microorganisms to generate nitrogen in reduced form from atmospheric nitrogen gas, and to identify particular strains of microorganisms as nitrogen-fixing or non-nitrogen-fixing.

In an example, the disclosure features systems for plant culture that include: a chamber including one or more walls enclosing a spatial volume internal to the chamber, where the one or more walls include a surface for supporting a plant within the enclosed spatial volume; a gas delivery apparatus, including at least one gas source; a nutrient delivery apparatus including a reservoir; a sampling apparatus connected to a port formed in the one or more walls; and a controller connected to the gas delivery apparatus and the nutrient delivery apparatus, and configured so that during operation of the system, with a plant entirely positioned within the enclosed spatial volume of the chamber, the controller activates the nutrient delivery apparatus to deliver an aqueous growth medium to the plant, and activates the gas delivery apparatus to deliver into the enclosed spatial volume a mixture of isotopically-substituted gases.

In one aspect, the present disclosure refers to a system for plant culture, comprising:

a chamber comprising one or more walls enclosing a spatial volume internal to the chamber, wherein the one or more walls comprise a surface for supporting a plant within the enclosed spatial volume;

a gas delivery apparatus, comprising at least one gas source;

a nutrient delivery apparatus comprising a reservoir;

a sampling apparatus connected to a port formed in the one or more walls; and

a controller connected to the gas delivery apparatus and the nutrient delivery apparatus, and configured so that during operation of the system, with a plant entirely positioned within the enclosed spatial volume of the chamber, the controller:

activates the nutrient delivery apparatus to deliver an aqueous growth medium to the plant; and

activates the gas delivery apparatus to deliver into the enclosed spatial volume a mixture of isotopically-substituted gases.

Examples of the systems can include any one or more of the following features.

A height of the enclosed spatial volume measured between the surface and a wall or wall portion opposite the surface can be at least 0.5 meters (e.g., at least 3.0 meters). The enclosed spatial volume can be at least 500 L (e.g., at least 1000 L). When the chamber is filled with a gas at a pressure of 1.5 atmospheres, a leakage rate of the gas from the chamber can be less than 0.5 L/day (e.g., less than 0.1 L/day). When the chamber is filled with a gas at a pressure p at a first time, the one or more walls of the chamber can be sufficiently impermeable so that the gas pressure within the chamber at a second time at least 7 days after the first time is $0.80p$ or more (e.g., $0.90p$ or more).

The gas delivery apparatus can include a valve connected to the controller, and during operation of the system, the controller can be configured to activate the valve to regulate gas delivery from the gas delivery apparatus. During operation of the system, the at least one gas source can include a source of nitrogen gas for which an isotopic ratio of ^{15}N to ^{14}N is greater than a ratio of ^{15}N to ^{14}N in atmospheric nitrogen gas. During operation of the system, the at least one gas source can include a source of nitrogen gas for which an isotopic ratio of ^{13}N to ^{14}N is greater than a ratio of ^{13}N to ^{14}N in atmospheric nitrogen gas. During operation of the system, the controller can be configured to adjust the isotopic ratio of ^{15}N to ^{14}N in the chamber. During operation of the system, the nitrogen gas mixture in the chamber can include at least 0.1 atom% ^{15}N (e.g., at least 0.5 atom% ^{15}N).

acetylene to the soil, after an elapsed measurement time, activating the ethylene detector to measure an amount of ethylene generated from the acetylene gas by the microorganism, and determining a rate of acetylene reduction based on the amount of ethylene generated and the elapsed time.

Examples of the systems can also include any of the other features described herein, including any combinations of features described in connection with different examples, except as expressly stated otherwise.

In another example, the disclosure features systems for plant culture that include: a chamber including one or more walls enclosing a spatial volume internal to the chamber, where the one or more walls include a surface for supporting a plant within the enclosed spatial volume; a gas delivery apparatus including a nitrogen gas source and a carbon dioxide gas source; a gas removal apparatus connected to a port formed in the one or more walls; a gas detection apparatus including one or more sensors configured to generate measurement signals including information about amounts of oxygen and carbon dioxide in the chamber; a nutrient delivery apparatus including a reservoir and a fluid conduit connected to the reservoir; and a controller connected to the gas delivery apparatus, the gas removal apparatus, the gas detection apparatus, and the nutrient delivery apparatus, and configured so that during operation of the system, the controller activates the nutrient delivery apparatus to deliver a nutrient medium to a plant within the chamber to facilitate growth of the plant, and activates the gas delivery apparatus and gas removal apparatus to adjust concentrations of oxygen, carbon dioxide, and nitrogen in the chamber, and to adjust an isotopic ratio of ^{15}N to ^{14}N in the chamber to a value greater than an isotopic ratio of ^{15}N to ^{14}N in atmospheric nitrogen gas.

In one aspect, the present disclosure refers to a system for plant culture, comprising:

a chamber comprising one or more walls enclosing a spatial volume internal to the chamber, wherein the one or more walls comprise a surface for supporting a plant within the enclosed spatial volume;

a gas delivery apparatus comprising a nitrogen gas source and a carbon dioxide gas source;

a gas removal apparatus connected to a port formed in the one or more walls;

a gas detection apparatus comprising one or more sensors configured to generate measurement signals comprising information about amounts of oxygen and carbon dioxide in the chamber;

a nutrient delivery apparatus comprising a reservoir and a fluid conduit connected to the reservoir; and

a controller connected to the gas delivery apparatus, the gas removal apparatus, the gas detection apparatus, and the nutrient delivery apparatus, and configured so that during operation of the system, the controller:

activates the nutrient delivery apparatus to deliver a nutrient medium to a plant within the chamber to facilitate growth of the plant; and

activates the gas delivery apparatus and gas removal apparatus to adjust concentrations of oxygen, carbon dioxide, and nitrogen in the chamber, and to adjust an isotopic ratio of ^{15}N to ^{14}N in the chamber to a value greater than an isotopic ratio of ^{15}N to ^{14}N in atmospheric nitrogen gas.

Examples of the systems can include any one or more of the following features.

A height of the enclosed spatial volume measured between the surface and a wall or wall portion opposite the surface can be at least 0.5 meters (e.g., at least 3.0 meters). The enclosed spatial volume can be at least 500 L (e.g., at least 1000 L). When the chamber is filled with a gas at a pressure of 1.5 atmospheres, a leakage rate of the gas from the chamber can be less than 0.5 L/day (e.g., less than 0.1 L/day). When the chamber is filled with a gas at a pressure p at a first time, the one or more walls of the chamber are sufficiently impermeable so that the gas pressure within the chamber at a second time at least 7 days after the first time can be $0.80p$ or more (e.g., $0.90p$ or more).

The gas delivery apparatus can include a valve connected to the controller, and during operation of the system, the controller can be configured to activate the valve to regulate gas

determining a rate of acetylene reduction based on the amount of ethylene generated and the elapsed time.

Examples of the systems can also include any of the other features described herein, including any combinations of features described in connection with different examples, except as expressly stated otherwise.

In another example, the disclosure features methods of detecting nitrogen incorporation in a plant, the methods including: positioning a test plant in a support medium within an enclosed chamber of a plant culture system; adjusting a composition of a nitrogen gas mixture within the chamber so that a ratio of at least two nitrogen isotopes is different from a naturally occurring atmospheric ratio of the isotopes; delivering an aqueous growth medium to the test plant to cause growth of the test plant over a growth period; performing an isotope analysis of a test plant tissue to determine relative amounts of the at least two nitrogen isotopes in the test plant tissue; and comparing the relative amounts of the at least two nitrogen isotopes in the test plant tissue to reference information to detect nitrogen incorporation in the test plant.

In one aspect, the present disclosure refers to a method of detecting nitrogen incorporation in a plant, the method comprising:

positioning a test plant in a support medium within an enclosed chamber of a plant culture system;

adjusting a composition of a nitrogen gas mixture within the chamber so that a ratio of at least two nitrogen isotopes is different from a naturally occurring atmospheric ratio of the isotopes;

delivering an aqueous growth medium to the test plant to cause growth of the test plant over a growth period;

performing an isotope analysis of a test plant tissue to determine relative amounts of the at least two nitrogen isotopes in the test plant tissue; and

comparing the relative amounts of the at least two nitrogen isotopes in the test plant tissue to reference information to detect nitrogen incorporation in the test plant.

Examples of the methods can include any one or more of the following features.

Adjusting the composition of nitrogen gas can include activating a gas delivery apparatus of the plant culture system to deliver nitrogen gas including a ratio of the at least two nitrogen isotopes that differs from a naturally occurring ratio of the at least two isotopes in atmospheric nitrogen gas. The at least two nitrogen isotopes can include ^{15}N and ^{14}N, or ^{13}N and ^{14}N, or ^{15}N, ^{14}N, and ^{13}N. The delivered nitrogen gas can include at least 20 atom% ^{15}N (e.g., at least 50 atom% ^{15}N, at least 90 atom% ^{15}N). Following adjustment of the composition of the nitrogen gas mixture, the nitrogen gas mixture can include at least 0.1 atom% ^{15}N or ^{13}N (e.g., at least 0.3 atom% ^{15}N or ^{13}N, at least 0.5 atom% ^{15}N or ^{13}N).

The aqueous growth medium can include a modified Hoaglund's solution. The growth period can include at least 7 days. The test plant tissue can includes root tissue and/or newly emerged whorl tissue and/or top-collared leaf tissue.

The methods can include harvesting the test plant tissue. The methods can include drying the harvested tissue for a drying time, grinding the dried, harvested tissue to form a powder, and performing the isotope analysis on the powder.

The reference information can be derived from tissue of a reference plant. The methods can include growing the reference plant with the test plant in the enclosed chamber of the plant culture system. Growing the reference plant can include positioning the

include inoculating the test plant with the at least one nitrogen-fixing bacterium after positioning the test plant within the enclosed chamber of the plant culture system.

The methods can include determining a relative measurement of nitrogen fixation by the at least one nitrogen-fixing bacterium. Determining the relative measurement of nitrogen fixation can includes: activating an acetylene gas source to deliver a quantity of acetylene to a portion of the support medium; after an exposure interval, measuring an amount of ethylene generated by the at least one nitrogen-fixing bacterium from the quantity of acetylene; and determining a rate of acetylene reduction by the at least one nitrogen-fixing bacterium based on the amount of ethylene generated.

Examples of the methods can also include any of the other features described herein, including any combinations of features described in connection with different examples, except as expressly stated otherwise.

In another example, the disclosure features methods of identifying a nitrogen-fixing bacterial strain, the methods including: inoculating a test plant or a seed of a test plant with a composition including at least one bacterium of a candidate bacterial strain; positioning the test plant in a support medium within an enclosed chamber of a plant culture system; positioning a reference plant in a support medium within the enclosed chamber; adjusting a composition of a nitrogen gas mixture within the chamber so that a ratio of at least two nitrogen isotopes is different from a naturally occurring atmospheric ratio of the isotopes; growing the test and reference plants over a growth period within the enclosed chamber; determining relative amounts of nitrogen isotopes in test and reference plant tissues; and identifying the candidate bacterial strain as a nitrogen-fixing bacterial strain or a non-nitrogen-fixing bacterial strain based on the relative amounts of at least one nitrogen isotope in the test and reference plant tissues.

In one aspect, the present disclosure refers to a method of identifying a nitrogen-fixing bacterial strain, the method comprising:

inoculating a test plant or a seed of a test plant with a composition comprising at least one bacterium of a candidate bacterial strain;

positioning the test plant in a support medium within an enclosed chamber of a plant culture system;

positioning a reference plant in a support medium within the enclosed chamber; adjusting a composition of a nitrogen gas mixture within the chamber so that a ratio of at least two nitrogen isotopes is different from a naturally occurring atmospheric ratio of the isotopes;

growing the test and reference plants over a growth period within the enclosed chamber;

determining relative amounts of nitrogen isotopes in test and reference plant tissues; and

identifying the candidate bacterial strain as a nitrogen-fixing bacterial strain or a non-nitrogen-fixing bacterial strain based on the relative amounts of at least one nitrogen isotope in the test and reference plant tissues.

Examples of the methods can include any one or more of the following features.

The reference plant and a seed of the reference plant are not inoculated with a bacterium of the candidate bacterial strain. Adjusting the composition of nitrogen gas can include activating a gas delivery apparatus of the plant culture system to deliver nitrogen gas including a ratio of the at least two nitrogen isotopes that differs from a naturally occurring ratio of the at least two isotopes in atmospheric nitrogen gas. The at least two nitrogen isotopes can include ^{15}N and ^{14}N. The at least two nitrogen isotopes can include ^{13}N and ^{14}N. The at least two nitrogen isotopes can include ^{15}N, ^{14}N, and ^{13}N.

The delivered nitrogen gas can include at least 20 atom% ^{15}N (e.g., at least 50 atom% ^{15}N, at least 90 atom% ^{15}N). Following adjustment of the composition of the nitrogen gas

heating element and a cooling element of the system according to one or more temperature reference values for the test and reference plants. The methods can include, during growth of the test and reference plants over the growth period, activating one or more light sources of the plant culture system to deliver light to the test and reference plants according to a illumination reference information for the test and reference plants.

The methods can include determining a relative measurement of nitrogen fixation by the at least one bacterium of the candidate strain. Determining the relative measurement of nitrogen fixation can includes activating an acetylene gas source to deliver a quantity of acetylene to a portion of the support medium in which the test plant is supported, after an exposure interval, measuring an amount of ethylene generated by the at least one bacterium from the quantity of acetylene, and determining a rate of acetylene reduction by the at least one bacterium based on the amount of ethylene generated.

Examples of the methods can also include any of the other features described herein, including any combinations of features described in connection with different examples, except as expressly stated otherwise.

In another example, the disclosure features genetically engineered bacteria having a modification in a gene regulating nitrogen fixation or assimilation, where the bacterium is capable of fixing atmospheric nitrogen substantially throughout the tissues of a plant. In one aspect, the present disclosure refers to a genetically engineered bacterium comprising a modification in a gene regulating nitrogen fixation or assimilation, wherein said bacterium is capable of fixing atmospheric nitrogen substantially throughout the tissues of a plant.

Examples of the genetically engineered bacteria can include any one or more of the following features.

The modification can include a deletion of all or a portion of the coding sequence of the *nifL* gene. All or a portion of the *nifL* coding sequence can be replaced by the promoter of the *csp*E gene. The modification can include a deletion of a portion of the coding sequence of the *glnE* gene.

The deletion of the portion of the coding sequence of the *glnE* gene can result in a truncated GlnE protein lacking an adenylyl-removing (AR) domain. The modification can include a mutant *ntrC* gene, where the mutant *ntrC* gene encodes a NtrC protein having an alanine residue at position 54.

The bacteria can include a deletion of all or a portion of the coding sequence of the *nifL* gene, a deletion of portion of the coding sequence of the *glnE* gene, and a mutant *ntrC* gene, where the mutant *ntrC* gene encodes a NtrC protein comprising an alanine residue at position 54.

The bacteria can be capable of fixing atmospheric nitrogen in one or more of the roots, roots, stems, leaves, fruits, flowers, seeds, initial growth tissue, and top growth. The bacteria can be diazotrophs.

The bacteria can be *Klebsiella* variicola, *Kosakonia* sacchari, *Klebsiella pneumonia*, *Azotobacter vinelandii*, or *Rahnella aquatilis* bacteria. The bacteria can be designated 137-3890 (genotype glnE _KO2, ΔnifL::Prm1.2, NtrC_D54A), represented by 138-3890 bacteria deposited as ATCC Accession No. PTA-126479.

Examples of the genetically engineered bacteria can also include any of the other features described herein, in any combination as appropriate, except as expressly stated otherwise.

In another example, the disclosure features genetically engineered bacteria which provide sufficient fixed nitrogen to a plant for the fixed nitrogen to be detectable in multiple plant tissues, the genetically engineered bacteria having a modification in a gene regulating nitrogen fixation or assimilation, where the modification in the gene regulating nitrogen fixation or assimilation results in one or more of: constitutive expression of a nifA gene in nitrogen limiting and non-nitrogen limiting conditions, activity of nifA in non-nitrogen limiting conditions, decreased uridylyl-removing activity of GlnD, decreased adenylyl-removing activity of GlnE, and increased ammonium excretion. In one aspect, the present disclosure refers to a genetically engineered bacterium which provides sufficient fixed nitrogen to a plant for the fixed nitrogen to be detectable in multiple plant tissues, the genetically engineered bacterium comprising a modification in a gene regulating nitrogen fixation or assimilation, wherein the modification in the gene regulating nitrogen fixation or assimilation results in one or more of: constitutive expression of a nifA gene in nitrogen limiting and non-nitrogen limiting conditions, activity of nifA in non-nitrogen limiting conditions, decreased uridylyl-removing activity of GlnD, decreased adenylyl-removing activity of GlnE, and increased ammonium excretion.

In another example, the disclosure features genetically engineered bacteria which provide sufficient fixed nitrogen to a plant for the fixed nitrogen to be detectable in multiple plant tissues, the genetically engineered bacteria having a modification in a gene regulating nitrogen fixation or assimilation that results in constitutive expression of a nifA gene in nitrogen limiting and non-nitrogen limiting conditions and optionally where the genetically engineered bacterium further includes a modification in a gene regulating nitrogen fixation or assimilation that results in one or more of: activity of nifA in non-nitrogen limiting conditions, decreased uridylyl-removing activity of GlnD, decreased adenylyl-removing activity of GlnE, and increased ammonium excretion. In one aspect, the present disclosure refers to a genetically engineered bacterium which provides sufficient fixed nitrogen to a plant for the fixed nitrogen to be detectable in multiple plant tissues, the genetically engineered bacterium comprising a modification in a gene regulating nitrogen fixation or assimilation that results in constitutive expression of a nifA gene in nitrogen limiting and non-nitrogen limiting conditions and optionally wherein the genetically engineered bacterium further comprises a modification in a gene regulating nitrogen fixation or assimilation that results in one or more of: activity of nifA in non-nitrogen limiting conditions, decreased uridylyl-removing activity of GlnD, decreased adenylyl-removing activity of GlnE, and increased ammonium excretion.

In another example, the disclosure features genetically engineered bacteria which provide sufficient fixed nitrogen to a plant for the fixed nitrogen to be detectable in multiple plant tissues, the genetically engineered bacteria having a modification in a gene regulating nitrogen fixation or assimilation that results in constitutive expression of a nifA gene in nitrogen limiting and non-nitrogen limiting conditions and where the genetically engineered bacteria further include a modification in a gene regulating nitrogen fixation or assimilation that results in one or more of: activity of nifA in non-nitrogen limiting conditions, decreased

uridylyl-removing activity of GlnD, decreased adenylyl-removing activity of GlnE, and increased ammonium excretion. In one aspect, the present disclosure refers to a genetically engineered bacterium which provides sufficient fixed nitrogen to a plant for the fixed nitrogen to be detectable in multiple plant tissues, the genetically engineered bacterium comprising a modification in a gene regulating nitrogen fixation or assimilation that results in constitutive expression of a nifA gene in nitrogen limiting and non-nitrogen limiting conditions and wherein the genetically engineered bacterium further comprises a modification in a gene regulating nitrogen fixation or assimilation that results in one or more of: activity of nifA in non-nitrogen limiting conditions, decreased uridylyl-removing activity of GlnD, decreased adenylyl-removing activity of GlnE, and increased ammonium excretion.

In another example, the disclosure features genetically engineered bacteria which provide sufficient fixed nitrogen to a plant for the fixed nitrogen to be detectable in multiple plant tissues, the genetically engineered bacteria including a mutation in the coding sequence of the bacteria's *ntrC* gene, where the coding sequence of the ntrC gene with the mutation encodes a NtrC protein with a D54A amino acid substitution, and where the genetically engineered bacteria are genetically engineered diazotrophs. In one aspect, the present disclosure refers to a genetically engineered bacterium which provides sufficient fixed nitrogen to a plant for the fixed nitrogen to be detectable in multiple plant tissues, the genetically engineered bacterium comprising a mutation in the coding sequence of the bacterium's *ntrC* gene, wherein the coding sequence of the ntrC gene comprising the mutation encodes a NtrC protein with a D54A amino acid substitution, and wherein the genetically engineered bacterium is a genetically engineered diazotroph.

In another example, the disclosure features genetically engineered bacteria which provide sufficient fixed nitrogen to a plant for the fixed nitrogen to be detectable in multiple plant tissues, the genetically engineered bacteria including a mutation in the coding sequence of the bacteria's *ntrC* gene, where the coding sequence of the ntrC gene having the mutation encodes a NtrC protein with a D54A amino acid substitution, and where the genetically engineered bacteria further includes at least one modification in a gene regulating nitrogen fixation or assimilation that results in one or more of constitutive expression of a nifA gene in nitrogen limiting and non-nitrogen limiting conditions, activity of nifA in non-nitrogen limiting conditions, decreased uridylyl-removing activity of GlnD, decreased adenylyl-removing activity of GlnE, and increased ammonium excretion. In one aspect, the present disclosure refers to a genetically engineered bacterium which provides sufficient fixed nitrogen to a plant for the fixed nitrogen to be detectable in multiple plant tissues, the genetically engineered bacterium comprising:

a mutation in the coding sequence of the bacterium's *ntrC* gene, wherein the coding sequence of the ntrC gene comprising the mutation encodes a NtrC protein with a D54A amino acid substitution,

wherein the genetically engineered bacterium further comprises at least one modification in a gene regulating nitrogen fixation or assimilation that results in one or more of constitutive expression of a nifA gene in nitrogen limiting and non-nitrogen limiting conditions, activity of nifA in non-nitrogen limiting conditions, decreased uridylyl-removing activity of GlnD, decreased adenylyl-removing activity of GlnE, and increased ammonium excretion.

Examples of any of the foregoing genetically engineered bacteria can include any one or more of the following features.

The mutation in the coding sequence of the *ntrC* can result in increased ammonium excretion. The modification in a gene regulating nitrogen fixation or assimilation that results in activity of nifA in non-nitrogen limiting conditions can include a deletion of all or a portion of the coding sequence of the bacterium's nifL gene. The deletion of all or a portion of the coding sequence of the nifL gene can result in the decreased expression of nifL.

All or a portion of the *nifL* coding sequence can be replaced by a promoter. The promoter can be a non-intergeneric promoter. The promoter can be a constitutive promoter. The promoter can be an *infC* gene promoter, an ompX gene promoter, or a *csp*E gene promoter.

The modification in a gene regulating nitrogen fixation or assimilation that results in the decreased adenylyl-removing activity of GlnE can include a deletion of a portion of the coding sequence of the glnE gene. The deletion of a portion of the coding sequence of the glnE gene can result in a truncated GlnE protein lacking an adenylyl-removing (AR) domain.

Pseudomonas putida, Pseudomonas syringae, Serratia entomophila, Serratia marcescens, Streptomyces colombiensis, Streptomyces galbus, Streptomyces goshikiensis, Streptomyces griseoviridis, Streptomyces lavendulae, Streptomyces prasinus, Streptomyces saraceticus, Streptomyces venezuelae, Xanthomonas campestris, Xenorhabdus luminescens, Xenorhabdus nematophila, Rhodococcus globerulus AQ719 (NRRL Accession No. B-21663), *Bacillus sp.* AQ175 (ATCC Accession No. 55608), *Bacillus sp.* AQ 177 (ATCC Accession No. 55609), *Bacillus sp.* AQ178 (ATCC Accession No. 53522), or *Streptomyces sp.* strain NRRL Accession No. B-30145.

The genetically engineered bacteria can be *Kosakonia sacchari* bacteria or *Klebsiella variicola* bacteria.

The genetically engineered bacteria can include a deletion of all or a portion of the coding sequence of the *nifL* gene, a deletion of a portion of the coding sequence of the *glnE* gene, and a point mutation in the coding sequence of the *ntrC* gene .

The genetically engineered bacteria can be represented by 137-3890 bacteria deposited as ATCC Accession No. PTA-126749.

The multiple plant tissues can include multiple tissues selected from the group consisting of root, leaf, and whorl tissues.

Examples of any of the foregoing genetically engineered bacteria can also include any of the other features described herein, in any combination as appropriate, except as expressly stated otherwise.

In another example, the disclosure features compositions that include any of the genetically engineered bacteria described herein. In one aspect, the present disclosure refers to a composition comprising any one of the genetically engineered bacteria disclosed herein. The compositions can include any of the features described herein, in any combination as appropriate, except as expressly stated otherwise.

The use of the terms “a” and “an” and “the” and similar referents in the context of describing the disclosure (especially in the context of the following claims) are to be construed to cover both the singular and the plural, unless otherwise indicated herein or clearly contradicted by context. The terms “comprising,” “having,” “including,” and “containing” are to be construed as open-ended terms (i.e., meaning “including, but not limited to,”) unless otherwise noted. Recitation of ranges of values herein are merely intended to serve as a shorthand method of referring individually to each separate value falling within the range, unless otherwise indicated herein, and each separate value is incorporated into the specification as if it were individually recited herein. For example, if the range 10-15 is disclosed, then 11, 12, 13, and 14 are also disclosed. All methods described

As used herein, "*in planta*" may refer to in the plant, on the plant, or intimately associated with the plant, depending upon context of usage (e.g. endophytic, epiphytic, or rhizospheric associations). The plant may comprise plant parts, tissue, leaves, roots, root hairs, rhizomes, stems, seed, ovules, pollen, flowers, fruit, etc.

In some examples, native or endogenous control sequences of genes of the present disclosure are replaced with one or more intrageneric control sequences.

As used herein, "introduced" refers to the introduction by means of modern biotechnology, and not a naturally occurring introduction.

In some examples, the bacteria of the present disclosure have been modified such that they are not naturally occurring bacteria.

Fertilizers and exogenous nitrogen of the present disclosure may comprise the following nitrogen-containing molecules: ammonium, nitrate, nitrite, ammonia, glutamine, etc. Nitrogen sources of the present disclosure may include anhydrous ammonia, ammonia sulfate, urea, diammonium phosphate, urea-form, monoammonium phosphate, ammonium nitrate, nitrogen solutions, calcium nitrate, potassium nitrate, sodium nitrate, etc.

As used herein, "exogenous nitrogen" refers to non-atmospheric nitrogen readily available in the soil, field, or growth medium that is present under non-nitrogen limiting conditions, including ammonia, ammonium, nitrate, nitrite, urea, uric acid, ammonium acids, etc.

In some examples, the nitrogen fixation and assimilation genetic regulatory network comprises polynucleotides encoding genes and non-coding sequences that direct, modulate, and/or regulate microbial nitrogen fixation and/or assimilation and can comprise polynucleotide sequences of the *nif* cluster (e.g., *nif*A, *nif*B, *nif*C,…….*nifZ*), polynucleotides encoding nitrogen regulatory protein C, polynucleotides encoding nitrogen regulatory protein B, polynucleotide sequences of the *gln* cluster (e.g. *gln*A and *glnD*), *dra*T, and ammonia transporters/permeases. In some cases, the Nif cluster may comprise NifB, NifH, NifD, NifK, NifE, NifN, NifX, hesa, and NifV. In some cases, the Nif cluster may comprise a subset of NifB, NifH, NifD, NifK, NifE, NifN, NifX, hesa, and NifV.

In some examples, the increase of nitrogen fixation and/or the production of 1% or more of the nitrogen in the plant are measured relative to control or reference plants, which have not been exposed to the bacteria of the present disclosure. All increases or decreases in bacteria are measured relative to control or reference bacteria. All increases or decreases in plants are measured relative to control or reference plants.

As used herein the term "plant" can include plant parts, tissue, leaves, roots, root hairs, rhizomes, stems, seeds, ovules, pollen, flowers, fruit, etc. Thus, when the disclosure discusses providing a plurality of corn plants to a particular locus, it is understood that this may entail planting a corn seed at a particular locus.

As used herein, when the disclosure discuses a particular microbial deposit by accession number, it is understood that the disclosure also contemplates a microbial strain having all of the identifying characteristics of said deposited microbe, and/or a mutant thereof.

The term "microbial consortia" or "microbial consortium" refers to a subset of a microbial community of individual microbial species, or strains of a species, which can be described as carrying out a common function, or can be described as participating in, or leading to, or correlating with, a recognizable parameter, such as a phenotypic trait of interest.

The term "microbial community" means a group of microbes comprising two or more species or strains. Unlike microbial consortia, a microbial community does not have to be carrying out a common function, or does not have to be participating in, or leading to, or correlating with, a recognizable parameter, such as a phenotypic trait of interest .

As used herein, "isolate," "isolated," "isolated microbe," and like terms, are intended to mean that the one or more microorganisms has been separated from at least one of the materials with which it is associated in a particular environment (for example soil, water, plant tissue, etc.). Thus, an "isolated microbe" does not exist in its naturally occurring environment; rather, it is through the various techniques described herein that the microbe has been removed from its natural setting and placed into a non-naturally occurring state of existence. Thus, the isolated strain or isolated microbe may exist as, for example, a biologically pure culture, or as spores (or other forms of the strain). In examples, the isolated microbe may be in association with an acceptable carrier, which may be an agriculturally acceptable carrier.

In certain examples of the disclosure, the isolated microbes exist as "isolated and biologically pure cultures." It will be appreciated by one of skill in the art, that an isolated and biologically pure culture of a particular microbe, denotes that said culture is substantially free of other living organisms and contains only the individual microbe in question. The culture can contain varying concentrations of said microbe. The present disclosure notes that isolated and biologically pure microbes often "necessarily differ from less pure or impure materials." *See, e.g., In re Bergstrom*, 427 F.2d 1394, (CCPA 1970)(discussing purified

prostaglandins), *see also, In re Bergy*, 596 F.2d 952 (CCPA 1979)(discussing purified microbes), *see also, Parke-Davis & Co. v. H.K. Mulford & Co*., 189 F. 95 (S.D.N.Y. 1911) (Learned Hand discussing purified adrenaline), aff'd in part, rev'd in part, 196 F. 496 (2d Cir. 1912), each of which are incorporated herein by reference. Furthermore, in some examples, the disclosure provides for certain quantitative measures of the concentration, or purity limitations, that must be found within an isolated and biologically pure microbial culture. The presence of these purity values, in certain examples, is a further attribute that distinguishes the presently disclosed microbes from those microbes existing in a natural state. *See, e.g., Merck & Co. v. Olin Mathieson Chemical Corp*., 253 F.2d 156 (4th Cir. 1958) (discussing purity limitations for vitamin B12 produced by microbes), incorporated herein by reference.

As used herein, "individual isolates" should be taken to mean a composition, or culture, comprising a predominance of a single genera, species, or strain, of microorganism, following separation from one or more other microorganisms.

As used herein the terms "microorganism" or "microbe" should be taken broadly. These terms, used interchangeably, include but are not limited to, the two prokaryotic domains, Bacteria and Archaea. The term may also encompass eukaryotic fungi and protists.

Microbes of the present disclosure may include spores and/or vegetative cells. In some examples, microbes of the present disclosure include microbes in a viable but non-culturable (VBNC) state. As used herein, "spore" or "spores" refer to structures produced by bacteria and fungi that are adapted for survival and dispersal. Spores are generally characterized as dormant structures; however, spores are capable of differentiation through the process of germination. Germination is the differentiation of spores into vegetative cells that are capable of metabolic activity, growth, and reproduction. The germination of a single spore results in a single fungal or bacterial vegetative cell. Fungal spores are units of asexual reproduction, and in some cases are necessary structures in fungal life cycles. Bacterial spores are structures for surviving conditions that may ordinarily be nonconducive to the survival or growth of vegetative cells.

The term "determining" encompasses a wide variety of actions and, therefore, "determining" can include calculating, computing, processing, deriving, investigating, looking up (e.g., looking up in a table, a database or another data structure), ascertaining and the like. Also, "determining" can include receiving (e.g., receiving information), accessing (e.g., accessing data in a memory) and the like. Also, "determining" can include resolving, selecting, choosing, establishing and the like.

The phrase "based on" does not mean "based only on," unless expressly specified otherwise. In other words, the phrase "based on" describes both "based only on" and "based at least on."

The term "processor" should be interpreted broadly to encompass a general purpose processor, a central processing unit (CPU), a microprocessor, a digital signal processor (DSP), a controller, a microcontroller, a state machine and so forth. Under some circumstances, a "processor" may refer to an application specific integrated circuit (ASIC), a programmable logic device (PLD), a field programmable gate array (FPGA), etc. The term "processor" may refer to a combination of processing devices, e.g., a combination of a DSP and a microprocessor, a plurality of microprocessors, one or more microprocessors in conjunction with a DSP core or any other such configuration.

The term "memory" should be interpreted broadly to encompass any electronic component capable of storing electronic information. The term memory may refer to various types of processor-readable media such as random access memory (RAM), read-only memory (ROM), non-volatile random access memory (NVRAM), programmable read-only memory (PROM), erasable programmable read only memory (EPROM), electrically erasable PROM (EEPROM), flash memory, magnetic or optical data storage, registers, etc. Memory is said to be in electronic communication with a processor if the processor can read information from and/or write information to the memory. Memory that is integral to a processor is in electronic communication with the processor.

The terms "instructions" and "code" should be interpreted broadly to include any type of computer-readable statement(s). For example, the terms "instructions" and "code" may refer to one or more programs, routines, sub-routines, functions, procedures, etc. "Instructions" and "code" may comprise a single computer-readable statement or many computer-readable statements.

Some examples described herein relate to a computer storage product with a nontransitory computer-readable medium (also can be referred to as a non-transitory processor-readable medium) having instructions or computer code thereon for performing various computer-implemented operations. The computer-readable medium (or processor-readable medium) is nontransitory in the sense that it does not include transitory propagating signals per se (*e.g.*, a propagating electromagnetic wave carrying information on a transmission medium such as space or a cable). The media and computer code (also can be referred to as code) may be those designed and constructed for the specific purpose or purposes. Examples of non-transitory computer-readable media include, but are not limited

to, magnetic storage media such as hard disks, floppy disks, and magnetic tape; optical storage media such as Compact Disc/Digital Video Discs (CD/DVDs), Compact Disc-Read Only Memories (CD-ROMs), and holographic devices; magneto-optical storage media such as optical disks; carrier wave signal processing modules; and hardware devices that are specially configured to store and execute program code, such as Application-Specific Integrated Circuits (ASICs), Programmable Logic Devices (PLDs), Read-Only Memory (ROM) and Random-Access Memory (RAM) devices. Other examples described herein relate to a computer program product, which can include, for example, the instructions and/or computer code discussed herein.

Some examples and/or methods described herein can be performed by software (executed on hardware), hardware, or a combination thereof. Hardware modules may include, for example, a general-purpose processor, a field programmable gate array (FPGA), and/or an application specific integrated circuit (ASIC). Software modules (executed on hardware) can be expressed in a variety of software languages (e.g., computer code), including C, C++, Java™, Ruby, Visual Basic™, and/or other object-oriented, procedural, or other programming language and development tools. Examples of computer code include, but are not limited to, micro-code or micro-instructions, machine instructions, such as produced by a compiler, code used to produce a web service, and files containing higher-level instructions that are executed by a computer using an interpreter. Example may be implemented using imperative programming languages (e.g., C, Fortran, etc.), functional programming languages (Haskell, Erlang, etc.), logical programming languages (e.g., Prolog), object-oriented programming languages (e.g., Java, C++, etc.) or other suitable programming languages and/or development tools. Additional examples of computer code include, but are not limited to, control signals, encrypted code, and compressed code.

Unless otherwise defined, all technical and scientific terms used herein have the same meaning as commonly understood by one of ordinary skill in the art to which this disclosure belongs. Although methods and materials similar or equivalent to those described herein can be used in the practice or testing of the subject matter herein, suitable methods and materials are described below. All publications, patent applications, patents, and other references mentioned herein are incorporated by reference in their entirety. In case of conflict, the present specification, including definitions, will control. In addition, the materials, methods, and examples are illustrative only and not intended to be limiting.

The details of one or more examples are set forth in the accompanying drawings and the description below. Other features and advantages will be apparent from the description, drawings, and claims.

DESCRIPTION OF DRAWINGS

FIG. 1 is a schematic diagram of an example of a system for measuring nitrogen incorporation in plants.

FIG. 2 is a schematic diagram of an example of a gas delivery apparatus.

FIG. 3 is a schematic diagram of an example of a gas detector.

FIG. 4 is a schematic diagram of an example of a temperature regulation apparatus.

FIG. 5 is a schematic diagram of an example of a support structure for localizing detectors in proximity to plants within a chamber.

FIG. 6 is a schematic diagram of an example of a growth monitoring apparatus.

FIG. 7 is a schematic diagram of an example of a fluid removal mechanism.

FIG. 8 is a schematic diagram of an example of an inoculation mechanism.

FIG. 9 is a flow chart showing a series of example steps for performing an assay to detect nitrogen incorporation in plant tissue.

FIG. 10 is a flow chart showing a series of example steps for identifying bacterial strains that perform biological nitrogen fixation.

FIG. 11A is a schematic diagram showing different portions of plant tissue.

FIGS. 11B-11E are plots showing changes in ^{15}N abundance ($\delta^{15}N$) in tissues harvested from plants that were inoculated with a nitrogen-fixing bacterial strain, relative to tissues harvested non-inoculated plants.

FIG. 12 is a schematic diagram of an example of a controller.

Like reference symbols in the various drawings indicate like elements.

DETAILED DESCRIPTION

Introduction

Biological nitrogen fixation (BNF) is a process by which plant-associated microbes such as bacteria are believed to be able to provide nitrogen to host plants. Nitrogen is an important nutrient that influences plant growth. In particular, nitrogen is present in both amino acids and chlorophyll pigments, and a wide variety of biological processes, including plant-based protein synthesis and photosynthesis, therefore depend on the availability of

WHAT IS CLAIMED IS:

1. A system for plant culture, comprising:

a chamber comprising one or more walls enclosing a spatial volume internal to the chamber, wherein the one or more walls comprise a surface for supporting a plant within the enclosed spatial volume;

a gas delivery apparatus, comprising at least one gas source;

a nutrient delivery apparatus comprising a reservoir;

a sampling apparatus connected to a port formed in the one or more walls; and

a controller connected to the gas delivery apparatus and the nutrient delivery apparatus, and configured so that during operation of the system, with a plant entirely positioned within the enclosed spatial volume of the chamber, the controller:

activates the nutrient delivery apparatus to deliver an aqueous growth medium to the plant; and

activates the gas delivery apparatus to deliver into the enclosed spatial volume a mixture of isotopically-substituted gases.

2. The system of claim 1, wherein a height of the enclosed spatial volume measured between the surface and a wall or wall portion opposite the surface is at least 0.5 meters.

3. The system of claim 1, wherein when the chamber is filled with a gas at a pressure p at a first time, the one or more walls of the chamber are sufficiently impermeable so that the gas pressure within the chamber at a second time at least 7 days after the first time is $0.80p$ or more.

4. The system of claim 1, wherein the gas delivery apparatus comprises a valve connected to the controller, and wherein during operation of the system, the controller is configured to activate the valve to regulate gas delivery from the gas delivery apparatus.

5. The system of claim 4, wherein during operation of the system, the at least one gas source comprises a source of nitrogen gas for which an isotopic ratio of ^{15}N to ^{14}N is greater than a ratio of ^{15}N to ^{14}N in atmospheric nitrogen gas.

6. The system of claim 4, wherein during operation of the system, the at least one gas source comprises a source of nitrogen gas for which an isotopic ratio of ^{13}N to ^{14}N is greater than a ratio of ^{13}N to ^{14}N in atmospheric nitrogen gas.

7. The system of claim 6, further comprising a gas detector connected to the controller and configured to generate a measurement signal in response to a presence of one or more gas species within the chamber.

8. The system of claim 4, wherein during operation of the system, the gas delivery apparatus comprises a source of carbon dioxide gas.

9. The system of claim 8, further comprising a gas detector connected to the controller and configured to generate a measurement signal representing an amount of carbon dioxide gas in the chamber.

10. The system of claim 1, further comprising a gas detector connected to the controller and configured to generate a measurement signal in response to a presence of one or more gas species within the chamber.

11. The system of claim 10, wherein the gas detector is configured to generate a measurement signal representing an amount of nitrous oxide in the chamber.

12. The system of claim 1, further comprising a growth monitoring apparatus connected to the controller and configured to generate a measurement signal comprising information about growth of a plant within the chamber.

13. The system of claim 12, wherein the growth monitoring apparatus comprises:

a radiation source configured to direct illumination light to be incident on a plant within the chamber; and

a detector configured to detect light emitted from the plant.

14. The system of claim 13, wherein the detector is configured to detect light emitted from the plant in multiple distinct spectral bands, each comprising a local maximum spectral wavelength.

15. The system of claim 1, further comprising at least one chemical sensor connected to the controller and configured to generate a measurement signal comprising information about an analyte within the chamber.

16. The system of claim 1, further comprising at least one sensor connected to the controller and configured to generate a measurement signal comprising information about a change in plant mass within the chamber.

17. The system of claim 1, further comprising a fluid removal mechanism comprising a conduit connected to or extending through a port formed in the one or more walls and configured to extract a fluid from the chamber.

18. The system of claim 4, wherein the gas delivery apparatus comprises an acetylene gas source, and wherein the system comprises an ethylene detector connected to the controller.

19. A system for plant culture, comprising:

a chamber comprising one or more walls enclosing a spatial volume internal to the chamber, wherein the one or more walls comprise a surface for supporting a plant within the enclosed spatial volume;

a gas delivery apparatus comprising a nitrogen gas source and a carbon dioxide gas source;

a gas removal apparatus connected to a port formed in the one or more walls;

a gas detection apparatus comprising one or more sensors configured to generate measurement signals comprising information about amounts of oxygen and carbon dioxide in the chamber;

a nutrient delivery apparatus comprising a reservoir and a fluid conduit connected to the reservoir; and

a controller connected to the gas delivery apparatus, the gas removal apparatus, the gas detection apparatus, and the nutrient delivery apparatus, and configured so that during operation of the system, the controller:

activates the nutrient delivery apparatus to deliver a nutrient medium to a plant within the chamber to facilitate growth of the plant; and

activates the gas delivery apparatus and gas removal apparatus to adjust concentrations of oxygen, carbon dioxide, and nitrogen in the chamber, and to adjust an isotopic ratio of ^{15}N to ^{14}N in the chamber to a value greater than an isotopic ratio of ^{15}N to ^{14}N in atmospheric nitrogen gas.

20. A method of detecting nitrogen incorporation in a plant, the method comprising:

positioning a test plant in a support medium within an enclosed chamber of a plant culture system;

adjusting a composition of a nitrogen gas mixture within the chamber so that a ratio of at least two nitrogen isotopes is different from a naturally occurring atmospheric ratio of the isotopes;

delivering an aqueous growth medium to the test plant to cause growth of the test plant over a growth period;

performing an isotope analysis of a test plant tissue to determine relative amounts of the at least two nitrogen isotopes in the test plant tissue; and

comparing the relative amounts of the at least two nitrogen isotopes in the test plant tissue to reference information to detect nitrogen incorporation in the test plant.

21. The method of claim 20, further comprising harvesting the test plant tissue.

22. The method of claim 20, wherein the reference information is derived from tissue of a reference plant.

23. The method of claim 22, further comprising growing the reference plant with the test plant in the enclosed chamber of the plant culture system.

24. The method of claim 23, wherein growing the reference plant comprises:

positioning the reference plant in a growth medium within the enclosed chamber of the plant culture system; and

delivering an aqueous growth medium to the reference plant to cause growth of the reference plant over the growth period.

25. The method of claim 24, further comprising, prior to positioning the test and reference plants within the enclosed chamber of the plant culture system, inoculating the test plant or a seed precursor of the test plant with a bacterial suspension.

26. The method of claim 20, further comprising, during growth of the test plant over the growth period:

measuring an oxygen concentration within the enclosed chamber of the plant culture system; and

activating a gas removal apparatus of the plant culture system to adjust the oxygen concentration according to a reference value for the test plant.

27. The method of claim 20, wherein at least one of the test plant and the support medium comprises at least one nitrogen-fixing bacterium.

28. The method of claim 27, further comprising determining a relative measurement of nitrogen fixation by the at least one nitrogen-fixing bacterium.

29. A method of identifying a nitrogen-fixing bacterial strain, the method comprising:

inoculating a test plant or a seed of a test plant with a composition comprising at least one bacterium of a candidate bacterial strain;

positioning the test plant in a support medium within an enclosed chamber of a plant culture system;

positioning a reference plant in a support medium within the enclosed chamber;

adjusting a composition of a nitrogen gas mixture within the chamber so that a ratio of at least two nitrogen isotopes is different from a naturally occurring atmospheric ratio of the isotopes;

growing the test and reference plants over a growth period within the enclosed chamber;

determining relative amounts of nitrogen isotopes in test and reference plant tissues; and

identifying the candidate bacterial strain as a nitrogen-fixing bacterial strain or a non-nitrogen-fixing bacterial strain based on the relative amounts of at least one nitrogen isotope in the test and reference plant tissues.

30. A genetically engineered bacterium comprising a modification in a gene regulating nitrogen fixation or assimilation, wherein said bacterium is capable of fixing atmospheric nitrogen substantially throughout the tissues of a plant.

31. The genetically engineered bacterium of claim 30, wherein said modification comprises a deletion of all or a portion of the coding sequence of the *nifL* gene.

32. The genetically engineered bacterium of claim 30, wherein said modification comprises a deletion of a portion of the coding sequence of the *glnE* gene.

33. A composition comprising the genetically engineered bacterium of any one of claims 30-32.

34. A genetically engineered bacterium which provides sufficient fixed nitrogen to a plant for the fixed nitrogen to be detectable in multiple plant tissues, the genetically engineered bacterium comprising a modification in a gene regulating nitrogen fixation or assimilation, wherein the modification in the gene regulating nitrogen fixation or assimilation results in one or more of: constitutive expression of a nifA gene in nitrogen limiting and non-nitrogen limiting conditions, activity of nifA in non-nitrogen limiting conditions, decreased uridylyl-removing activity of GlnD, decreased adenylyl-removing activity of GlnE, and increased ammonium excretion.

35. A genetically engineered bacterium which provides sufficient fixed nitrogen to a plant for the fixed nitrogen to be detectable in multiple plant tissues, the genetically engineered bacterium comprising a modification in a gene regulating nitrogen fixation or assimilation that results in constitutive expression of a nifA gene in nitrogen limiting and non-nitrogen limiting conditions and optionally wherein the genetically engineered bacterium further comprises a modification in a gene regulating nitrogen fixation or assimilation that results in one or more of: activity of nifA in non-nitrogen limiting conditions, decreased uridylyl-removing activity of GlnD, decreased adenylyl-removing activity of GlnE, and increased ammonium excretion.

36. A genetically engineered bacterium which provides sufficient fixed nitrogen to a plant for the fixed nitrogen to be detectable in multiple plant tissues, the genetically engineered bacterium comprising a modification in a gene regulating nitrogen fixation or assimilation that results in constitutive expression of a nifA gene in nitrogen limiting and non-nitrogen limiting conditions and wherein the genetically engineered bacterium further comprises a modification in a gene regulating nitrogen fixation or assimilation that results in one or more of: activity of nifA in non-nitrogen limiting conditions, decreased uridylyl-removing activity of GlnD, decreased adenylyl-removing activity of GlnE, and increased ammonium excretion.

37. A genetically engineered bacterium which provides sufficient fixed nitrogen to a plant for the fixed nitrogen to be detectable in multiple plant tissues, the genetically engineered bacterium comprising a mutation in the coding sequence of the bacterium's *ntrC* gene, wherein the coding sequence of the ntrC gene comprising the mutation encodes a NtrC protein with a D54A amino acid substitution, and wherein the genetically engineered bacterium is a genetically engineered diazotroph.

38. A genetically engineered bacterium which provides sufficient fixed nitrogen to a plant for the fixed nitrogen to be detectable in multiple plant tissues, the genetically engineered bacterium comprising:

a mutation in the coding sequence of the bacterium's *ntrC* gene, wherein the coding sequence of the ntrC gene comprising the mutation encodes a NtrC protein with a D54A amino acid substitution,

wherein the genetically engineered bacterium further comprises at least one modification in a gene regulating nitrogen fixation or assimilation that results in one or more of constitutive expression of a nifA gene in nitrogen limiting and non-nitrogen limiting conditions, activity of nifA in non-nitrogen limiting conditions, decreased uridylyl-removing activity of GlnD, decreased adenylyl-removing activity of GlnE, and increased ammonium excretion.