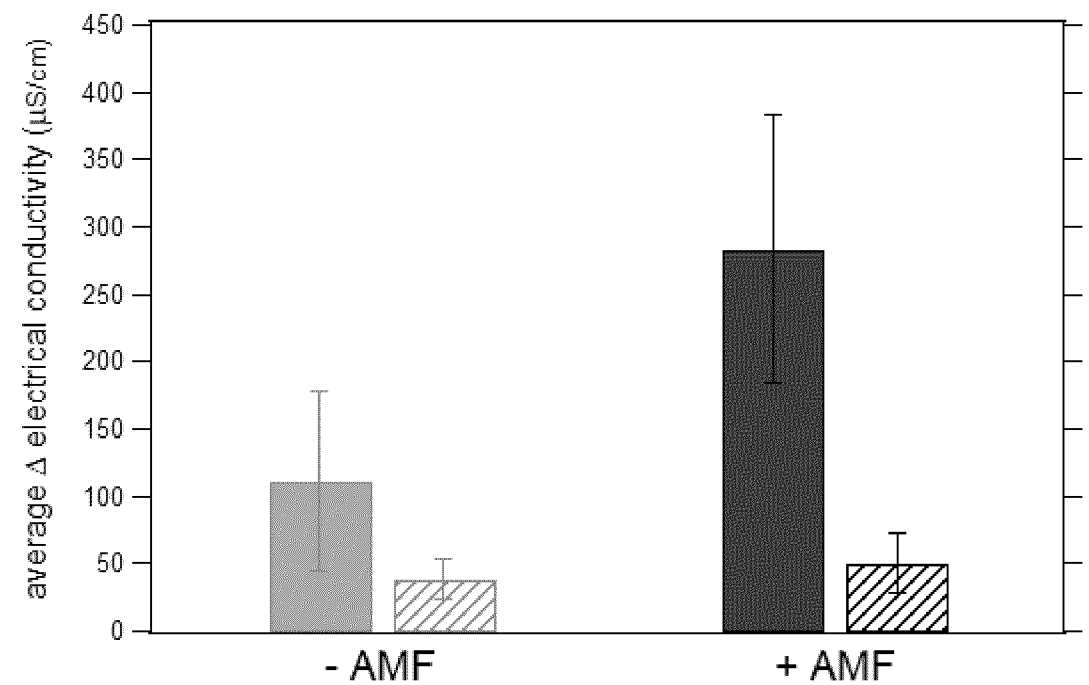


Fig. 12

INTERNATIONAL SEARCH REPORT

International application No

PCT/EP2024/065657

A. CLASSIFICATION OF SUBJECT MATTER

INV. G01N33/24

ADD.

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)

G01N

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practicable, search terms used)

EPO-Internal

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	MAFLA-ENDARA PAOLA MICAELA ET AL: "Microfluidic chips provide visual access to in situ soil ecology", COMMUNICATIONS BIOLOGY, vol. 4, no. 1, 889, 20 July 2021 (2021-07-20), pages 1-12, XP093097280, DOI: 10.1038/s42003-021-02379-5	1, 4, 6, 8, 11, 12
Y	abstract page 2, left-hand column, paragraph 3 - page 3, left-hand column, paragraph 1 page 4, right-hand column, paragraph 1 page 8, left-hand column, paragraph 2 - page 10, left-hand column, paragraph 3 figure 1 - / - -	2, 3, 5, 7, 9, 10, 13-15



Further documents are listed in the continuation of Box C.



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Date of the actual completion of the international search

30 August 2024

Date of mailing of the international search report

16/09/2024

Name and mailing address of the ISA/

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INTERNATIONAL SEARCH REPORT

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PCT/EP2024/065657

C(Continuation). DOCUMENTS CONSIDERED TO BE RELEVANT		
Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
	- & MAFLA-ENDARA PAOLA MICAELA ET AL: "Microfluidic chips provide visual access to in situ soil ecology - supplemental information", COMMUNICATIONS BIOLOGY, vol. 4, no. 1, 20 July 2021 (2021-07-20), pages 1-7, XP093097326, DOI: 10.1038/s42003-021-02379-5 figure 1 -----	
Y	SYLVAIN MATTHIEU ET AL: "The EcoChip: A Wireless Multi-Sensor Platform for Comprehensive Environmental Monitoring", IEEE TRANSACTIONS ON BIOMEDICAL CIRCUITS AND SYSTEMS, IEEE, US, vol. 12, no. 6, 1 December 2018 (2018-12-01), pages 1289-1300, XP011695337, ISSN: 1932-4545, DOI: 10.1109/TBCAS.2018.2878404 [retrieved on 2019-01-01] abstract page 1290, right-hand column, paragraph 4 - page 1292, left-hand column, paragraph 1 page 1294, left-hand column, paragraph 1 page 1296, left-hand column, paragraph 2 - right-hand column, paragraph 1 figures 1,7,12 -----	2,13,14
Y	BORER BENEDICT ET AL: "Spatial organization of bacterial populations in response to oxygen and carbon counter-gradients in pore networks", NATURE COMMUNICATIONS, vol. 9, no. 1, 769, 22 February 2018 (2018-02-22), pages 1-11, XP093008780, DOI: 10.1038/s41467-018-03187-y abstract page 2, right-hand column, paragraph 3 - page 3, right-hand column, paragraph 1 figures 1,2 ----- -/-	3,5

INTERNATIONAL SEARCH REPORT

International application No

PCT/EP2024/065657

C(Continuation). DOCUMENTS CONSIDERED TO BE RELEVANT		
Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
Y	RICHTER FELIX ET AL: "Fungi-on-a-Chip: microfluidic platforms for single-cell studies on fungi", FEMS MICROBIOLOGY REVIEWS, vol. 46, no. 6, 24 August 2022 (2022-08-24), pages 1-29, XP093097271, DOI: 10.1093/femsre/fuac039 abstract page 3, right-hand column, paragraph 1 page 9, right-hand column, paragraph 2 page 14, left-hand column, paragraph 5 page 21, right-hand column, paragraph 3 figure 10 -----	7, 15
Y	ANNE KAKOURIDIS ET AL: "Routes to roots: direct evidence of water transport by arbuscular mycorrhizal fungi to host plants", NEW PHYTOLOGIST, WILEY-BLACKWELL PUBLISHING LTD, GB, vol. 236, no. 1, 1 July 2022 (2022-07-01), pages 210-221, XP072537317, ISSN: 0028-646X, DOI: 10.1111/NPH.18281 abstract page 211, right-hand column, paragraph 1-2 figure 1 -----	9
Y	STANLEY CLAIRE E. ET AL: "Soil-on-a-Chip: microfluidic platforms for environmental organismal studies", LAB ON A CHIP, vol. 16, no. 2, 27 November 2015 (2015-11-27), pages 228-241, XP093097498, UK ISSN: 1473-0197, DOI: 10.1039/C5LC01285F abstract page 230, right-hand column, paragraph 1 page 235, right-hand column, paragraph 2 page 238, left-hand column, paragraph 2 figures 1,6 -----	10
A	OZOE KATSUYA ET AL: "Early characterization method of plant root adaptability to soil environments", PROCEEDINGS OF THE 2015 28TH IEEE INTERNATIONAL CONFERENCE ON MICRO ELECTRO MECHANICAL SYSTEMS (MEMS), 18 January 2015 (2015-01-18), pages 702-705, XP032740913, DOI: 10.1109/MEMSYS.2015.7051054 abstract page 702, right-hand column, paragraphs 2,3 figures 1,2,4,6 -----	1-15