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Review

Underexplored microbial metabolisms for enhanced nutrient recycling in agricultural soils

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Abstract: Worldwide, arable soils have been degraded through erosion and exhaustive cultivation, and substantial proportions of fertilizer nutrients are not taken up by crops. A central challenge in agriculture is to understand how soils and resident microbial communities can be managed to deliver nutrients to crops more efficiently with minimal losses to the environment. Throughout much of the twentieth century, intensive farming has caused substantial loss of organic matter and soil biological function. Today, more farmers recognize the importance of protecting soils and restoring organic matter through reduced tillage, diversified crop rotation, cover cropping, and increased organic amendments. Such management practices are expected to foster soil conditions more similar to those of undisturbed, native plant-soil systems by restoring soil biophysical integrity and re-establishing plant-microbe interactions that retain and recycle nutrients. Soil conditions which could contribute to desirable shifts in microbial metabolic processes include lower redox potentials, more diverse biogeochemical gradients, higher concentrations of labile carbon, and enrichment of carbon dioxide (CO_2) and hydrogen gas (H_2) in soil pores. This paper reviews recent literature on generalized and specific microbial processes that could become more operational once soils are no longer subjected to intensive tillage and organic matter depletion. These processes include heterotrophic assimilation of CO_2; utilization of H_2 as electron donor or reactant; and more diversified nitrogen uptake and dissimilation pathways. Despite knowledge of these processes occurring in laboratory studies, they have received little attention for their potential to affect nutrient and energy flows in soils. This paper

explores how soil microbial processes could contribute to in situ nutrient retention, recycling, and crop uptake in agricultural soils managed for improved biological function.

Keywords: microbial metabolisms; redox potential; reduced tillage; cover cropping; organic amendments; efficient biological nutrient cycling; greenhouse gas; hydrogen consumption; atypical *nosZ*; *nrfA*; *euknr* gene

1. Introduction

Native soil ecosystems have been converted for agricultural use since the dawn of human civilization. During the past century, global food demands have intensified land conversion, as well as use of fertilizers, irrigation, and mechanization [1]. Modern agriculture is dominated by large-scale, continuously mono-cropped fields that have incurred significant losses of soil and organic matter and require increasing amounts of fertilizers [2,3]. Reliance on synthetic fertilizer has increased due to decoupling of crop and livestock production and less use of manures and legume rotations to restore soil fertility. On a worldwide basis, less than 50% of fertilizer nitrogen (N), regardless of source, is taken up by crops [4]. Nutrient imbalances are exacerbated as livestock production becomes more concentrated, resulting in further losses of unused reactive N to the environment [5].

While modern agriculture has helped address the daunting challenges of increased population and food demand, it has also led to deterioration of the soil's capacity to sustain plant and microbial biodiversity and perform ecosystem services [6,7]. Native soils contain accumulated organic matter from decades to centuries of successional vegetation and decomposed litter, as well as intricate root-microbial networks belowground. Conversion of native soils to agriculture destroys the biological linkages between roots, mycelial networks, and interacting microorganisms, thus rendering soils more vulnerable to erosion [8]. Continuous agriculture precludes most plant residues from being returned to the soil, and repeated tillage further depletes soil organic matter through physical disruption and oxidation [9].

Awareness is growing, however, that a sustainable food supply calls for reversing decades of soil erosion and organic matter loss. More farmers are attempting to achieve this by reducing tillage, rotating crops, cover cropping, and returning more organic amendments to soils [3,10,11,12]. Reduced- or no-tillage helps restore soil biophysical integrity and stabilizes microbial habitats to facilitate nutrient exchanges among microbes and between microbes and plants. Less disturbed soils may support development of lower soil oxidation-reduction potentials to enable microbial metabolic diversification. Crop rotations and cover cropping introduce a wider variety of organic compounds through greater root densities. Such management practices could foster adaptive microbial diversity in soils for better nutrient reutilization and fewer losses to the environment [13].

This paper highlights beneficial microbial metabolisms that could become more operational once soils are no longer subjected to intensive tillage and organic matter depletion. It describes how management-induced soil conditions (i.e., improved physical structure, higher organic matter content) could promote such microbial processes as heterotrophic CO_2 consumption, H_2 utilization, and diversified N respiratory pathways in soils (Figure 1). The rationale for this review is that

biologically based agricultural management is expected to improve soil microbial habitat and increase microbial growth and diversity. When promoted in agricultural soils through management, these microbial processes could speed soil organic carbon (C) accretion, increase nutrient reuse, and reduce N losses to the environment.

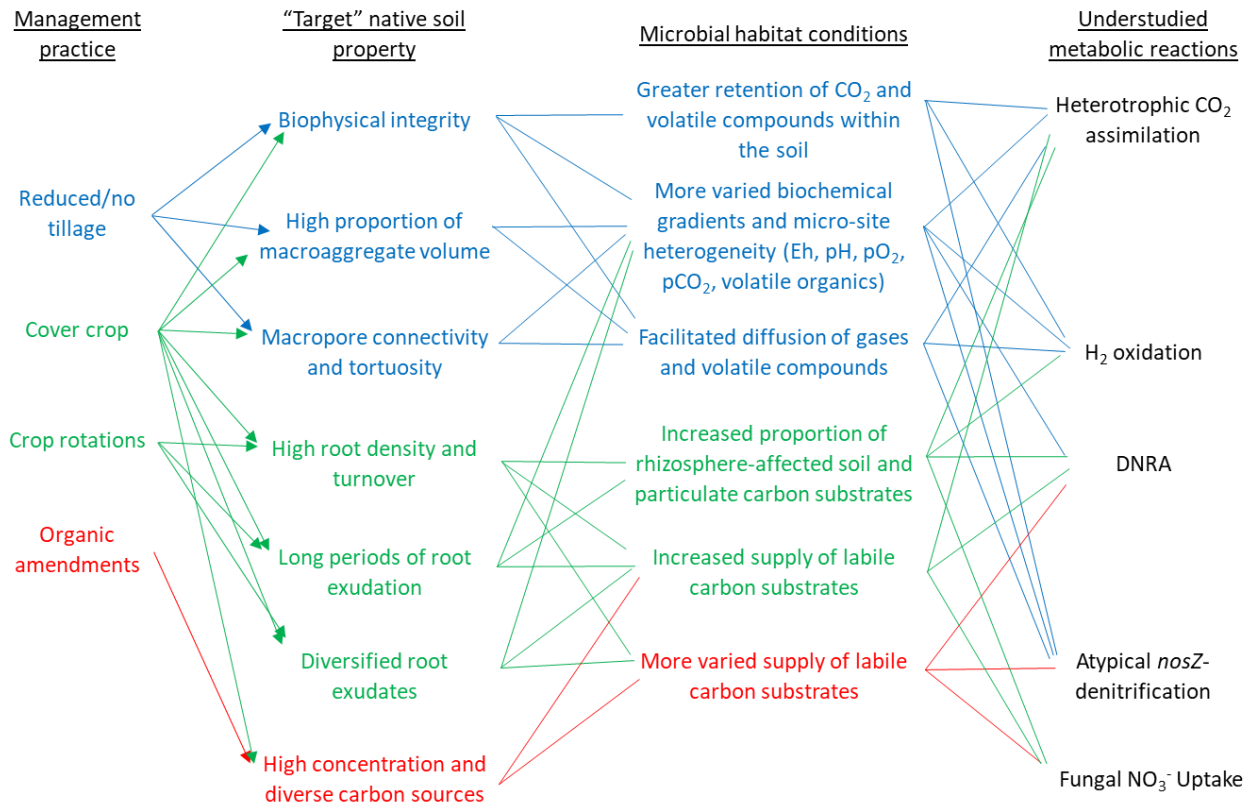

Figure 1. Schematic of agricultural management practices that aim to re-establish more native soil properties and create habitat conditions conducive to the microbial metabolisms highlighted in this review: heterotrophic CO_2 assimilation, H_2 oxidation, dissimilatory nitrate reduction to ammonium (DNRA), non-denitrifier N_2O reduction, and fungal NO_3^- uptake. Respective colors of text, connecting arrows, and lines are blue (for physical structure improvement); green (for increased plant inputs); and red (for more diverse organic inputs).

2. Heterotrophic CO_2 Consumption

Most soil microorganisms are aerobic heterotrophs and obtain their cell material and energy from reduced C atoms in organic matter. Soil microbes have estimated C use efficiencies (CUEs) ranging from 30 to 50%, with aerobic heterotrophic metabolism resulting in 50 to 70% of substrate-C being released as CO_2 [14]. It has long been recognized that heterotrophic growth is enhanced in the presence of CO_2 [15]. Growth enhancement by CO_2 is attributable at least in part to the carboxylation of pyruvate and phosphoenolpyruvate (forming oxaloacetate) in anaplerotic reactions of the tricarboxylic acid cycle [16]. Anaplerotic uptake of CO_2 is unlikely to be limited in undisturbed soils, where partial pressures of CO_2 in soil pores can be up to 1000 times higher than

they are in the atmosphere. This general metabolic process, however, could be affected when physical disruption of soils during plow tillage causes release of large amounts of soil CO_2 to the atmosphere [17].

Microbial assimilation of CO_2 can therefore result from heterotrophic as well as autotrophic metabolisms in soils. In mesocosm experiments using agricultural soils, CO_2 assimilation accounted for 1–8.6% of total microbial biomass [18]. In pure culture studies with common heterotrophic soil bacteria (e.g., *Pseudomonas putida*), between 1.4–6.5% of cellular biomass can result from CO_2 assimilation [19]. During studies of CO_2 uptake by other heterotrophic bacteria, Roslev and coauthors showed strong correlations between growth and CO_2 assimilation, with CO_2-C mainly recovered in bacterial lipids [19]. In studies using stable isotope enrichments of soil, added ^{13}C was recovered in amino acids, amino sugars and fatty acids of bacteria and actinomycetes, demonstrating the ability of microbes to incorporate CO_2-C into multiple cellular compounds [20,21].

In addition to generalized anaplerotic uptake, CO_2 can also be consumed as a reactant during fermentative metabolisms. Several heterotrophic bacterial species in the phylum Firmicutes (e.g., *Clostridium spp.*, *Ruminococcus spp.* and *Butyribacterium rettgeri*) produce acetate from two CO_2 molecules [22,23]. Although acetogenic reactions in soils would be expected to occur only under suboxic or anoxic conditions, anaplerotic reactions by heterotrophs occur more widely. Moreover, CO_2 assimilation can be stimulated by increased H_2 availability. A recent study by Jones et al. [24] observed CUE close to 100% when *Clostridium spp.* were grown as mixotrophs using H_2 as a reducing agent during fermentation. Likewise, CUE increased for other known acetogens like *Eubacterium limosum* and *Moorella thermoacetica* during fermentation with H_2 additions [24].

Heterotrophic assimilation of extracellular CO_2 could therefore increase CUE of soil microbial communities, speed accretion of microbial biomass and organic matter, and decrease greenhouse CO_2 losses to the atmosphere as a greenhouse gas. This process may play an increasingly pivotal role in global C cycles as temperatures and respiration increase. Thus, understanding CO_2 assimilation by heterotrophic microbes and the environmental cues that stimulate it may become more critical for maintaining biological functions in agricultural soils.

3. Utilization of H_2

Dihydrogen gas, H_2, is a widely available source of energy and reducing power for microorganisms that can utilize it under suitable conditions. Despite low-energy yields from coupling H_2 oxidation with O_2 reduction, H_2 in soils may be important for maintaining viability of microbes with depleted C supplies [25,26]. It has been estimated that for every gram of soil, oxidation of available H_2 in soils could provide enough energy to maintain the viability of 10^7 starved bacteria [27]. Analogous to substantial losses of soil CO_2 observed after tillage, H_2 losses also would be expected to occur as a result of physical disturbance. Although comparisons of H_2 partial pressures in tilled and undisturbed soil have yet to be reported in the literature, it is reasonable to propose that H_2 availability to resident microorganisms is higher and more consistent when soils remain undisturbed.

Many soils act as net sinks for H_2, and it has been estimated that approximately 88 ± 11 Tg ha^{-1} of atmospheric H_2 is taken up by soils each year [28]. Uptake of atmospheric H_2 occurs at the soil-air interface, with H_2 having a tropospheric half-life of ca. 1.4 yr [28] at an estimated concentration of 530 ppb [29]. Consumption of H_2 is also a common metabolic reaction in rhizospheres of legumes,

where symbiotic rhizobia produce H_2 as a byproduct of N_2 fixation [29,30]. Concentrations of H_2 at soil-nodule interfaces can be up to 20,000 times higher in the rhizosphere compared to the troposphere. Steep gradients in H_2 concentrations occur with distance from nodules (decrease to sub-atmospheric levels within 4.5 cm from nodule), and measurable H_2 consumption rates in soil have been observed [31,32,33].

Bacteria that use extracellular H_2 as a reducing agent occur in diverse phyla [26,34,35,36]. These bacteria can further be categorized into different groups based on their affinity for H_2, which is dependent upon the specific [Ni-Fe] hydrogenase enzyme used by the bacteria. Group 1 [Ni-Fe] hydrogenases are membrane-bound enzymes that generally belong to species within the Proteobacteria phylum and are characterized as having a low-affinity for H_2 [27]. In contrast, high-affinity hydrogenases can be either membrane bound or referred to as "abiontic hydrogenases" (exoenzymes), which can oxidize H_2 at low concentrations [27]. Thus, it is hypothesized that H_2 concentrations can influence the activity of different hydrogenase enzymes, specifically at the soil-atmosphere and soil-root nodule interfaces, which could favor and select for specific H_2-oxidizing bacteria.

Oxygen serves as an important electron acceptor for H_2 oxidation. In pure culture studies, *Actinobacteria* spp. were unable to oxidize H_2 under anoxic conditions whereas H_2 oxidation was stimulated as oxygen availability increased [27]. Other energy-yielding H_2 oxidation reactions also could occur along gradients and interfaces, specifically the "Knallgas" reaction, where electrons are transferred between H_2 and O_2 to form H_2O in oxic/suboxic zones [27]. Thus, H_2 utilization expands energy supplies when reduced C sources become limiting. Other microorganisms under fermentative conditions can metabolize H_2 and CO_2 simultaneously [22], such as the acetogen *Acetobacterium woodii* [37]. *Clostridium themoaceticum* can also grow with either H_2 or CO_2, but the growth of *C. themoaceticum* was highest when both H_2 and CO_2 were supplied [38].

Mixotrophic and syntrophic growth strategies are also recognized as means by which some bacteria can enhance growth. *Mycobacterium smegmatis*, for example, can co-oxidize H_2 and organic compounds simultaneously. If H_2 is the sole electron donor, *M. smegmatis* growth is impeded [27]. Syntrophy can rely on transfers of H_2 between different taxa. An example is the relationship between *Desulfovibrio alaskensis* and *Syntrophomonas wolfei*. Growth of *S. wolfei*, which was inhibited by the accumulation of H_2, was increased in the presence of bacteria that oxidize H_2, such as *D. alaskensis* [39]. In fact, expression of the [Ni-Fe] hydrogenase by *D. alaskensis* under syntrophic growth with *S. wolfei* was over 40 times higher than its expression when *D. alaskensis* was grown axenically [39], suggesting that the relationship stimulates H_2 oxidation by *D. alaskensis*.

Expression of [Ni-Fe] hydrogenase enzymes by mixotrophs also may be increased during periods of energy limitation (i.e. oligotrophic environments). Under C limitations, *M. smegmatis* can upregulate the group 5 [Ni-Fe] hydrogenase enzyme and downregulate other hydrogenase enzymes that are not associated with H_2 oxidation. Thus, it has been hypothesized that microbes will supplement their energy requirements by oxidizing H_2 during periods of dormancy. Gene knockout studies have demonstrated the importance of H_2 oxidation for viability of dormant cells of *M. smegmatis* and *Streptomyces avermitilis* [36]. It has been estimated that 95–99.9% of all microbial cells in soils are dormant [27], but it is not known what proportion of those cells might depend on the oxidation of H_2 during those periods. Still, this information indicates that H_2-oxidizing metabolic reactions are important in helping maintain microbial activity and diversity in soils [27]. In undisturbed soils, H_2 can be recycled to keep soil microorganisms in more active states, thus serving to reduce lag times for mineralization of newly added organic substrates and facilitating release of

inorganic nutrients to plants.

The study of H_2 oxidation in soils has focused on the rhizosphere, where steep concentration gradients exist due to H_2 release during N_2 fixation by rhizobia [33]. It is likely that H_2 oxidation occurs in other soil microhabitats, as evidenced by H_2 uptake measurements [40,41,42], but much more needs to be learned about how soil management affects microbial use of H_2 as an energy source for enhanced soil biological function. A potential concern in agroecosystems is the use of H_2 by methanogens to reduce CO_2 to methane (CH_4). Supplementing agricultural soils with cow manure, for example, results in increased methanogen abundance and CH_4 production [43]. In fact, manure addition can change the relationship between CH_4 consumption and production in soils so that more CH_4 is produced than consumed [43]. On the other hand, increased methanogenesis could be counterbalanced by more methanotrophic consumption of CH_4 in rhizosphere soils. Therefore, development of soil microenvironments to foster bacterial consumption of CH_4, CO_2, and H_2 could be one means to enhance nutrient use efficiency. It is reasonable to hypothesize that soils having varied microsites and redox potentials would possess greater gas uptake capacities than highly disturbed and degraded soils. Indeed, measurement of gas uptake capacity has the potential to be developed as an indicator of soil biological function.

Studying relationships between microbial metabolisms, gas production, and soil management remain problematic due to the spatial heterogeneity of microbial assemblages and microhabitats. Historically soil microbiological methods have involved removing samples from intact pedons, sieving/mixing, and co-mingling organisms that have had no spatial or physiological relationships whatsoever in the original soil. Therefore, a prerequisite for learning about relationships between microbial metabolisms and soil management will be to devise reliable methods for the study of gas exchange in intact soils. To test hypotheses regarding the relationships between gas metabolism and soil structural integrity, analysis methods enabling assessment of in situ metabolic processes will need to be developed and applied.

4. Diversified N Transformation Pathways

Fertilized soils are major sources of reactive N in the environment, and the many possible fates of nitrate (NO_3-N) make it the most pivotal reactive N species in soil. Since NO_3^- is a soluble anion that is repelled by negatively charged sites on soil particles, it is highly mobile and transported readily through soil by mass flow. If not assimilated into crops or soil microorganisms, NO_3^- can be leached readily through the soil profile, lost in runoff, or denitrified (dissimilated) and lost to the atmosphere as different gaseous N species. While losses of inert N_2 gas contribute to inefficient N use, they do not contribute directly to the greenhouse effect. Soil N losses as N_2O, however, are environmentally more problematic, since N_2O is 300 times more potent than CO_2 as a greenhouse gas and speeds depletion of ozone [43]. In the United States, agriculture accounts for the majority (i.e., 75–80%) of anthropogenic N_2O emissions, with fertilized soils and livestock wastes contributing about 60% and 30% of that total [4]. Lowering net N_2O emissions is therefore crucial for mitigating agriculture's impact on global warming.

Heterotrophic (classic) denitrification is considered to be the main process responsible for N_2O losses from most agricultural soils. Denitrification occurs when soils become wet, causing denitrifiers to switch from using O_2 as an electron acceptor to NO_3-N for anaerobic respiration. The classic denitrification sequence consists of stepwise N reductions from NO_3^- to NO_2^- to NO to N_2O

to N_2 (Figure 2). Each step is carried out by a specific inducible enzyme, namely dissimilatory nitrate reductase (dNar), nitrite reductase (Nir), nitric oxide reductase (Nor) and nitrous oxide reductase (Nos) [44]. The entire sequence can take place within one organism possessing all requisite enzymes or by multiple organisms, necessitating the exchange of N intermediates. Denitrifiers are phylogenetically diverse, and many do not possess all enzymes needed to completely reduce NO_3^- to N_2 via N_2O as an intermediate [45]. Thus, the main end products from denitrification (N_2 or N_2O) will depend not only on soil O_2 content and electron donor availability, but also on microbial community structure and enzyme induction.

Although classic denitrification is the most well-studied N dissimilation process in soil, it is not the only means by which biological NO_3^- reduction can occur. Some bacteria reduce NO_3^- to NH_4^+ (Figure 2) in a process known as dissimilatory nitrate reduction to ammonium (DNRA). Soil conditions that favor DNRA over denitrification are poorly understood, but it is reasonable to expect that DNRA would extend N residence times in soils by preventing or delaying losses of N gases to the atmosphere. Reduction of N_2O to N_2 by non-denitrifiers is another process that could help lower net N_2O emissions (Figure 2). Recent studies have described novel N_2O-reducing enzymes present in a wide diversity of organisms which lack the enzymes to produce N_2O [46]. Both DNRA and non-denitrifier N_2O reduction represent N dissimilation pathways that could serve as N_2O sinks in agricultural soils [47].

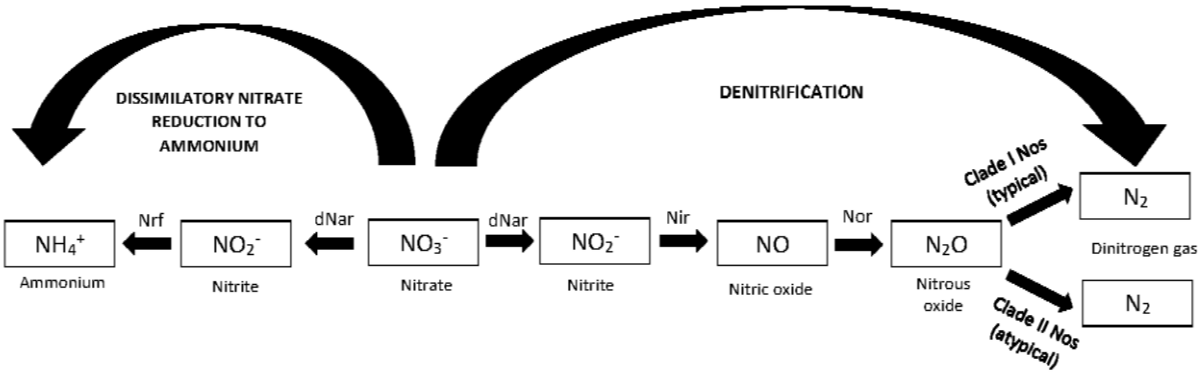

Figure 2. A depiction of respiratory nitrogen (N)-reducing enzymes involved in dissimilatory nitrate reduction to ammonium viz. dNar (dissimilatory nitrate reductase) and Nrf (cytochrome c nitrite reductase) and denitrification viz. dNar, Nir (nitrite reductase), Nor (nitric oxide reductase) and Nos (nitrous oxide reductase).

4.1. Dissimilatory nitrate reduction to ammonium

The DNRA process can be carried out by phylogenetically diverse bacteria and consists of two steps [44,48]. Even though DNRA has been demonstrated in marine fungi and other marine eukaryotes [49], the discussion here focuses solely on DNRA by bacteria. The first of the two steps in DNRA is the initial reduction of NO_3^--N to NO_2^--N, which is similar to what occurs in classic denitrification. The first step can be catalyzed either by a periplasmic nitrate reductase complex (Nap) or a membrane-bound nitrate reductase (Nar) [50]. The second, more distinctive step is the reduction of NO_2^--N to NH_4^+-N by cytochrome c nitrite reductase by formate (Nrf). From a thermodynamic

standpoint, denitrification yields more energy than DNRA during respiration, but the inefficiencies of energy conservation during multiple denitrification steps makes the actual energy yield of DNRA higher than that of denitrification in pure cultures [51]. Most DNRA organisms use a non-membrane bound nitrite reductase for conversion of NO_2^--N to NH_4^+-N and thus do not conserve energy but use the reaction as a sink for excess electrons. However, *Wolinella succinogenes* and other enteric species in the gammaproteobacteria use a membrane-bound nitrite reductase, which enables this reaction to be coupled to energy production [52]. In some cases, bacteria can perform DNRA for either electron disposal or to produce energy, as in *E. coli* [53]. An alternative benefit of DNRA is that the process is a means to detoxify NO_2^- [54,55,56].

Recent advances in molecular detection of DNRA bacteria indicate surprisingly high genetic diversity and widespread distribution in the environment. Indeed, many enteric bacteria present in animal wastes (e.g., *E. coli*) are known to carry out this process using the DNRA, pentaheme cytochrome c nitrite reductase (Nrf), which requires Ca^{2+} for its activity [57,58]. Enzymes other than Nrf may carry out dissimilative N reductions, such as multi-heme cytochrome proteins (e.g., octaheme tetrathionate reductase (Ota)) in *Shewanella oneidensis* [59]. However, validation of primers or probes for conserved sites in these genes has not yet been reported [60].

Genes encoding the Nrf enzymes provide genetic markers for DNRA populations and processes [61,62,63]. The study by Mohan et al. [61] was the first to describe primers for *nrfA* (a gene that encodes for the enzyme) based on extant DNA sequence accessions. These highly degenerate primers, which were designed by aligning amino acid sequences from *E. coli* K-12, *Sulfurospirillum deleyianum* and *Wolinella succinogenes*, amplified a 490-bp fragment of *nrfA*. Subsequently, it was found that these primers did not amplify *nrfA* from many DNRA organisms, which are now recognized to be quite phylogenetically diverse [48]. Thus, it was not surprising that the first published primer sets failed in other studies to yield amplicons from soil, despite the known occurrence of *nrfA* genes in common bacterial taxa found in soil.

With the objective of detecting other DNRA populations in a broader set of soils, Welsh et al. [65] designed a different forward primer for *nrfA*. The new forward primer was designed by aligning nucleotide regions that were conserved in at least 75% of the 474 newly reported NrfA amino acid sequences from the FUNGENE database (http://fungene.cme.msu.edu/ (accessed 10 July 2017)). When combined with one of the reverse primers of Mohan et al. [61], the new forward primer amplified a 269 bp fragment from soils collected from agricultural field sites in Illinois [64]. This new primer set was applied by Song et al. [63] to demonstrate the co-occurrence of higher DNRA rates and higher *nrfA* gene abundances in estuaries using stable isotope probing. That study was the first to show that *nrfA* gene abundance can be used as a potential genetic proxy for DNRA rates.

Another recent study using the primers of Welsh et al. [64] was conducted on DNA and cDNA in soils from tilled potato fields planted with different cover crops [65]. In that study, *nrfA* transcripts were detected in low-temperature soils during two successive winter seasons, but transcript abundances were not affected by cover crop type. In other studies, the amounts of available C in agricultural soils clearly altered the fate of NO_3-N [10,66]. Thus, C availability also is expected to influence the kinds of N dissimilation processes that occur in soils, and this has important practical implications for N conservation (Figure 1). Fazzolari et al. [55] reported that available C, rather than O_2, was the main factor regulating DNRA in soils and that the effect of variable O_2 concentrations depended on the ratios of available C to NO_3-N.

Different C sources influence N dissimilation processes and their transient intermediates and end products. Although N_2O is a known byproduct of DNRA activity [67], classic denitrification and nitrifier denitrification are still considered to be the major N_2O-producing processes in oxic and suboxic soils, respectively [68,69]. Thus, soil conditions favoring DNRA over denitrification are expected to have lower net N_2O emissions [70]. Another factor that should favor DNRA over other N dissimilation processes is soil redox state [71]. Reduced or no-till management minimizes soil disturbance, improves soil water-holding capacity, and results in lower redox potentials than those observed in intensively tilled soils [72]. All these properties are expected to favor DNRA populations in soils [73]. Since other studies have shown that DNRA is less sensitive to fluctuating soil redox conditions [74,75], DNRA activity may be easier to sustain in soils with appropriate management.

The fact that the DNRA process involves a transfer of three additional electrons (compared to denitrification during the reduction of NO_3^--N) implies that DNRA will be favored when the supply of electron donors is high and when soil conditions are strongly reduced. While it is true that NH_4^+ products of DNRA are still subject to re-oxidation to NO_3-N by nitrification, the specific N atoms involved will be held longer in soils due to their greater probability of being taken up by crops or assimilated by soil microorganisms before or after subsequent nitrification.

4.2. N_2O reduction to N_2 by non-denitrifiers

Another means by which net N_2O emissions from soils could be lowered is by promoting activity of non-denitrifier populations that reduce N_2O to N_2 [76]. These populations employ N_2O reductase [46] enzymes that differ from those used by complete denitrifiers in the final step of classical denitrification (Figure 2). Early molecular research assessing the potential of soil microbial communities to reduce N_2O was based on the use of PCR primers for the *nosZ* gene [47]. However, substantial discrepancies have been observed in studies where shifts in *nosZ* communities were used to link N_2O consumption rates during denitrification in an ecosystem, indicating the existence of one or more unaccounted sinks for N_2O. More recently, two distinct groups of Nos proteins were identified based on expanded sequence databases for *nos*Z genes [46].

Most classic or complete denitrifiers belonging to alpha-, beta-, and gammaproteobacteria possess *nos*Z genes which group within Clade I, whereas other taxa possess atypical *nos*Z gene sequences grouping in Clade II [46]. Bioinformatics analyses have revealed that atypical *nosZ* sequences exhibit regulatory and functional components distinct from typical *nosZ* sequences [46]. So far, most environmental studies have used primers for typical *nosZ* genes to amplify environmental DNA to estimate abundance and activity of N_2O-reducing populations [77,78]. These primers, however, were found to be unsuitable for amplifying atypical *nosZ* genes found in other diverse taxa. Using a database of five *Anaeromyxobacter* genomes, Sanford et al. [46] developed primers for atypical *nos*Z to amplify 880-bp fragments from DNA of agricultural soils. They also demonstrated presence of atypical *nosZ* sequences in many additional taxa by screening 126 bacterial and 7 archaeal genomes. Jones et al. [76] subsequently developed primers for quantitative PCR assay for atypical *nosZ* gene abundances from environmental soil, freshwater sediments and activated sludge samples.

To date, half of the organisms screened for atypical *nosZ* genes are incomplete denitrifiers, including *Anaeromyxobacter* spp., which lack genes encoding nitrite reductase (Nir) enzymes [76]. Managing soils for lower N_2O emissions would be aided by the activity of N_2O-reducers like

Anaeromyxobacter spp., which convert N_2O to N_2, thereby counteracting classic denitrification in the presence of high NO_3^- concentrations [46]. Promoting populations of non-denitrifier N_2O reducers could be an effective tool to address the potential for N_2O production by fungi, which are more likely to flourish in no-till than in tilled soils. Although NO_3-N reduction as an electron-accepting process is less common among fungi than it is among bacteria, the major fungal product is N_2O rather than N_2 [79]. Members of the Ascomycota, Basidiomycota and Mucoromycota are capable of using NO_3^- as an electron acceptor, although this trait does not appear to be widely distributed within each of these phyla [80]. In a study by Maeda et al. [79], 70 of 207 fungal isolates tested were capable of producing N_2O from NO_3^-, and this capability was most frequently observed in members of the order *Hypocreales*. Potential production of N_2O by fungi in no-till soils thus could be offset by enhanced activity of non-denitrifier N_2O reducers.

In an experiment conducted by Orellana et al. [81], atypical *nosZ* gene-carrying N_2O reducers were dominated by *Anaeromyxobacter* spp. and outnumbered the typical *nosZ* carrying microorganisms in Illinois corn-belt soils. Atypical *nosZ*-containing bacteria appear to have diverse N metabolisms, with some, such as *Anaeromyxobacter* spp., possessing *nrfA* genes to potentially perform DNRA [82]. Other bacteria containing atypical *nosZ* genes, such as *Wolinella succinogens*, *Geobacillus thermodenitrificans*, and several *Bacillus* spp. from soils, have been shown to reduce N_2O to N_2. Different management practices could be used to shape the relative abundances and activities of populations with typical and atypical *nosZ* genes and affect the capacity of soil to act as a sink for N_2O [83]. Atypical *nosZ* genes were found to be most prominent in soil samples taken directly from the field, whereas incubation experiments following nitrate and glucose addition resulted in a bottleneck effect and selected for typical *nosZ* carrying bacteria [84]. Estimation of the potential for N_2O reduction by non-denitrifiers should aid in modeling efforts and better projections of N_2O fluxes from agricultural management systems.

4.3. Fungal uptake of NO_3-N

Molecular analyses of the soil N cycle have focused mostly on bacterial genes involved in dinitrogen fixation (*nif*), nitrification (*amo*) and the more downstream steps of nitrate respiration or dissimilation (*nir*, *nor*, *nos*) [85]. Since these genes are found in fewer, more specialized taxa, they have been considered more ecologically informative than the more widely distributed *nar* and *nap* genes for dissimilatory nitrate reduction [86]. Even more universal among bacteria are genes for assimilatory nitrate reduction (*nas*), which have been virtually ignored as a functional N cycle indicator. On the other hand, nitrate assimilation genes (*euknr*) in different fungal taxa might have potential as indicators of fungal N cycling activity in response to agricultural management.

The involvement of fungi in the N cycle of agricultural soils, although acknowledged, is not well-studied, and the distinctive functionalities of fungal saprotrophs and biotrophs add to the research challenge. Since fungal biomass is often (but not always) higher in no-till than in intensively tilled soils [87,88], fungal contributions to N cycling are expected to become more important after soil disturbance ceases, due to less breakage of hyphal networks and less damage to mycorrhizal spores [89,90]. Fungi would be expected to play greater roles in N cycling in surface residues and upper layers of no-till soils (0–5 cm), where larger increases in fungal biomass have been reported relative to deeper soils [90,91]. Abundance and/or expression of fungal *euknr* could be an important indicator of fungal activity and nitrate uptake in less-disturbed soil strata.

Most fungi are capable of nitrate assimilation [92]. Fungi gained the ability to assimilate NO_3^- following horizontal gene transfer of the *euknr* gene from oomycetes. Through the study of *Aspergillus nidulans* and *Neurospora crassa*, the enzymes and/or co-factors involved in nitrate assimilation and its regulation have been well characterized in ascomycetes [93]. Less is known about nitrate assimilation in basidiomycetes, although the enzyme for nitrate reductase, EukNR, is the same in each phylum [80].

Over the last 10 years several groups have created primer sets to specifically detect and amplify fungal nitrate reductases from soil communities. Nygren et al. [94] created primers to amplify nitrate reductases from Basidiomycetes, while Gorfer et al. [95] focused on nitrate reductase from ascomycetes, which are often the most abundant fungal taxa in agricultural soils. Neither of the primer sets were able to amplify nitrate reductases from all nitrate reductase-carrying ascomycetes or basidiomycetes. Primer sets designed by Gorfer et al. [95] showed a bias towards Pezizomycotina, while primers constructed by Nygren et al. [94] excluded species within the Russulacea family and *Amanita* genus. Still, these primer sets were able to amplify nitrate reductase sequences from either forest or agricultural soils. Despite the bias associated with fungal nitrate reductase primers, nitrate reductase sequence classifications and quantifications from agricultural soil were similar to fungal abundance and community structure based on ribosomal intergenic transcribed spacer sequencing [96].

Nitrate reductase fungal activity can be measured using several techniques; qPCR with appropriate primer sets or stable isotope probing and have been used to identify parameters that influence nitrate assimilation. Primers designed by Gorfer et al. [95] were used to quantify fungal expression of the nitrate reductase gene in microcosms using agricultural soil. Expression levels of *euknr* were only measurable after C addition, demonstrating the sensitivity of nitrate reductase activity in fungi towards C availability. Regulation of nitrate assimilation by C availability is most likely due to the energy intensive reduction processes required in NO_3^- assimilation [97,98,99]. Identifying management practices that promote fungal growth and activity, therefore, could enhance NO_3^- assimilation, with widespread implications for water and air quality, as less NO_3^- would be lost through leaching or reduced to N_2O by denitrifiers.

5. Facilitating Diverse Metabolic Processes Through Soil Management

Determining conditions that facilitate one or more of the aforementioned microbial processes in situ can be challenging due to the complexity and diversity of microbes, substrates, and soil properties. A recent study conducted by Raynaud and Nunan [100] in agricultural soils indicated that the potential of microbes to interact with each other was positively correlated with increased bacterial density. Agricultural management practices like minimizing tillage, integrating cover crops and diverse crop rotations along with animal amendments that help achieve "low-disturbance-higher-C" soils would promote higher bacterial density/activity, thereby stimulating metabolic reactions that could capitalize on wastes from other metabolic reactions (Figure 3).

At any given time, only 1–5% of bacteria in soil are thought to be actively metabolizing [25,27]. Management strategies employing mixed cover species and crop rotations, which in turn increase microbial activity and metabolic diversity, will increase root densities and lengthen periods of live root activity. The C added to soil through various root exudates, rhizo-deposits, crop residues or animal amendments stimulates microbial activity, thereby increasing concentrations of reactants for CO_2 assimilators, H_2 oxidizers and populations that carry out DNRA. Higher DNRA rates, for

example, were measured after addition of either rice straw or Chinese milk vetch residues to soils incubated under greenhouse conditions as compared to control soils without crop residues [101]. They also found DNRA rates to be positively correlated with the concentration of dissolved organic C during the incubation study. Addition of alfalfa residues yielded higher DNRA activity as compared to straw residues in another soil incubation experiment [102]. Labile C availability can also increase CO_2 assimilation into organic matter by microbes [103]. It therefore follows that microbial growth and mineralization would be favored in the presence of higher CO_2 concentrations in low-disturbance-higher C soil. Another example of a C amendment that can affect N transformations is biochar. In one study, adding biochar to soils resulted in changes in atypical *nosZ* transcripts and lower N_2O emissions compared to unamended soils [77,104].

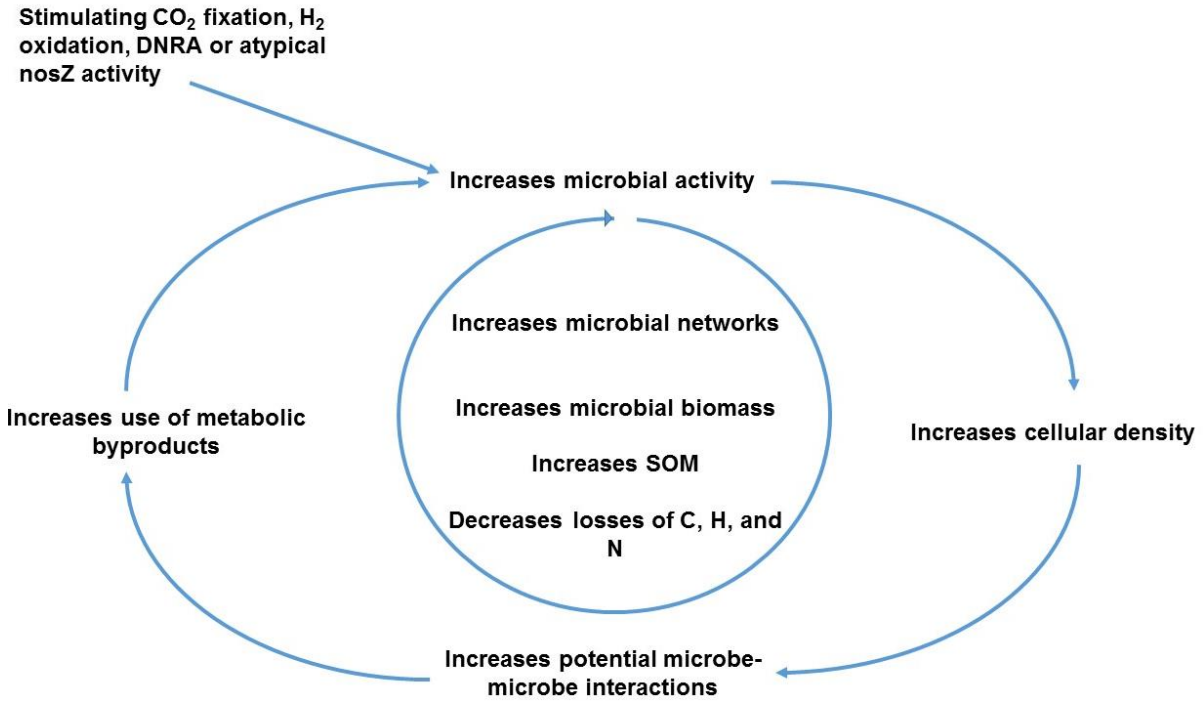

Figure 3. Microbial feedback responses resulting from conditions that stimulate underexplored diverse microbial metabolisms in agricultural soil.

In addition to C availability, NH_4^+ has been thought to regulate NO_3^- assimilation by fungi. However, Inselsbacher et al. [105], reported that fungal NO_3^- assimilation was unaffected by high concentrations of NH_4^+ in agricultural soil mesocosms assayed with stable isotopes. This led to their proposal that fungi would assimilate NO_3^- faster than bacteria [105]. Increasing fungal growth and activity, especially glomalin production [106], also helps build soil structure, which in turn regulates gaseous exchange and oxidation-reduction potentials of microsites. Moreover, when soil disturbance is reduced, and fungi are allowed to establish viable networks, higher labile C supplies could stimulate fungal uptake of nitrate. Besides direct assimilation, another fate for NO_3^- in fungal biomass is cytoplasmic translocation, since cytoplasm inside hyphal networks can move bidirectionally (i.e., to and from patches of high nutrient concentrations and to and from roots). Promoting viable fungi in soils therefore allows nitrates to be translocated in soil within "contained

networks", which would serve to enhance N retention and reduce leaching. Endophytic fungi could play an especially important role in improving N use efficiency by delivering NO_3^- to plant roots.

The addition of animal wastes enriches soils with more anaerobic and microaerophilic organisms that are favored by lower redox conditions. Wastes from the gastrointestinal tracts of mammals contain enteric bacteria like *E. coli* that are capable of conducting DNRA [107]. It is therefore expected that soils receiving repeated manure additions would be enriched in DNRA bacteria. It is also known that at high soil pH, nitrite accumulation facilitates DNRA [108]. Agronomic practices which combine liming with manure additions can raise soil pH > 6.5 which could favor DNRA [109] and promote NO_2^- accumulation [110]. However, more research needs to be conducted to assess the diversity and activity of DNRA organisms in manure amended soils.

Reduced-tillage soils managed with manures are expected to harbor more microaerophilic and anaerobic populations that could exploit syntrophic relationships facilitated by lower redox potentials. Restoring biophysical integrity and microsite heterogeneity through reduced soil disturbance should permit maintenance of higher CO_2 and H_2 concentrations over longer periods of time, thereby expanding the soil energy supply overall. Soil aggregation along with root architecture also controls soil bulk density as well as macropore connectivity and tortuosity. Identifying plant traits that provide favorable rhizosphere habitats could be important in designing crop management strategies to promote these microbial interactions. A study by Hansel et al. [111] demonstrated how anaerobic microenvironments within soil aggregates provide conditions that are conducive to both anaerobic- and aerobic-based metabolisms. Co-occurrence of aerobic and anaerobic microbial habitats due to microscale heterogeneity [111] could therefore promote the understudied microbial metabolisms highlighted in this paper.

6. Conclusions

In this review we have attempted to relate management practices intended to mimic native soil conditions with insights from the literature on CO_2 assimilation, H_2 oxidation, alternative N transformation pathways, and enhanced fungal involvement in N cycling. We have the properties of undisturbed, native soils as management targets for plant-soil systems that could result in less nutrient loss. At the same time, we acknowledge that native soils are not capable of delivering needed amounts of nutrients on a sustained basis for agricultural production as we know it. In order for agricultural systems to be assisted by soil microbial processes, appropriate plant choices, organic amendments, and soil management practices will be needed to establish soil conditions that permit sustained activity of diverse microbial metabolisms. We also acknowledge that soils which have undergone organic matter depletion for long periods will not return quickly to the conditions once extant in native soils. Nevertheless, less disturbed soils are expected to become more spatially and temporally heterogeneous eventually over time.

One of the approaches to assess the importance of specific microbial metabolisms in increasing nutrient cycling efficiency will be to couple next generation sequencing and metabolomics with biogeochemical process measurements applied to soils with well-characterized management histories and field records. Such studies will help to identify taxa that play critical roles in improving nutrient cycling function and relate these agricultural management practices. Deeper understanding of potential soil microbial metabolisms is needed so that agricultural soils can be managed in a more ecologically and environmentally sustainable manner. Gaining insights into conditions that lead to

ecologically beneficial microbial metabolisms will help to align agricultural management practices with efficient nutrient cycling and lower environmental impact.

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Conflict of Interest

All authors declare no conflict of interest in this paper.

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