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Title page

Microbial nitrous oxide emissions in dryland ecosystems: mechanisms, microbiome and mitigation

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Running title: Microbial regulation of dryland N_2O emissions

Originality-significance statement

Drylands occupy 41.3% of Earth's land surface and contribute 30% of global gaseous nitrogen emissions. This article synthesizes the latest knowledge of the key biological pathways underpinning dryland nitrous oxide emissions and their potential responses to emerging global changes. We propose a conceptual framework to precisely manipulate the dryland microbiome to mitigate nitrous oxide emissions *in situ* using emerging technologies, which may enable the development of environmental-friendly microbiome-based solutions to future mitigation of climate change.

Summary

Globally, drylands represent the largest terrestrial biome and are projected to expand by 23% by the end of this century. Drylands are characterized by extremely low levels of water and nutrients and exhibit highly heterogeneous distribution in plants and biocrusts which make microbial processes shaping the dryland functioning rather unique compared with other terrestrial ecosystems. Nitrous oxide (N_2O) is a powerful greenhouse gas with ozone depletion potential. Despite of the pivotal influences of microbial communities on the production and consumption of N_2O, we have limited knowledge of the biological pathways and mechanisms underpinning N_2O emissions from drylands, which are estimated to account for 30% of total gaseous nitrogen emissions on Earth. In this article, we describe the key microbial players and biological pathways regulating dryland N_2O emissions, and discuss how these processes will respond to emerging global changes such as climate warming, extreme weather events, and nitrogen deposition. We also provide a conceptual framework to precisely manipulate the dryland microbiome to mitigate N_2O emissions *in situ* using emerging technologies with great specificity and efficacy. These cross-disciplinary efforts will enable the development of novel and environmental-friendly microbiome-based solutions to future mitigation strategies of climate change.

Keywords: dryland, nitrous oxide, nitrogen cycling, climate change; microbiome; mitigation

Introduction: overview of nitrous oxide emissions from drylands

Nitrous oxide (N_2O) is a powerful greenhouse gas with 298 times greater global warming potential of CO_2 on an equivalent mass basis (IPCC, 2013), and is also a potent stratospheric ozone depleting substance (Ravishankara *et al.*, 2009). Over the past several decades, human activities have substantially enhanced atmospheric N_2O concentrations by increasing the amount of reactive nitrogen (N) in the soil environment as a consequence of extensive use of synthetic N-based fertilizers (*ca.* 140 Tg N per year) and cultivation of N-fixing forages and crops (Del Grosso and Parton, 2012). Globally, terrestrial ecosystems emit approximately 6.8 Tg N_2O-N per year into the atmosphere, and is the largest contributor (~65%) to the global N_2O budget (IPCC, 2013). An emerging body of evidence highlights that soil microbial communities are key drivers of terrestrial N_2O emissions and N transformations, and modulate the effects of climate change on ecosystem functioning (Richardson *et al.*, 2009; Singh *et al.*, 2010; Hu *et al.*, 2015a). A mechanistic understanding of these microbial modulators and their direct links to N_2O fluxes, therefore, is a prerequisite for improved estimation of global N_2O emissions and development of innovative mitigation strategies.

Drylands (including hyper-arid, arid, semi-arid, and dry sub-humid ecosystems) are water-limited and drought-prone regions with an aridity index (defined as the ratio of the mean annual precipitation to potential evapotranspiration) less than 0.65 (Safriel and Adeel, 2005). Drylands occupy approximately 41.3% of the Earth's land surface (Figure 1), representing the largest terrestrial biome on the planet (Safriel and Adeel, 2005). It provides a variety of essential ecosystem services (e.g. maintenance of biodiversity, and production of wood-fuel, food, and fibre) to more than 38% of the global population (Reynolds *et al.*, 2007). However, drylands are considered highly vulnerable to climate change and desertification processes (Reynolds *et al.*, 2007), owing to its unique climatic features (e.g., infrequent rainfall and intense solar radiation) and extremely low levels of water and nutrients (Delgado-Baquerizo *et al.*, 2013; Maestre *et al.*, 2016b). The most recent climatic projections expect a further 23% increase in the total area of global drylands by the end of this century, as a result of population growth, global climate change, and land cover alternations (Huang *et al.*, 2016). These predicted changes would have unknown consequences for future atmospheric N_2O concentrations and mitigation strategies, if microbial processes and biological pathways mediating dryland N_2O emissions are not properly accounted for in experimental and modelling efforts.

The past two decades have seen an increasing effort devoted to understanding the microbial N_2O pathways from diverse ecosystems at multiple spatial scales (Singh *et al.*, 2010; Hu *et al.*, 2015a), which has substantially improved our knowledge of how terrestrial ecosystems contribute to greenhouse gas emissions. The majority of understanding, however, has been gained through studies dominated by temperate and humid ecosystems where water and nutrients are not scarce, with N_2O production from drylands being documented less frequently. Although severe water and nutrient stresses restrict the net primary productivity and biological activity (Delgado-Baquerizo *et al.*, 2014), emerging studies have reported the wide prevalence of N transformations and N_2O emissions in arid and semiarid environments (Austin *et al.*, 2004; Barton *et al.*, 2013; Zaady *et al.*, 2013), particularly in dryland agricultural and forest plantation soils with N fertilization and irrigation (Hu *et al.*, 2015b; Martins *et al.*, 2015). It was found that a constant level of N_2O fluxes is generally observed during the dry seasons, with considerable increases in the amounts of N_2O emissions occuring as soon as drylands are wetted following rainfall or irrigation events (Barton *et al.*, 2013). This "wetting-pulse" pattern of N_2O fluxes during brief periods can account for the majority of annual N_2O emissions, making dryland N_2O patterns rather unique compared with other terrestrial ecosystems. Drylands, therefore, have been considered a massive contributor to the global N budget, accounting for approximately 30% of the gaseous N emissions on Earth (Bowden, 1986) but dominant mechanisms that drive the N_2O flux remain poorly understood. With global drylands projected to continuously expand this century (Huang *et al.*, 2016), it is imperative to gain a better understanding of the major microbial predictors of dryland N_2O emissions, and their interactions with various abiotic and biotic factors.

In order to combat the steady increase in atmospheric N_2O concentrations, various strategies have been proposed to manipulate soil physico-chemical conditions through land-management practices (e.g., amendment of agrochemicals and optimization of agricultural practices) either to reduce formation of N_2O, or to increase its conversion to N_2 (Richardson *et al.*, 2009; Thomson *et al.*, 2012). However, these technologies had inconsistent mitigation effects, and some of them were even reported to be ineffective in drylands. For example, minimal tillage had limited effects on N_2O reduction in semi-arid agricultural soils (Cookson *et al.*, 2008), and the nitrification inhibitor DMPP had no significant effect on N_2O emissions in acidic dryland pasture soils (Shi *et al.*, 2016). In addition, the use of excessive agrochemicals (e.g., the synthetic materials in polymer-coated or encapsulated fertilizers) as the main mitigation strategy has negative environmental impacts, resulting in accumulation of

undesired residues in fields (Singh and Trivedi, 2017). With the development of omics-based technologies, a typical microbiome was found to be closely associated with the plant species (termed as phytomicrobiome) (Singh and Trivedi, 2017; Teste *et al.*, 2017), opening up new opportunities to manipulate the soil microbiome for mitigation purposes. In recent years, the *in situ* microbiome engineering approach has been suggested as a new paradigm of genetic and microbial engineering (Sheth *et al.*, 2016), which has the potential to revolutionize current mitigation strategies by precisely manipulating the soil microbiome with positive environmental outcomes.

This article aims to identify the major microbial pathways and abiotic and biotic factors that mediate the production and consumption of N_2O in drylands, and discuss their possible feedback responses to emerging global changes. We also describe the limitations of current physico-chemical mitigation strategies, and explore the opportunities for utilizing emerging technologies to manipulate dryland soil microbiome *in situ* for potential N_2O mitigation. This integration is critical to bridge knowledge gaps for a more confident simulation of future dryland N_2O emissions, and may accelerate the development of novel mitigation strategies for reducing N_2O emission *in situ*.

Key biological pathways of N_2O emissions in drylands

The conventional N_2O emission is thought to be largely the result of bacterial pathways, however recent novel molecular techniques like metagenomics and transcriptomics combined with nitrification inhibitors and stable isotope probing techniques have enabled microbiologists to identify unexpectedly diverse N_2O-relevant metabolic pathways within other microbial domains (e.g., fungi and archaea), resulting in paradigm shifts in our understanding of the terrestrial N transformation processes (Baggs EM, 2011; Shoun *et al.*, 2012; Orellana *et al.*, 2014; Stieglmeier *et al.*, 2014; Hink *et al.*, 2016). Multiple pathways, such as ammonia oxidation, nitrifier denitrification, heterotrophic denitrification, anaerobic ammonium oxidation, and dissimilarity nitrate reduction to ammonium, are now known to generate or consume N_2O in terrestrial ecosystems (Hu *et al.*, 2015a). Nevertheless, it is widely accepted that heterotrophic denitrification and nitrification-related pathways constitute the principal sources of soil N_2O emissions (Wrage *et al.* 2001). These biological pathways are subjected to the unique climatic features of drylands (scarcity of water and nutrients during the dry seasons and a pulse of water and nutrients following precipitation), which may

selectively favour the growth and functioning of specific microbial players under different conditions.

Recent studies have shown that fungi contribute substantially to N_2O production, which is a common trait amongst fungal taxa (Crenshaw *et al.*, 2008; Laughlin *et al.*, 2009; Marusenko *et al.*, 2013). By using fungal inhibitor cycloheximide and bactericide streptomycin to distinguish the relative contribution of fungi and bacteria to N_2O production, fungal denitrifiers were found to be the dominant sources of dryland N_2O emissions during the dry seasons across diverse environmental conditions (Crenshaw *et al.*, 2008; Laughlin *et al.*, 2009; Marusenko *et al.*, 2013). For example, in laboratory microcosms combined with inhibition techniques, fungi contribute >70% of the total N_2O production in semi-arid grassland and desert soils (Marusenko *et al.*, 2013), 89% in grassland soils from Ireland (Laughlin and Stevens, 2002), 85% in grassland soils from New Mexico (Crenshaw *et al.*, 2008), and 79% in riparian soils from Arizona (McLain and Martens, 2006).

Genomic analysis revealed that the fungal denitrification system is characterized by a copper-containing NO_2^- reductase and a cytochrome P450 NO reductase to reduce NO_2^- to N_2O (Shoun *et al.*, 2012). However, fungi generally lack the *nosZ* gene, encoding the N_2O reductase, to further reduce N_2O to N_2 (Philippot *et al.*, 2011), making N_2O as the end product of fungal denitrification (Crenshaw *et al.*, 2008). Members of the groups *Ascomycota* and *Basidiomycota* are typical fungal communities (Shoun *et al.*, 2012) found within the lichen-dominated biocrusts and mycorrhizal perennial grasses in drylands (Marusenko *et al.*, 2013). Some groups of endophytic fungi such as dark septate endophytes are also known to survive under dry conditions with high temperatures, and arbuscular mycorrhizal fungi are often associated with desert-adapted plants (Porras-Alfaro *et al.*, 2008; Marusenko *et al.*, 2013). The competitive advantage of some fungi relative to bacteria in drylands is attributed to their strong adaptation capacity, including sporulation to enhance survival, association with primary producers (e.g., soil biocrusts and plants) to increase nutrient acquisition, lower N requirements than bacteria, and higher efficiency to degrade recalcitrant organic molecules that are unavailable to bacteria (Austin *et al.*, 2004; Porras-Alfaro *et al.*, 2008; Schneider *et al.*, 2012). These observations suggest that fungal denitrification is a key mediator of dryland N_2O emissions during the dry seasons (Figure 2). In contrast, fungi play a minor role in N_2O production in irrigated and wet dryland soils in which classic bacterial denitrification, a multistep reaction which can reduce NO_3^- and NO_2^- to N_2O under oxygen-limited conditions

(Philippot *et al.*, 2007), was thought to dominate in anaerobic microsites, caused by intensive respiration following wetting-up events (Figure 2) (Abed *et al.*, 2013).

The nitrification-related pathways (including ammonia oxidation and nitrifier denitrification) regulated by ammonia-oxidizing archaea (AOA) and bacteria (AOB) are also considered to be an important source of dryland N_2O emissions (Barton *et al.*, 2008; Martins *et al.*, 2015). AOA may substantially contribute to N_2O formation through the ammonia oxidation pathway either with the intermediate HNO, rather than hydroxylamine used by AOB, as a direct precursor of N_2O (Walker *et al.*, 2010) or through a novel hybrid formation mechanism combining one N atom from NO or NO_2^- with another N atom from hydroxylamine, HNO, amines or NH_4^+ in an enzymatic reaction (Stieglmeier *et al.*, 2014). There is increasing evidence suggesting the cellular, genomic, and physiological differences between AOA and AOB: AOA ammonia monooxygenase possesses a significantly higher affinity for N substrates than AOB (Martens-Habbena *et al.*, 2009); AOA cells have a higher density of NH_4^+ transporters which can facilitate substrate uptake (Urakawa *et al.*, 2011); and AOA's autotrophic pathway of assimilating inorganic carbon (C) via the hydroxypropionate/hydroxybutyrate cycle is far more energy efficient than AOB's costly Calvin-Benson cycle (Könneke *et al.*, 2014). Therefore, the high efficiency of metabolism and strong ability to compete for substrates perfectly suit the oligotrophic lifestyle of AOA to thrive in drylands with a constantly low-level energy supply (He *et al.*, 2012; Hu *et al.*, 2015b). The majority of AOB, however, stay less active or dormant under dry conditions (Hu *et al.*, 2015b, 2016) and in nutrient-poor environments (He *et al.*, 2012), thus presumably contributing less to N_2O production in drylands. Although the number of studies is small, it appears that, apart from fungal denitrification, AOA ammonia oxidation may also account for a significantly large portion of dryland N_2O production during the dry seasons (Figure 2).

In addition to the ammonia oxidation pathway, ammonia oxidizers can also produce N_2O through the nitrifier denitrification pathway ($NH_3 \rightarrow NH_2OH \rightarrow NO_2^- \rightarrow NO \rightarrow N_2O$) catalysed by the NO_2^- and NO reductases, which was thought to be restricted within AOB under oxygen-limited conditions (e.g., in anaerobic microsites of dryland soils under wet conditions) (Wrage *et al.* 2001). However, physiological studies expanded their niches to aerobic conditions and identified nitrifier denitrification as a universal trait of AOB (Shaw *et al.*, 2006). Although AOA harbour homologous genes of a NO_2^- reductase (Bartossek *et al.*, 2010), they lack genes encoding a potential NO reductase (Spang *et al.*, 2012), which is required for nitrifier denitrification. Stieglmeier *et al.* (2014) provided evidence that the soil

AOA strain *Nitrososphaera viennensis* could produce N_2O under aerobic conditions but was unlikely to be capable of nitrifier denitrification. In contrast to AOB ammonia oxidation which is favoured under N-rich conditions (He *et al.*, 2012), AOB nitrifier denitrification was thought to be of significant importance under unfavourable conditions such as scarcity in water and nutrients (Wrage *et al.* 2001; Hu *et al.*, 2015b). Therefore, it is likely that AOB might also contribute to N_2O production during the dry seasons via the nitrifier denitrification pathway (Figure 2), and the proportional contribution of which can be examined by using available approaches such as dual-isotope (^{18}O and ^{15}N) labelling technique (Kool *et al.*, 2011).

Water is the most fundamental abiotic factor influencing N transformation processes (Austin *et al.*, 2004) and leading to niche separation of N_2O-revelant microorganisms in drylands (Delgado-Baquerizo *et al.*, 2016a). During the dry seasons, water deficit can cause disconnection of soil capillaries and reduction of soil water films, which affects diffusion paths for substrates and result in declined rates of substrate transport to microbes (Manzoni *et al*., 2012). In addition, water deficit induces low soil water potentials, which can reduce activity of intracellular enzymes, and result in negative physiological effects associated with cell dehydration (Schimel *et al*., 2007). Therefore, only certain groups of fungi (Marusenko *et al.*, 2013) and AOA (Hu *et al.*, 2015b) with strong adaptation abilities to such extreme drought and starvation stresses can function during the dry seasons, while the majority of soil microbial communities might remain inactive or dormant. However, wetting of dry soils can stimulate the activity of microbes by removing water stress, and improve soil hydraulic conditions by connecting microbes with substrates (Schaeffer *et al*., 2013). Such changes in water potentials were reported to cause bursts of respiration and mineralization associated with intracellular solutes release from microbial cells undergoing osmotic stress, resulting in a high flux of nutrients into soils (Schimel *et al*., 2007; Zaady *et al.*, 2013). The high bursts of respiration can be strong enough to rapidly deplete soil oxygen levels, allow anaerobic processes (i.e., heterotrophic denitrification) to occur, and lead to substantial N_2O emissions. Indeed, studies in drylands reported that nitrification (Hu *et al.*, 2015b) and denitrification (Martins *et al.*, 2015) were driven more by water than substrate availability. These findings suggest that the drying-rewetting events can lead to niche separation of N_2O-relevant microorganisms, potentially with fungal denitrification and AOA ammonia oxidation as the dominant sources under dry conditions, and heterotrophic bacterial denitrification under wet conditions (Figure 2).

Biotic forces, ranging from diversity and spatial distribution of vascular plants and biocrusts to livestock grazing are also likely to influence dryland N_2O emissions. Drylands are highly heterogeneous ecosystems characterized by a sparse distribution of plants, which are separated by open areas often covered by biocrusts and inhabited by soil arthropods (Delgado-Baquerizo *et al.*, 2016a). Biocrusts are surface components typical of natural drylands, and constitute bacteria, fungi, algae, lichens and mosses that are of great importance for the regulation of N cycling processes (Delgado-Baquerizo *et al.*, 2014; Liu *et al.*, 2016). These biotic attributes and their interactions have unique effects on N-cycling microorganisms and ecosystem functioning in drylands (Delgado-Baquerizo *et al.*, 2016a) due to their ability to capture and cycle water and nutrients and attract a variety of microbiota (Maestre *et al.*, 2016). The species identity of biocrust-forming lichens has been reported to be a key driver modulating the response of soil N cycling and N_2O emissions to global change drivers (e.g., climate warming, altered rainfall frequency and N deposition) (Delgado-Baquerizo *et al.*, 2014; Liu *et al.*, 2016). Vascular plants could modulate the responses of the AOA and AOB abundances to changes in aridity and soil properties (Delgado-Baquerizo *et al.*, 2013). Despite the importance of the interactions among plants and biocrusts as drivers of dryland functionality (Delgado-Baquerizo *et al.*, 2014), there is a lack of knowledge on their feedback responses to abiotic factors (e.g., aridity, rainfall and high temperature), which in turn can alter abiotic attributes and ultimately dryland N_2O emissions. Future manipulative research should simultaneously evaluate the relative importance of biotic and abiotic variables and their interactions, as drivers of N-cycling microorganisms and associated N_2O emissions.

Effects of emerging global changes on the biological pathways of dryland N_2O emissions

Climate changes including climate warming, increased aridity and climatic extremes

Impacts of climate changes on N cycling and N_2O emissions are assumed to be particularly relevant within drylands (Reynolds *et al.*, 2007; Maestre *et al.*, 2012), because these ecosystems are highly vulnerable and characterized by scarcity of water and N (Delgado-Baquerizo *et al*., 2013, 2014). There is a growing consensus that the future climate of drylands will be characterized by (1) increase in frequency and severity of extreme weather events (such as droughts, storms and heat waves); (2) elevated temperatures and altered rainfall regimes; and (3) increasing aridity levels (IPCC, 2013; Maestre *et al.*, 2016b). These climate changes will alter existing patterns of water and nutrient with a range of different

consequences for N-cycling microorganisms, and in return due to the different metabolic requirements and adaptations of microbes, the biological N_2O pathways may have different feedback responses to climate changes.

Global temperatures are predicted to increase by between 1.2~4.8°C by the end of this century (IPCC, 2013). Dryland-specific predictions are not universal and readily available, but it has been reported that global warming can reduce the diversity and cover of lichen-dominated biocrusts, and alter N transformation rates, inorganic N pools (Delgado-Baquerizo *et al.*, 2014) and N_2O emissions in drylands, and thus induces reinforcing (positive) or stabilizing (negative) feedbacks (Del Grosso and Parton, 2012). An increase in available N pools (Delgado-Baquerizo *et al.*, 2014) as well as enhanced metabolic activity of N-cycling microorganisms (Hu *et al.*, 2016) under climate warming may further contribute to increased dryland N_2O emissions. In addition, it was found that the abundance and metabolic activity of AOA and AOB responded differently to warming, with increasing dominance of AOA in nitrification, and thus potentially increasing contribution to N_2O production, under experimental warming within dryland forest (Hu *et al.*, 2016) and grassland soils (Tourna *et al.*, 2008). These observations are supported by the lack of shifts in AOB community structure under warming in soil microcosms (Tourna *et al.*, 2008) and open-top chamber experiments (Yergeau *et al.*, 2012). Therefore, climate warming is more likely to favour the growth of AOA, which has an ecological advantage in drylands over their counterparts AOB (Hu *et al.*, 2015b), and thus AOA ammonia oxidation might be an increasingly important pathway for dryland N_2O production under elevated temperature. Apart from climate warming, elevated CO_2 may also indirectly influence N-cycling microbes and N_2O emissions. Some studies have reported enhanced N_2O emissions under elevated CO_2 levels, but only under the conditions with excess N (Baggs *et al.*, 2003), which is supported by the findings that N_2O emissions, nitrification rates, and ammonia oxidizers did not respond significantly to elevated CO_2 in three dryland forest soils in the short term (Hu *et al.*, 2016; Martins *et al.*, 2016). However, in the long term, elevated CO_2 may drive progressive N limitation through enhancing C and N sequestration in soil organic matter (SOM) and plant biomass (Luo *et al.*, 2004), promoting further scarcity in available N pools in drylands, which favours the growth of AOA and fungal denitrifiers with stronger adaptation to oligotrophic environments. Currently, mechanistic knowledge on how climate warming and elevated CO_2 jointly affect dryland N_2O emissions in the long-term is entirely lacking, and this knowledge gap should be targeted by future field and pot experiments.

Recent studies suggest that the projected increase in aridity for most global drylands in the late 21st century (Huang *et al.*, 2016) will likely alter the relative abundance of microsites (e.g., vascular plants, biocrusts, and open areas) through increasing water stress, reducing the diversity and cover of vascular plants and expanding the area occupied by biocrusts and open areas (Maestre *et al.*, 2012). In drylands, the highest SOM content and N availability are generally observed under tree canopies, while open areas have negative impacts on SOM and N availability (Delgado-Baquerizo *et al.*, 2016a). Therefore, the changes in these microsites will lead to shifts in nutrient availability and SOM content, with consequences for microbial communities and ecosystem functioning (Maestre *et al.*, 2016). Indeed, a global survey of dryland soil processes concluded that increasing aridity would decouple the N and C cycles and decrease N and C availability (Delgado-Baquerizo *et al.*, 2013b), which will likely increase AOA abundance and their contribution to N_2O production due to their high resistance to drought and starvation stresses (Delgado-Baquerizo *et al.*, 2013a). In Australian drylands, it has been reported that AOA abundance significantly increased with increasing aridity levels under vascular plants and in open areas and biocrusts, while the response of AOB abundance to aridity was microsite-dependent (Delgado-Baquerizo *et al.*, 2016a). Increases in aridity can also reduce the diversity of bacteria and fungi in drylands, but increase the abundance of fungi and the fungal:bacterial ratios (Delgado-Baquerizo *et al.*, 2014; Maestre *et al.*, 2015). These results further strengthen the notion that AOA and fungi usually outcompete their bacterial counterparts in niches with more extreme conditions (Austin *et al.*, 2004; He *et al.*, 2012), and may become an increasingly important pathway for dryland N_2O emissions with increasing aridity.

There is increasing recognition that a greater frequency of extreme climatic events such as droughts, heatwaves, storms, and heavy precipitation events may have fundamental impacts on the structure, composition and functioning of drylands (IPCC, 2013). Extreme climatic events last a few hours to several days but can contribute up to 80% of the annual N_2O emissions from an ecosystem, because they are key elements driving pulsed resource dynamics in drylands (Ussiri and Lal, 2012). These climate extremes are generally considered a massive disturbance, and will diminish the capacity of drylands to provide ecosystem services such as primary production via impacting N transformation rates and abundances of AOA and AOB, and may drive into irreversible changes in the N cycle (Fuchslueger *et al.*, 2014). Among the various influences that increases in climate extremes have on biotic and abiotic parameters are alterations in the relative abundances of plant types, shifts in plant

species interactions, and reductions in total plant and biocrust cover, soil organic C and total N, loss of soil structure, and imbalance in nutrient stoichiometry (Delgado-Baquerizo *et al.*, 2013, 2014; Maestre *et al.*, 2016b). These changes in biotic and abiotic attributes will likely influence the abundance and diversity of soil microbes, and have direct or indirect impacts on dryland N_2O emissions (Maestre *et al.*, 2015). Simultaneously, other global changes such as climate warming and land-use changes can alter ecosystem responses to climate extremes (e.g., amplifying or moderating heatwaves) (Bahn *et al.*, 2015). All these climatic variations together with human activities (e.g., overgrazing and poor cultivation practices) can cause drylands to become unable to properly sustain its ecological and economic functions, which is defined as the desertification process (Reynolds *et al.*, 2007; Maestre *et al.*, 2016). It is estimated that severe desertification is present on 10-20% of global drylands (Safriel and Adeel, 2005), the consequences of which are predicted to cause land degradation and a significant reduction in net primary productivity (Reynolds *et al.*, 2007). Therefore, to address the knowledge gaps in multi-trophic interactions and their ecological consequences, we propose a conceptual framework (Figure 3) to target all biotic components and their interactions with current and future climate changes and soil properties. An improved understanding of the responses of microbial players and biological N_2O pathways to the interactions of these factors can facilitate the development of microbiome-based technologies (by manipulating soil abiotic/biotic factors and soil microbiome) for future mitigation of climate change (Figure 3).

N deposition and dryland farming

In parallel with climate change, increased release of reactive N due to intensive anthropogenic perturbations (e.g., fertilization and combustion of fossil fuels) is enhancing N deposition in global drylands (Delgado-Baquerizo *et al.*, 2016b), with current global annual N deposition rates estimated at approximately 120 Tg N $year^{-1}$ (Maestre *et al.*, 2016 and references therein). Impacts of N deposition on soil N_2O emissions can be particularly significant in drylands, because N is, after water, the most important factor limiting net primary productivity and biological activity (Del Grosso and Parton, 2012). It has been demonstrated that N deposition has already increased the amount of inorganic N in drylands (Delgado-Baquerizo *et al.*, 2016b), and negatively influenced plant diversity and phosphorous availability, with important consequences for nutrient cycling and primary production in drylands (Maestre *et al.*, 2016). Increased N availability can enhance nitrification, denitrification and potentially N_2O emission in drylands (Liu *et al.*, 2016), and a global meta-

analysis found that drylands showed greater N_2O emission increase in response to N deposition than other terrestrial ecosystems (Aronson and Allison, 2012). N deposition can also result in different magnitudes of changes in biological N_2O pathways, with microbial groups well-adapted to N-rich environments are favoured. For example, fungal communities are often stimulated by N addition in N-limited environments (Strickland and Rousk 2010; Maestre *et al.*, 2016b) and AOB are more dominant in nitrification of N-rich soils (He *et al.*, 2012), suggesting that contribution of fungal denitrifiers and AOB to N_2O production might be more dominant under N deposition scenarios in non-managed dryland soils. However, it should be noted that elevated CO_2 and N deposition are changing the inorganic N inputs in opposite directions (elevated CO_2 drives N limitation in the long term while N deposition increases it), and meanwhile the accumulation of N due to N deposition in global drylands can be offset by increases in aridity (Delgado-Baquerizo *et al.*, 2016b). Therefore, future efforts should be devoted to address the combined effects of multiple climate change drivers and N deposition on dryland N_2O emissions and ecosystem structure.

Apart from atmospheric N deposition, it is estimated that 25% of global drylands are used for agricultural production which receive a large amount of N fertilizers (Safriel and Adeel, 2005). An extensive body of studies have reported that N fertilization strikingly increased N_2O fluxes from arid and semi-arid agricultural soils, with drylands under both dry and wet conditions as hotspots of N_2O production (Barton *et al.*, 2008; Martins *et al.*, 2015; Homyak *et al.*, 2016). This pattern of N_2O emission in dryland agriculture can be explained by three possible mechanisms. Firstly, plants compete for soil N, which can limit N availability for N_2O production during the plant growth seasons. However, plant N uptake is slow under dry conditions, which can prolong N exposure and increase N supply to N_2O producing microorganisms and lead to increased N_2O production from drylands (Homyak *et al.*, 2016 and references therein). Therefore, compared with non-managed drylands and other terrestrial ecosystems, agricultural drylands are more vulnerable to N loss through N_2O emissions due to the decoupling between plant N uptake and soil N cycling during the dry seasons. Secondly, although microorganisms are generally sensitive to drought, some ammonia oxidizers (in particular AOA) and fungal denitrifiers can still remain active in thin water films (Sullivan *et al.*, 2012; Hu *et al.*, 2015b), providing the microbial catalysts for the high N_2O emissions from dryland agricultural soils during the dry seasons. Thirdly, when dryland agricultural soils are wetted following irrigation and fertilized with N fertilizers, the constraints of water and nutrients that limit growth of microbes are removed, and therefore all

N-cycling microorganisms can potentially contribute to N_2O production, with their relative contributions depending on soil properties and climatic factors (Hu *et al.*, 2015a). These mechanisms suggest that, in contrast to non-managed drylands, agricultural drylands with N fertilizers may operate as N_2O hotspots under both dry and wet conditions, which requires the integrated management of soil, water and crop N demand to ensure sustainable production and reduce N_2O emissions.

Dryland biomes have a wide range of variation in terms of water, nutrient availability, soil health status and contain different plant and microbial diversity which usually decline progressively from sub-humid to hyper arid ecosystems. Therefore, it is expected that these ecosystem types (semi-humid to hyper-arid) will respond differently to climate and anthropogenic disturbance. Indeed, recent studies in global drylands suggest progressive decline in plant diversity and microbial diversity across aridity gradient which directly influence ecosystem functions (Maestre *et al.*, 2012, 2015; Delgado-Baquerizo *et al.*, 2016). Therefore, a systematic study on annual fluctuation in N_2O emissions and their biotic and abiotic modulators are needed across the global drylands to identify their contribution to global inventory but also to identify the dominant mechanisms of emission under semi-humid to hyper-arid ecosystems. Such a study should explicitly consider arable and natural land to factor out the influence of farming, N fertilization and irrigation on total N_2O emissions. Manipulative experiments to test the impact of climate change including extreme weather events with explicit consideration of ecosystem and soil types will provide the crucial knowledge needed to improve and validate simulation model prediction and to inform policy and management decisions for mitigation and sustainable use of drylands (Figure 3).

Current approaches to manipulate microbiomes for N_2O mitigation.

Nitrous oxide emission and consumption are microbial mediated processes, therefore, technologies and interventions developed, tested and trialled are targeted either at *in situ* manipulation of activities and community composition of microbiomes or reduce accessibility to substrate. Currently used technologies/ interventions are mainly based on utilizing chemicals; agronomic practices to manipulate microbiome activities and community composition while emerging technologies for *in situ* microbiome manipulation are focused towards use of biochemical and molecular means.

Physico-chemical technologies-based mitigation approaches: Currently, mitigation of N_2O emissions from agro-ecosystems is mostly based on physico-chemical technologies-based approaches, the underlying principle of which is to increase the N use efficiency by crops and decrease the amount of N accessible to soil microorganisms (Ussiri and Lal, 2012). A range of physico-chemical strategies have been formulated to manipulate soil conditions to eliminate emission of N_2O and/or to promote its reduction to N_2 (Figure 2). These include: (1) manipulation of soil pH by liming (Bakken *et al.*, 2012; Barton *et al.*, 2013); (2) use of efficiency-enhanced fertilizers (e.g., slow- and controlled-release fertilizers) that better synchronize N release and crop demand (Hatfield and Venterea, 2014); (3) use of chemical additives such as urease (e.g., N-(n-butyl) thiophosphoric triamide (NBPT)) and nitrification inhibitors (e.g., DCD and DMPP) (Shi *et al.*, 2016); (4) manipulation of overall soil properties by biochar amendment (Harter *et al.* 2013); (5) proper manipulation of soil water content to reduce anaerobic conditions through improved management of irrigation and drainage (Singh *et al.* 2010); (6) manipulation of soil C availability by incorporating plant residues with high C:N ratio to increase microbial N immobilization and decrease the amount of inorganic N available to soil microbes (Fisk *et al.*, 2015); (7) optimization of SOM application; (8) optimization of split fertilizer N application to better match crop N demand in timing and amount (Reay *et al.*, 2012); and (9) manipulation of soil properties (e.g., soil bulk density, water content, temperature) by changing tillage practices from conventional tillage to minimal or no tillage (Cookson *et al.*, 2008).

Effects of these physico-chemical strategies prove highly variable across soil types, owing to the complex biological pathways that control N_2O emissions, and the influences of a myriad of biotic and abiotic factors. For example, the impact of biochar addition on N_2O emissions strongly depends on soil hydrology, where N_2O emissions varied from 89% reduction in very wet soils to a 51% increase in dry soils (Yanai *et al.*, 2007). Short-term inputs of plant residues enhance N immobilization and reduce inorganic N pools, but long-term inputs of plant residues can increase SOM, up-regulate the entire N cycle and lead to increase dryland N_2O emissions (Fisk *et al.*, 2015). Tillage practices changing from conventional tillage to minimal or no tillage had no significant effect on reducing dryland N_2O emission after seven years of treatment (Cookson *et al.*, 2008). In humid climates, no tillage resulted in lower N_2O emissions compared to conventional tillage, while in drier environments no tillage increased N_2O emissions (Six *et al.*, 2004). The use of the nitrification inhibitor DMPP is effective in alkaline dryland soils through inhibiting the

growth of AOB, but has no effects on N_2O emissions from acidic dryland soils (Shi *et al.*, 2016). Meanwhile, although nitrification inhibitors can generally decrease N_2O emissions by 8-57%, they increase ammonia volatilization by 3-65% (Lam *et al.*, 2016), suggesting that all the N loss pathways must be considered when evaluating the inhibitors as a mitigation strategy. There are no reported urease and nitrification inhibitors that can consistently eliminate N_2O emissions without other environmental N losses. Other drawbacks of nitrification inhibitors in agriculture include: short half-life in soils, possible influences on beneficial soil microorganisms, and not specifically blocking an enzymatic reaction (Shi *et al.*, 2016). In addition, it has been recognized that the use of additional agrochemicals (e.g., inhibitors and synthetic materials in polymer-coated or encapsulated fertilizers) has negative biological and environmental impacts, resulting in accumulation of undesired residues in the field and negatively affecting the association of plant and soil microbiota (Singh and Trivedi, 2017). Considering the principal roles of soil microorganisms in the processes of N_2O production and consumption, we argue that an in-depth understanding of the key functional genes, enzymes and regulatory mechanisms, as well as their relationships with N_2O fluxes, should be central to improvement of future physico-chemical mitigation strategies. We also need to improve our understanding of the interactions between different climatic, soil and biotic properties that influence soil N cycling, which is currently limited.

Plant community technologies-based mitigation approaches: Our ability to manipulate N transformation rates and decrease the risk of soil N_2O emissions greatly depends on the synchronization of N supply and plant N demand and, therefore, reduced substrate availability for microbial emissions. Plant community-based mitigation approaches mainly focus on controlling N supply through plant breeding or engineering to improve plant N use efficiency, immobilize excess inorganic N in plant biomass and/or release biological nitrification inhibitors (Thomson *et al.*, 2012; Fisk *et al.*, 2015). Some other plant-based mitigation practices that complement plant breeding techniques include crop rotations, and using cover crops and deep-rooted crops to recover and retain residual N (Ussiri and Lal, 2012). Other studies also suggested that increasing the extent and duration of actively growing plant roots, by incorporation of perennials into the current annual cropping system during fallow periods, can mitigate N loss from dryland soils (Crews and Peoples, 2005). Root growth may also be increased in the early growing season by managing soil constraints that restrict root growth

and by crop breeding for selective traits such as increased root branching and early growth vigour, which can also increase root capture of inorganic N (Fisk *et al.*, 2015).

Plant traits can also be used to directly manipulate soil microbiomes *in situ*. Some plant roots, such as those of *Brachiaria humidicola*, can release a substantial amount of exudates containing inhibitory organic compounds which could inhibit the ammonia monooxygenase and hydroxylamine oxidoreductase of ammonia oxidizers (Subbarao *et al.*, 2009). Other plants were reported to reduce the abundance of both nitrifying and denitrifying microbes. Screening dryland plants with similar properties can greatly enhance our ability to reduce N_2O emissions from this biome by using those plants directly for *in situ* microbiome engineering. Plants and their associated microbial communities (particularly of rhizosphere) have developed evolutionary relationships, and a typical microbiome was found to be associated with the plant species, selected by the ability of microbes to utilize root exudates and/or to provide benefits for plant nutrition (Teste *et al.*, 2017). There is evidence that the presence of arbuscular mycorrhizal fungi, which form symbiotic relationships with the majority of plants, can induce a reduction of 34–42% in soil N_2O emissions, possibly through changing the abundances of N_2O-relevant genes, increasing N immobilization into plant and microbial biomass, and reducing N resources for N_2O formation (Bender *et al.* 2014). Traditional plant breeding programmes, however, do not consider their associated microbiota, which might result in their poor resistance to biotic and abiotic factors (Singh and Trivedi, 2017), loss of beneficial microbes, disruption of symbiosis associations (Bender *et al.* 2014), and unknown consequences for other ecosystem processes (Singh and Trivedi, 2017). Therefore, future plant breeding programmes should consider the impacts of associated soil microbiota to ensure sustainable N_2O mitigation without losing beneficial microbiota and other ecosystem functions.

The potential of emerging microbiome-based technologies for N_2O mitigation

Despite the principal role in all the processes of N_2O emissions, the soil microbiome is still rarely considered in designing practical mitigation approaches to control N_2O emissions. Our ability to manipulate the microbiome for improved mitigation effect was limited to land-use and land-management practices (for the establishment of microbial communities that favour N_2O reduction) or addition of microbial inoculants (e.g., bio-fertilizers) (Singh and Trivedi, 2017). However, the underlying mechanisms for responses of microorganisms to

agricultural practices remain largely unknown, and the use of microbial inoculants has limited success under field conditions, mainly due to competition with the indigenous soil microbes in natural settings. Although the N_2O reductase harbouring denitrifier phenotype, *Paracoccus denitrificans*, can effectively reduce N_2O to N_2 in batch cultures (Bakken *et al.* 2012), and inoculation of *Bradyrhizobium japonicum* resulted in a significant decrease of N_2O emissions from soybean root systems in pot experiments (Itakura *et al.* 2013), their mitigation effects have yet to be verified under field conditions.

Novel *in situ* microbiome manipulation approaches have been proposed to improve animal and plant fitness by artificially selecting microbiomes (Mueller and Sachs, 2015), and to sustainably improve farm productivity and food quality with positive environmental and biological effects (Singh and Trivedi, 2017). Recently, Sheth *et al.* (2016) highlighted the possibility of emerging *in situ* microbiome molecular engineering approaches to directly manipulate the genomic content of native microbial communities with greater specificity and efficacy. Harnessing soil microbiomes, therefore, has the potential to revolutionize current N_2O mitigation strategies by integrating soil health with more efficient approaches to reduce the use of agrochemicals and keep mitigation performance under various soil and climatic conditions. A variety of approaches, based on biochemical (e.g., use of xenobiotics and nutritional variation), cellular (e.g., probiotics and engineered probiotics, microbiota transplants or synthetic communities), and DNA methods (e.g., phages and mobile DNA), have been applied to manipulate microbial communities in their native context (Table 1). Biochemical manipulations by applying xenobiotics are specific to particular microbial strains and biochemical processes and cannot be broadly applied. For example, antibiotics, as a widely used class of xenobiotics, can lead to persistent alterations in microbiota composition by modulating cellular machinery, and can further select for antibiotic-resistance genes (Kopmann *et al.*, 2013). On the other hand, interfering with plant immune responses through exogenous application of phytohormones that alter the microbiome in a predictable fashion can be an easy applied approach to manipulate the microbiome for desired benefits (Lebeis *et al.*, 2015). Cellular approaches require transplantation of foreign live bacterial strains or synthetic communities into an ecologically competitive environment, which may lead to unwanted consequences or interactions (Panke-Buisse *et al.*, 2015; Tompson *et al.*, 2015). DNA-based methods, such as phages and engineered mobile DNA, can yield perturbations of microbiomes over a greater range of magnitudes and specificity (Sheth *et al.*, 2016). Instead of introducing foreign live bacterial strains into the ecosystem, it is possible to directly add a

metabolic pathway to the native microbiome (Silva *et al.*, 2014; Perera *et al.*, 2015), by which high-specificity and large-magnitude manipulation can be achieved (Sheth *et al.*, 2016). Therefore, engineered mobile DNA may be an effective method for manipulating microbial communities for improved N_2O mitigation strategy. We envision that with the rapid development of *in situ* genome engineering techniques, a suite of novel genetic tools will be available to precisely manipulate the genetic content of complex soil microbial communities. This will provide the basic knowledge to genetically engineer desired bacteria and manipulate native soil microbial community to enhance N_2O reduction and/or reduce N_2O formation. However, other non-molecular approaches (e.g. use of plant traits, signal molecules, microbial cocktails) of *in situ* microbiome manipulation are also proposed which seem more practical given regulatory requirements and public perception regarding genetically modified organisms (Singh and Trivedi, 2017).

The microbiome-based mitigation strategy has enormous potential to reduce N_2O emission from drylands and other ecosystems, but there are some key components that need to be developed to form the foundation for the *in situ* genome engineering approach. Successful *in situ* microbiome engineering will require integrative and cross-disciplinary research, and an expanded understanding of fundamental ecological principles and synthetic biology.

(1) To understand the complex soil microbial communities and manipulate them *in situ* for enhanced N_2O reduction, the starting point is to characterize soil microbiomes in typical dryland ecosystems, and identify the core microbiomes and their potential functionality. The rapidly developing high-throughput sequencing technologies have enabled a comprehensive investigation of the enormous diversity of soil microbiota including global drylands (Fierer *et al.*, 2012; Maestre *et al.*, 2015), but a functional consequence of these largely unexplored microbiota is lacking. Emerging technologies such as metagenomics, metatranscriptomics, and metaproteomics can enable a fine-scale understanding of the specific functional potential of *in situ* microbiome (Eyice *et al.*, 2015; Singh and Trivedi, 2017) and the genetic inventory of known and novel N_2O-relevant genes (Orellana *et al.*, 2014) in the context of different environmental and climatic conditions. These omics-based technologies combined with well-defined, controlled experiments and stable-isotope probing techniques will facilitate a better mechanistic understanding of the core dryland microbiomes and their linkage with N_2O emissions, and provide the basic scientific foundation for developing potentially sustainable means to modulate microbiome growth and function with high specificity. For example,

consumption of N_2O was attributed to bacteria encoding "typical" N_2O reductase (NosZ) (Richardson *et al.*, 2009), however, recent whole-genome shotgun metagenomes from agricultural soils identified previously uncharacterized atypical NosZ proteins encoded in genomes of diverse bacterial groups (Orellana *et al.*, 2014). The atypical *nosZ* genes outnumbered typical *nosZ* genes in most publicly available soil metagenomes (Fierer *et al.*, 2012), indicating that atypical NosZ proteins might be more important than their typical counterparts in controlling N_2O fluxes in soils (Orellana *et al.*, 2014). These findings advance our understanding of the diversity of microbes involved in the N cycle, and provided the means (e.g., gene sequences for primer design and engineering) to facilitate future mitigation of N_2O.

(2) Identification of signal molecules used by microbes for communication. Soil consists of complex and dynamic microbiomes with highly inter-connected networks of metabolic and ecological interactions, which are also influenced by climate, land use and land management practices (Mueller and Sachs, 2015). There is evidence for strong and constant associations for N-cycling microbes in dryland ecosystems (Hu *et al.*, 2015b), and they closely interact with each other to produce or reduce soil N_2O emissions (Butterbach-Bahl *et al.* 2013). However, we have limited knowledge on the signal molecules and chemicals used by these microbes for communication. Identifying these signal molecules can potentially provide an effective tool for manipulating microbe interactions and artificially selecting upon microbiomes for maximizing the activity of N_2O consumption. For example, signal molecules (or their inhibitors) could be used to specifically promote the activity of N_2O-reducing microbes or to inhibit the activity of N_2O-producing microbes to mitigate N_2O emissions. Using a novel quorum quenching coupled with mRNA sequencing approach, Mellbye *et al.* (2016) revealed that QS signaling influences production and consumption of NO, NO_2 and N_2O in a model nitrite oxidizer, *Nitrobacter winogradskyi*. QS mediated expression of genes can also regulate inter/intra kingdom signaling in microbiome. For example, QS mediated expression of *nnrS* gene is postulated to result in cross-talk between nitrite-oxidizers and AOB through NO signaling (Mellbye *et al.*, 2016). These findings have implications for the development of engineered bacteria to modulate soil microbiomes to secret chemicals to stimulate the activities of N_2O reduction to N_2. The same is true for plant based technologies to manipulate microbial activities and communities. However, there is a significant challenge to characterize these signal molecules by using available technology, given the complexities of the soil microbiota and the variety of signal molecules they utilize. Also we have a very

limited knowledge on the genomic circuits and cascades of signal transductions for difficult-to-work-with organisms mediating N_2O transformations. Along with the increasing sensitivity of spectroscopies, an integrated approach of metagenomics, metatranscriptomics and metabolomics will be needed to characterize signal molecules, and their diversity and specificity to harness them for improving mitigation effects. Furthermore, advanced genomic techniques such as generating selected knockout mutants can further define regulatory networks and metabolic pathways in model strains. This information can be used to design small molecules that target specific proteins in communication pathways to mitigate N_2O production.

(3) There is a need to develop novel and sustainable engineering technologies to effectively add a metabolic pathway (e.g., N_2O conversion to N_2) to the genome of native dryland microbiomes over a large range of magnitudes and specificities. The emerging field of synthetic biology will play an important role in engineering predictable functions and pathways in bacteria which upon addition to soils will manipulate the native microbiomes and their activities in a predicted manner (Esvelt and Wang, 2013). This would require the development of synthetic biology and genome editing tools to precisely engineer mobile DNA (e.g., phages, plasmids and transposons) for targeted community manipulations of N_2O reduction, for example, by adding N_2O-reducing genes (Figure 4). Recent studies suggest that plasmids are prevalent in microbial communities, and can be transferred to native soil bacteria from different phyla with high efficiencies (Klumper *et al.*, 2015), and mobile genetic element-mediated transfer is considered a tractable approach to manipulate diverse communities (Sheth *et al.*, 2016). Synthetic communities with N_2O reduction capacity could enhance natural communities in drylands to enable predictability and control over the N_2O reduction processes. Delivery of mobile genetic elements and subsequent transfer between endogenous microbiota *in situ* could minimize perturbations to the overall structure of a given community. A better understanding of the function and dynamics of natural mobile genetic elements will enable strategies to ensure their efficient delivery, transfer, propagation, and long-term stability in natural settings.

(4) The manipulation and engineering of dryland microbiomes for enhanced N_2O reduction will require significant advances in our ability to reliably regulate the engineering outcomes. After introducing these engineered bacterial cells into soil environments, we need to precisely control their behaviours and develop techniques to monitor their survival and ability across the dryland microbial communities. Development of tools for bacterial immune evasion is

667 required to enable efficient transfer and propagation of engineered DNA, and to predictably
668 manipulate efficiencies of gene transfer *in situ* (Sheth *et al.*, 2016). Gene regulation systems
669 such as programmable transcriptional and post-transcriptional regulators, combined with
670 community-level measurement strategies and chemical sensing pathways (Sheth *et al.*, 2016)
671 need to be developed to better control engineered functions in complex communities. Finally,
672 these technologies should be validated in field conditions for sustainable mitigation of N_2O
673 from various dryland ecosystems.

674 5. The use of genetically modified organisms remains an important regulatory and social
675 issue that need to be addressed. In addition, monitoring growth, dispersal and containment of
676 bacterial GMOs in soils will be a significant challenge, therefore, in the short- and medium-
677 term, solutions including the emerging biochemical (plant- based signal molecules, microbial
678 cocktails) technologies will be the focus of technological development and utilization.

679

680 **Conclusions and perspectives**

681 There is consensus that the global dryland is continuously expanding and global climate
682 change is happening, however uncertainty remains in predictions of future N_2O emissions
683 from drylands in a changing world. To help tackle this uncertainty, there is an urgent need to
684 improve the mechanistic understanding of the microbial N_2O sources and sinks in drylands,
685 the interactive influences of biotic and abiotic factors, and the potential to mitigate N_2O
686 emission via manipulating soil microbiomes. We also need to better understand the interactive
687 effects of global change on plants, biocrusts, and soil microorganisms, and the role of their
688 interactions in modulating the feedback responses of dryland N_2O emission to global change.
689 This integrative understanding will help to develop frameworks to parameterize complex
690 microbial systems (abundance, diversity, structure, and activity) into process-based ecosystem
691 models to improve prediction performance (BOX 1), which could ultimately reveal
692 underlying ecological interactions.

693 The emerging *in situ* microbiome approaches offer exciting opportunities for the
694 sustainable mitigation of N_2O from drylands. If this is to be achieved, we need to develop
695 multidisciplinary approaches to include microbial ecology, metagenomics, soil science, plant
696 science, synthetic biology and ecosystems modelling (Figure 5). These cross-disciplinary
697 efforts will reveal the mechanisms underlying natural microbial ecosystems, and will
698 correspondingly suggest new strategies to manipulate the soil microbiome with high

specificity and efficacy. We envisage that increased knowledge and functional annotation of microbial genomes, coupled with advances in modelling techniques to predict the effects of particular genomic manipulations *in situ*, will be necessary to realize the true potential of the microbiome-molecular engineering tools in mitigation approaches. However, the use of existing (e.g., physico-chemical, agronomic interventions) and emerging biochemical (e.g. signal molecules, plant traits) technologies will remain the focus of technological development and use for mitigation of N_2O emissions. Meanwhile, we need to pay attention to social policies and regulatory requirements associated with these emerging tools, and the use of these microbiome-manipulation technologies in natural settings should be sufficiently communicated with all stakeholders including public to ensure successful implementation.

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1029 **Table 1** Selected studies using microbiome- and plant-based technologies to manipulate microbiomes, which have implications for mitigation
1030 strategies for reducing N_2O emission from dryland ecosystems.

Types of microbiome	Engineering strategies	Technical steps	Outcomes	References
Phytomicrobiome	Microbiome-based, microbial inoculants	The field trial evaluated effects of inoculants including plant growth-promoting rhizobacteria and/or arbuscular mycorrhiza fungi on plant growth and yield.	Microbial inoculants promoted plant growth and yield, and enhanced N content in grain tissues.	Adesemoye *et al.*, 2008
Human gastrointestinal microbiome	Microbiome-based, xenobiotics	A small molecule structural analog of choline inhibited reduction of trimethylamine (TMA) in cultured microbes and reduced levels of TMA N-oxide (TMAO), which is associated with cardiac disease.	Manipulation of gut microbial production of TMA can be a potential therapeutic approach for the treatment of cardiometabolic diseses.	Wang *et al.*, 2015
Mammalian gut microbiome	Microbiome-based, bacterium	The enteric bacterium, *Escherichia coli* was engineered to manipulate the quorum sensing signal in the mouse intestine and the effect on antibiotic-induced gut microbiota dysbiosis was investigated.	The engineered *E. coli* altered the composition of the antibiotic-treated gut microbiota, and significantly increased the Firmicutes/Bacteroidetes ratio, the balance of which is known to influence human health.	Tompson *et al.*, 2015
Phytomicrobiome	Microbiome-based, recombinant strains	Poplar, a plant species, was inoculated with the endophyte *Burkholderia cepacia* VM1468 expressing toluene degradation.	Inoculation of poplar had a positive effect on plant growth in the presence of toluene and reduced the amount of toluene released through evaporanspiration.	Taghavi et al., 2005
Phytomicrobiome	Microbiome-based, microbiota transplantation	Soils suppressive to a fungal pathogen *Rhizoctonia solani* was mixed with disease conducive soils.	The microbiota transplantation successfully suppressed the infection in sugar beet, which was attributed to the core-microbiome in the soil in disease suppression.	Mendes *et al.*, 2011
Soil microbiome	Microbiome-based, microbiota transplantation	Soil microbiomes selected by *Arabidopsis thaliana* host plants were inoculated into soils of three *A. thaliana* genotypes and a related crucifer.	Plant hosts showed a shift in flowering time corresponding with the inoculation of early- or late-flowering microbiomes.	Panke-Buisse *et al.*, 2015
Soil microbiome	Microbiome-based, microbiota transplantation	*Bradyrhizobium japonicum* mutant with higher N_2O reduction activity was inoculated into soybean rhizosphere.	Inoculation with N_2O reductase-containing *B. japonicum* effectively reduced N_2O emissions from soybean root systems in pot experiments.	Itakura *et al.*, 2013
Various food microbiomes	Microbiome-based, bacteriophage	A bacteriophage cocktail was evaluated as a bio-control agent for *Listeria monocytogenes* in various ready-to-eat foods.	The bacteriophage treatment can significantly reduce *L. monocytogenes* contamination in lettuce, cheese, apples, smoked salmon, and frozen foods.	Perera *et al.*, 2015

Aquaculture water microbiome	Microbiome-based, bacteriophage	The study investigated the effect of physical and chemical properties of aquaculture waters on the efficiency of phage therapy on inactivation of bacterial pathogens.	Phage therapy can effectively inactivate bacterial pathogens in aquaculture systems, but the efficiency was mostly affected by salinity and organic matter content.	Silva *et al.*, 2014
Phytomicrobiome	Plant-based, cultivar selection	Wheat cultivars were compared for their capacity to stimulate disease suppression by enhancing populations of specific antagonist (Pseudomonads) against *Rhizoctonia solani*.	Wheat cultivars that stimulate disease suppression can enhance populations of specific genotypes with antagonistic activity toward this pathogen.	Mozzola, 2002
Phytomicrobiome	Plant-based, cultivar selection	The effects of four chickpea cultivars on the soil microbiome were compared in the semiarid grasslands of North America	Certain chickpea cultivars can select more beneficial microbiomes for the subsequent wheat growing and were associated with the antagonist species.	Ellouze *et al.*, 2013
Phytomicrobiome	Plant-based, genetic modification	The transgenic tobacco (*Nicotiana tabacum*) plants were investigated for their ability to express plasma membrane proton pump ATPase which can activate ion and nutrient transport and is involved in salt tolerance.	The transgenic plants displayed increased salt tolerance during germination and seedling growth.	Gevaudant *et al.*, 2007
Phytomicrobiome	Plant-based, genetic modification	The transgenic canola (*Brassica napus*) plants overexpressing a mitochondrial citrate synthase were examined for their aluminium tolerance.	The transgenic plants showed enhanced levels of citrate exudation, and had enhanced levels of aluminium tolerance.	Anoop *et al.*, 2003
Phytomicrobiome	Plant-based, genetic modification	Plants expressing acyl-homoserine lactonase quenched pathogen quorum sensing signals and showed enhanced resistance to *Erwinia carotovora* infections	Generation of transgenic plants producing quorum-sensing signals can be used as a potential tool for disease control.	Dong *et al.*, 2001

1031

Box 1 Microbial communities and biogeochemical process-oriented N_2O models

Process-oriented simulation model, as a simplified representation of complex physical, chemical, or biological processes, can integrate a range of climate and soil variables as well as various N cycling processes for quantitative prediction of N_2O emissions (Del Grosso *et al.*, 2006). The underlying assumption in process-oriented models is that N_2O emission is controlled by comparable factors across various biomes and climates, and that the temporal changes of N_2O fluxes can be predicted by capturing the major N-cycling processes. To date, a number of process-oriented models, such as NGAS-DAYCENT (Del Grosso *et al.*, 2000) and PnET-N-DNDC (Butterbach-Bahl *et al.*, 2001), have been developed to predict site-specific or regional-scale N_2O fluxes. However, these models had large inconsistencies in simulation performance and rarely considered microbial communities as an important predictor, which might be attributed to the previous assumption that microbial communities would have little impact on large-scale N_2O emissions (Singh *et al.*, 2010), and the lack of evidence on the direct links between microbial community structure and ecosystem functioning (Trivedi *et al.*, 2016). Recent modelling efforts have demonstrated that the inclusion of microbial communities (e.g., enzyme, biomass, bacterial:fungal ratios, and growth kinetics) into ecosystem models can improve projections: for example, the Community Land Model incorporated microbial dynamics as a new module to improve simulation performance for soil C cycling (Wieder *et al.*, 2013), and estimation of microbial biomass C decomposition was improved by 21-71% by incorporating the microbial parameters into the Microbial Enzyme-mediated Decomposition model (Wang *et al.*, 2014). However, these parameters can only capture microbial variations at coarse levels, with diversity and composition of microbial communities seldom considered, even though recent work has demonstrated strong correlations between microbial diversity and ecosystem functioning (Powell *et al.*, 2015; Trivedi *et al.*, 2016). To further improve process descriptions and modelling accuracy, it is essential to parameterize data on diversity and community structure of microbial taxa (Bakken *et al.*, 2012; Powell *et al.*, 2015). Although it is challenging to generate a simplified set of microbial parameters for complex N_2O pathways, we argue that some important steps should be taken to facilitate the identification of key microbial metrics for N_2O emissions under varying levels of complexity, and incorporation of them into process-based models.

(1) Culture-dependent studies are highly necessary to cultivate more representative strains of N-cycling microbes (e.g., enriched or pure isolates of AOA, AOB, fungal and bacterial

denitrifiers from drylands), to determine their specific rates of N_2O production or consumption, and to link their genomic contents with functioning. For example, the soil AOA strain, *Nitrososphaera viennensis* can produce N_2O via a pathway of N-nitrosating hybrid formation at a rate of 4.6 amol N_2O $cell^{-1}$ h^{-1}, in the range as those of the marine AOA strain *Nitrosopupumilus maritimus* and the AOB strain *Nitrosospira multiformis* under oxic growth conditions (Stieglmeier *et al.*, 2014). These studies can provide valuable information for species-specific N_2O production/reduction rates, and serve as a basis for a better mechanistic understanding of the microbial mechanisms underpinning biological N_2O pathways.

(2) Manipulative microcosm/pot experiments can be conducted to manipulate soil physico-chemical conditions (e.g., soil water content, N levels and soil pH) to quantify the kinetic responses of soil N_2O emissions as a function of the abundance, diversity, or expression levels of the key N_2O-relevant microorganisms. The combined use of quantitative PCR, omics-based approaches, and DNA/RNA-stable isotope probing techniques might allow direct linkage between the taxonomic, physiological and functional properties of microbial communities with rates of N_2O production. For example, in laboratory soil microcosms with or without nitrification inhibitors acetylene and/or 1-octyne, kinetics of nitrification and N_2O production were directly linked with activities of AOA and AOB, suggesting that AOB dominate N_2O production under conditions of high inorganic ammonia inputs, while AOA produce N_2O resulting from mineralized ammonia (Hink *et al.*, 2016). Another microcosm study with water-saturated soils directly linked biochar-reduced N_2O emission with the increased gene and transcript copy numbers of the *nosZ*-encoded bacterial N_2O reductase (Harter *et al.* 2013). Recent studies by experimentally manipulating soil microbial diversity using a dilution approach, the denitrifier diversity was directly linked with potential denitrification activity (Philippot *et al.*, 2013) and N_2O production (Philippot *et al.*, 2011) in soil microcosms. The multi-model inference approach and regression analyses suggested that the abundance of N_2O-reducing bacteria is an important predictor of dryland N_2O fluxes in climate-controlled glasshouse studies and fitted well into the biogeochemical models (Martins *et al.*, 2016).

(3) At the plot and landscape scale, continuous measurements of N_2O fluxes under field conditions (by using automated chamber systems, open-path Fourier transform infrared spectroscopy, and quantum cascade laser absorption spectrometer) as well as high-frequency monitoring of key N_2O-relevant biomarkers (by targeting their abundance, diversity, structure and distribution patterns) are highly recommended. Such information is particularly lacking in

drylands, but is of considerable value to establish the quantitative relationships between N_2O fluxes and microbial communities. It is urgently needed to develop cheap and reliable flux measurement techniques to improve the long-term temporal resolution of observations in drylands particularly following rainfall and irrigation events prone to high N_2O emissions. These efforts might provide an overview of the distribution of functional microbial groups in soils, and enable the identification of indicator genes of soil N_2O fluxes with potential to be incorporated into biogeochemical N_2O models. The inclusion of the evenness of *nirS*-denitrifying communities and the abundance of a specific *nirS* genotype had a substantial effect on model precision on denitrification potential across two agricultural production systems under field conditions (Powell *et al.*, 2015). At the landscape scale, the variations in the activity of enzymes involved in C degradation were predicted by the corresponding functional gene abundance across three geographical dryland regions of Australia in structural equation modelling (Trivedi *et al.*, 2016). In addition, there has been strong evidence for the significant relationships between the key N-cycling genes with soil nitrification/denitrification rates and N_2O fluxes at the plot and landscape scales (e.g., Ma *et al.*, 2008; Morales *et al.*, 2010; Nemeth *et al.*, 2014).

(4) The final step should practically parameterize the identified N_2O indicator genes as a new module into models, and rigorously compare across models to quantify the benefit of incorporating these microbial parameters (Todd-Brown *et al.*, 2012). Toward this end, empirical and statistical approaches (such as machine learning techniques) could provide valuable insights into emergent scaling relationships, such as between microscopic and the macroscopic scales, and how they vary in time and space. Advances in the availability of empirical data and modeling methodologies will assist progress in this area. The abundance, diversity and structure of AOA and AOB *amoA* genes together with their specific N_2O production rates could be promising attributes to improve the estimation of N_2O emitted from the nitrification pathway, while dynamics of the key denitrification genes such as *nirK*, *nirS* and *nosZ* together with their specific N_2O production/consumption rates could be used to improve the simulation of N_2O emitted from the denitrification pathway. It should be noted that the framework proposed here is not necessarily unique to drylands, but the relevant information is more limited in these ecosystems. Nevertheless, including aspects of microbial community diversity and structure in process-based models offers a great opportunity to improve prediction of dryland N_2O emission and enhance our understanding of ecosystem functionality and its feedback responses to global changes.

Figure legends

Figure 1 Global map of estimated aridity index (AI) with a spatial resolution of 10 arc minutes. The AI is defined as the ratio of yearly precipitation to average yearly potential evapotranspiration by the United Nations Environmental Programme. The classification of drylands is: hyper-arid AI < 0.05; arid 0.05 < AI < 0.20; semi-arid 0.20 < AI < 0.50, and dry sub-humid 0.50 < AI < 0.65. Data of the AI were obtained from the global aridity map of the FAO (2014) (Available at: http://www.fao.org/nr/aquastat.).

Figure 2 Hypothetical flow diagram for the key N_2O production and consumption pathways in drylands under dry and wet conditions. Different pathways are denoted by different colours, and arrow thickness indicates the relative magnitude of N flow through the pathway. Under dry conditions, fungal denitrification and AOA ammonia oxidation are considered as the dominant N_2O sources, while under wet conditions following rainfall events, bacterial denitrification predominates the N_2O production and consumption processes.

Figure 3 The relationships and interactions among N-cycling microorganisms, biotic factors, abiotic factors, global change factors, mitigation strategies, and dryland N_2O emissions. Soil microbial communities (including ammonia oxidizers, bacterial and fungal denitrifiers) and their abundance, diversity and community structure are key drivers of dryland N_2O emissions. These microbial players and biological pathways are affected by a wide range of biotic, abiotic, and emerging global change factors as well as their interactions. An improved understanding of the responses of N_2O-relevant microbial communities to these factors can facilitate the development of physico-chemical technologies (by manipulating soil abiotic factors), plant community-based technologies (by manipulating soil biotic factors) and microbiome-based technologies (by manipulating soil microbiome *in situ*) for future mitigation of climate change.

Figure 4 *In situ* manipulation of genomic content of native dryland microbial communities to mitigate N_2O emissions. Mobile genetic elements (e.g., phages, plasmids and transposons) can be used to deliver and transfer engineered N_2O-reducing gene sequences to donor cells, via processes such as transduction, transformation, and conjugation. Donor cells carrying engineered DNA can be introduced into dryland soils to manipulate the native microbiome content communities to acquire the ability to reduce N_2O to N_2 *in situ*, and would propagate over time within the dryland microbiome with replication, integration, and optimized immune evasion strategies.

1163 **Figure 5** Knowledge gaps and priority challenges for sustainable mitigation of N_2O from
1164 drylands.

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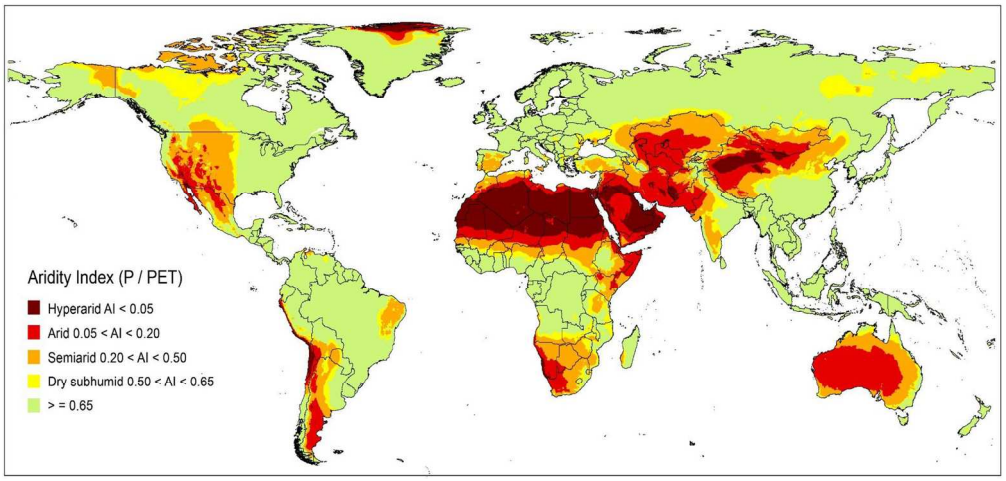

Fig 1

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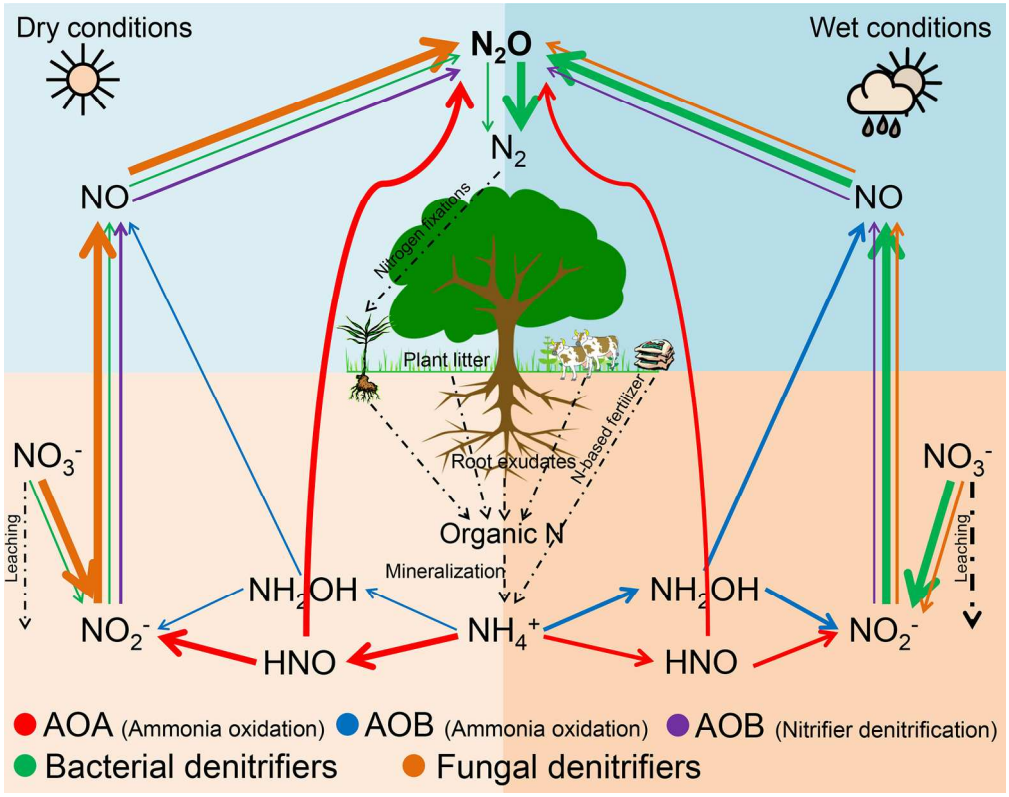

Fig 2

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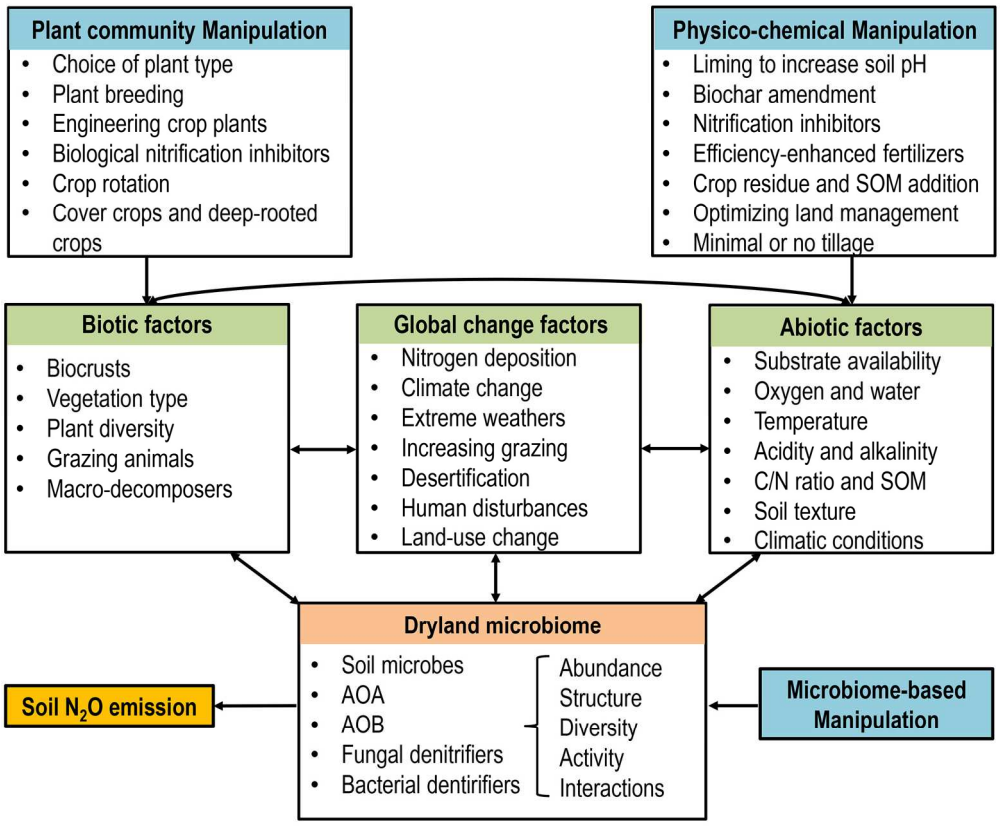

Fig 3.

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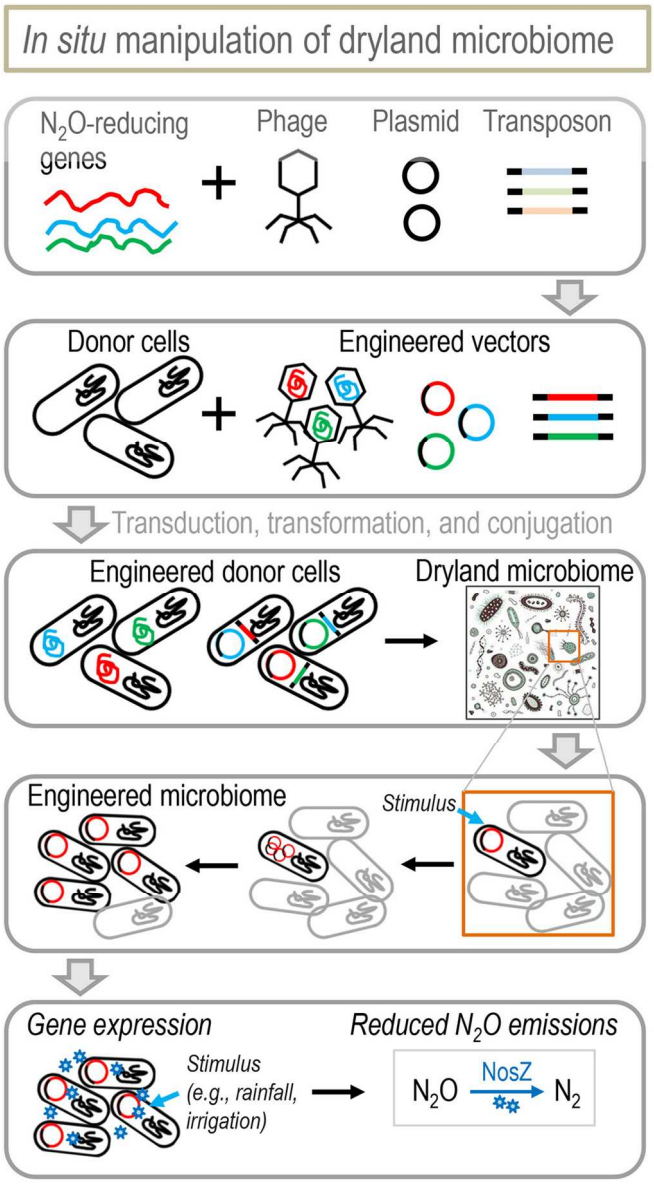

Fig 4.

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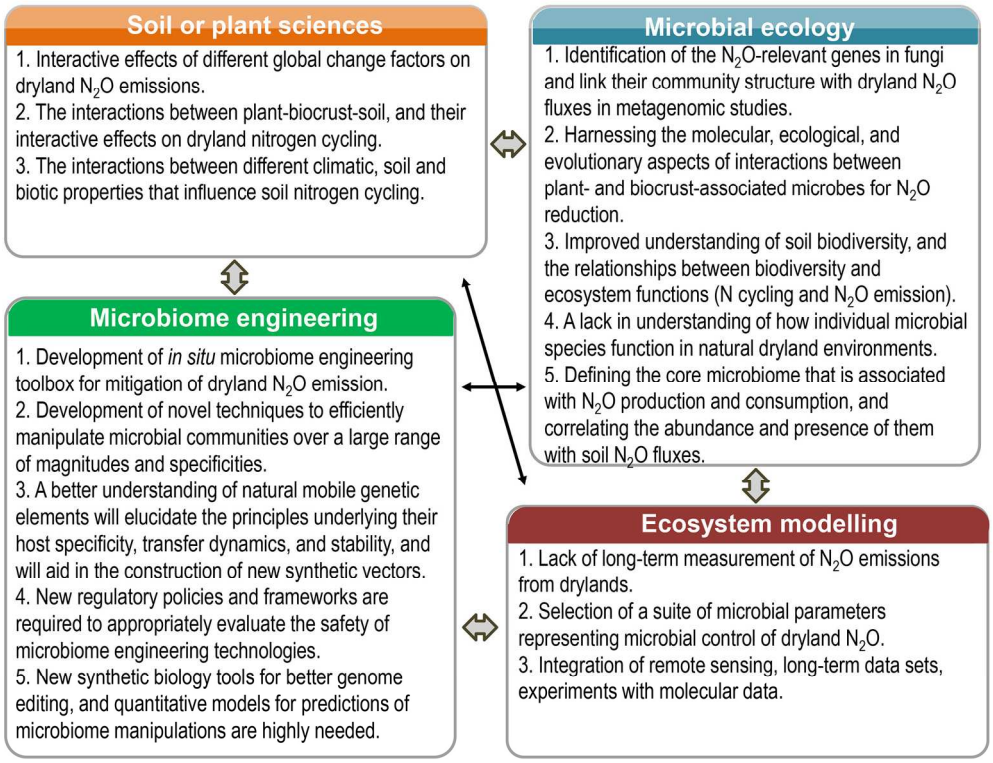

Fig 5.

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