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 (CO₂) and hydrogen gas (H₂) 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 CO₂; utilization of H₂ 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 CO₂ consumption, H₂ 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$_2$O 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 CO₂ to the atmosphere [17].

Microbial assimilation of CO₂ can therefore result from heterotrophic as well as autotrophic metabolisms in soils. In mesocosm experiments using agricultural soils, CO₂ 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 CO₂ assimilation [19]. During studies of CO₂ uptake by other heterotrophic bacteria, Roslev and coauthors showed strong correlations between growth and CO₂ assimilation, with CO₂-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 CO₂-C into multiple cellular compounds [20,21].

In addition to generalized anaplerotic uptake, CO₂ 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 CO₂ 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, CO₂ assimilation can be stimulated by increased H₂ availability. A recent study by Jones et al. [24] observed CUE close to 100% when *Clostridium spp.* were grown as mixotrophs using H₂ as a reducing agent during fermentation. Likewise, CUE increased for other known acetogens like *Eubacterium limosum* and *Moorella thermoacetica* during fermentation with H₂ additions [24].

Heterotrophic assimilation of extracellular CO₂ could therefore increase CUE of soil microbial communities, speed accretion of microbial biomass and organic matter, and decrease greenhouse CO₂ 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 CO₂ 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 H₂**

Dihydrogen gas, H₂, 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 H₂ oxidation with O₂ reduction, H₂ 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 H₂ in soils could provide enough energy to maintain the viability of 10⁷ starved bacteria [27]. Analogous to substantial losses of soil CO₂ observed after tillage, H₂ losses also would be expected to occur as a result of physical disturbance. Although comparisons of H₂ partial pressures in tilled and undisturbed soil have yet to be reported in the literature, it is reasonable to propose that H₂ availability to resident microorganisms is higher and more consistent when soils remain undisturbed.

Many soils act as net sinks for H₂, and it has been estimated that approximately 88 ± 11 Tg ha⁻¹ of atmospheric H₂ is taken up by soils each year [28]. Uptake of atmospheric H₂ occurs at the soil-air interface, with H₂ having a tropospheric half-life of ca. 1.4 yr [28] at an estimated concentration of 530 ppb [29]. Consumption of H₂ is also a common metabolic reaction in rhizospheres of legumes,
where symbiotic rhizobia produce H2 as a byproduct of N2 fixation [29,30]. Concentrations of H2 at soil-nodule interfaces can be up to 20,000 times higher in the rhizosphere compared to the troposphere. Steep gradients in H2 concentrations occur with distance from nodules (decrease to sub-atmospheric levels within 4.5 cm from nodule), and measurable H2 consumption rates in soil have been observed [31,32,33].

Bacteria that use extracellular H2 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 H2, 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 H2 [27]. In contrast, high-affinity hydrogenases can be either membrane bound or referred to as “abiotic hydrogenases” (exoenzymes), which can oxidize H2 at low concentrations [27]. Thus, it is hypothesized that H2 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 H2-oxidizing bacteria.

Oxygen serves as an important electron acceptor for H2 oxidation. In pure culture studies, Actinobacteria spp. were unable to oxidize H2 under anoxic conditions whereas H2 oxidation was stimulated as oxygen availability increased [27]. Other energy-yielding H2 oxidation reactions also could occur along gradients and interfaces, specifically the “Knallgas” reaction, where electrons are transferred between H2 and O2 to form H2O in oxic/suboxic zones [27]. Thus, H2 utilization expands energy supplies when reduced C sources become limiting. Other microorganisms under fermentative conditions can metabolize H2 and CO2 simultaneously [22], such as the acetogen Acetobacterium woodii [37]. Clostridium thermoaceticum can also grow with either H2 or CO2, but the growth of C. thermoaceticum was highest when both H2 and CO2 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 H2 and organic compounds simultaneously. If H2 is the sole electron donor, M. smegmatis growth is impeded [27]. Syntrophy can rely on transfers of H2 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 H2, was increased in the presence of bacteria that oxidize H2, 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 H2 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 H2 oxidation. Thus, it has been hypothesized that microbes will supplement their energy requirements by oxidizing H2 during periods of dormancy. Gene knockout studies have demonstrated the importance of H2 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 H2 during those periods. Still, this information indicates that H2-oxidizing metabolic reactions are important in helping maintain microbial activity and diversity in soils [27]. In undisturbed soils, H2 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 H₂ oxidation in soils has focused on the rhizosphere, where steep concentration gradients exist due to H₂ release during N₂ fixation by rhizobia [33]. It is likely that H₂ oxidation occurs in other soil microhabitats, as evidenced by H₂ uptake measurements [40,41,42], but much more needs to be learned about how soil management affects microbial use of H₂ as an energy source for enhanced soil biological function. A potential concern in agroecosystems is the use of H₂ by methanogens to reduce CO₂ to methane (CH₄). Supplementing agricultural soils with cow manure, for example, results in increased methanogen abundance and CH₄ production [43]. In fact, manure addition can change the relationship between CH₄ consumption and production in soils so that more CH₄ is produced than consumed [43]. On the other hand, increased methanogenesis could be counterbalanced by more methanotrophic consumption of CH₄ in rhizosphere soils. Therefore, development of soil microenvironments to foster bacterial consumption of CH₄, CO₂, and H₂ 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 (NO₃⁻-N) make it the most pivotal reactive N species in soil. Since NO₃⁻ 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, NO₃⁻ 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 N₂ gas contribute to inefficient N use, they do not contribute directly to the greenhouse effect. Soil N losses as N₂O, however, are environmentally more problematic, since N₂O is 300 times more potent than CO₂ 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 N₂O emissions, with fertilized soils and livestock wastes contributing about 60% and 30% of that total [4]. Lowering net N₂O emissions is therefore crucial for mitigating agriculture’s impact on global warming.

Heterotrophic (classic) denitrification is considered to be the main process responsible for N₂O losses from most agricultural soils. Denitrification occurs when soils become wet, causing denitrifiers to switch from using O₂ as an electron acceptor to NO₃⁻-N for anaerobic respiration. The classic denitrification sequence consists of stepwise N reductions from NO₃⁻ to NO₂⁻ to NO to N₂O.
to N₂ (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 NO₃⁻ to N₂ via N₂O as an intermediate [45]. Thus, the main end products from denitrification (N₂ or N₂O) will depend not only on soil O₂ 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 NO₃⁻ reduction can occur. Some bacteria reduce NO₃⁻ to NH₄⁺ (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 N₂O to N₂ by non-denitrifiers is another process that could help lower net N₂O emissions (Figure 2). Recent studies have described novel N₂O-reducing enzymes present in a wide diversity of organisms which lack the enzymes to produce N₂O [46]. Both DNRA and non-denitrifier N₂O reduction represent N dissimilation pathways that could serve as N₂O 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 NO₃⁻-N to NO₂⁻-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 NO₂⁻-N to NH₄⁺-N by cytochrome c nitrite reductase by formate (Nrf). From a thermodynamic

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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 \( \text{NO}_2^- \) to \( \text{NH}_4^+ \) 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 \( \text{NO}_2^- \) [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 \( \text{Ca}^{2+} \) 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 \( \text{NO}_3^- \) [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 \( \text{O}_2 \), was the main factor regulating DNRA in soils and that the effect of variable \( \text{O}_2 \) concentrations depended on the ratios of available C to \( \text{NO}_3^- \).
Different C sources influence N dissimilation processes and their transient intermediates and end products. Although N$_2$O is a known byproduct of DNRA activity [67], classic denitrification and nitrifier denitrification are still considered to be the major N$_2$O-producing processes in oxic and suboxic soils, respectively [68,69]. Thus, soil conditions favoring DNRA over denitrification are expected to have lower net N$_2$O 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$_2$O emissions from soils could be lowered is by promoting activity of non-denitrifier populations that reduce N$_2$O to N$_2$ [76]. These populations employ N$_2$O 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$_2$O 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$_2$O consumption rates during denitrification in an ecosystem, indicating the existence of one or more unaccounted sinks for N$_2$O. More recently, two distinct groups of Nos proteins were identified based on expanded sequence databases for nosZ genes [46].

Most classic or complete denitrifiers belonging to alpha-, beta-, and gammaproteobacteria possess nosZ genes which group within Clade I, whereas other taxa possess atypical nosZ 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$_2$O-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 nosZ 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 archaеal 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$_2$O emissions would be aided by the activity of N$_2$O-reducers like...
Anaeromyxobacter spp., which convert N₂O to N₂, thereby counteracting classic denitrification in the presence of high NO₃⁻ concentrations [46]. Promoting populations of non-denitrifier N₂O reducers could be an effective tool to address the potential for N₂O production by fungi, which are more likely to flourish in no-till than in tilled soils. Although NO₃-N reduction as an electron-accepting process is less common among fungi than it is among bacteria, the major fungal product is N₂O rather than N₂ [79]. Members of the Ascomycota, Basidiomycota and Mucoromycota are capable of using NO₃⁻ 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 N₂O from NO₃⁻, and this capability was most frequently observed in members of the order Hypocreales. Potential production of N₂O by fungi in no-till soils thus could be offset by enhanced activity of non-denitrifier N₂O reducers.

In an experiment conducted by Orellana et al. [81], atypical nosZ gene-carrying N₂O 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 N₂O to N₂. 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 N₂O [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 N₂O reduction by non-denitrifiers should aid in modeling efforts and better projections of N₂O fluxes from agricultural management systems.

4.3. Fungal uptake of NO₃-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$_2$O 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 CO₂ assimilation into organic matter by microbes [103]. It therefore follows that microbial growth and mineralization would be favored in the presence of higher CO₂ 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 N₂O emissions compared to unamended soils [77,104].

![Microbial feedback responses](image)

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

In addition to C availability, NH₄⁺ has been thought to regulate NO₃⁻ assimilation by fungi. However, Inselsbacher et al. [105], reported that fungal NO₃⁻ assimilation was unaffected by high concentrations of NH₄⁺ in agricultural soil mesocosms assayed with stable isotopes. This led to their proposal that fungi would assimilate NO₃⁻ 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 NO₃⁻ 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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