

Nitrogen: the double-edged sword

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Nitrogen is a component of protein and DNA and as such, is essential to all living things. Prior to the Industrial Revolution, around 97% of the nitrogen supporting life on earth was fixed biologically. Over the last century, intensification of farming, coupled with a lack of understanding of soil microbial communities, has resulted in reduced biological activity and an increased application of industrially produced forms of nitrogen to agricultural land.

In 2013, Australian grain-growers expended close to \$3 billion on inorganic nitrogen (Marino 2014). Between 10 and 40% of the applied N was taken up by the crop. The other 60-90% was leached into water, volatilised into the air or immobilised in soil.

Impacts of inorganic nitrogen

The application of high rates of inorganic nitrogen in agricultural systems has had many unintended negative consequences for soil function and environmental health. Data from North America's longest running field experiment on the impacts of farm production methods on soil quality have revealed that high nitrogen inputs deplete soil carbon, impair soil water-holding capacity - and ironically, also deplete soil N (Khan *et al.* 2007, Larson 2007).

Taken together, these factors have been implicated as the underlying cause of widespread reports of yield stagnation around the world (Mulvaney *et al.* 2009).

The evidence suggests that although nitrogen is essential to plant growth, the application of large amounts of N in an inorganic form is detrimental to soil.

Fortunately, the news is not all bad. Rates of fertiliser application have decreased in recent years in some developed countries. France, Germany, and the United Kingdom have achieved success in this area, maintaining high yields with forty to fifty percent less fertiliser than used in the 1980s (Krietsch 2014).

Cost-effective nitrogen management is the key to profitable and productive farming. It is also the key to building soil carbon. Stable forms of soil carbon (such as humus) cannot form in the presence of high levels of inorganic nitrogen, due to the inhibition of the microbes essential to sequestration.

Biological nitrogen fixation (BNF)

On a global scale, biological nitrogen fixation accounts for around 65% of the nitrogen used by crops. There is scope for considerable increase. The supply of nitrogen is inexhaustible, as dinitrogen (N₂) comprises almost 80% of the earth's atmosphere. The key is to transform inert nitrogen gas to a biologically active form.

Much of the nitrogen currently used in agriculture derives from the Haber-Bosch process, developed in the early 1900s. This process catalytically combines atmospheric nitrogen with hydrogen derived from natural gas to produce ammonia under conditions of high temperature and pressure. The Haber-Bosch process uses non-renewable resources, is energy intensive and expensive.

Fortunately - thanks to some 'enzymatic magic' - atmospheric nitrogen can be transformed to ammonia by a wide variety of nitrogen-fixing bacteria and archaea - for free.

Ideally, newly fixed ammonia will be rapidly incorporated into organic molecules such as amino acids and humus. These stable molecules are vital to soil fertility and cannot be volatilised or leached from the soil system. Importantly, the stabilisation of nitrogen requires a steady supply of carbon - also fixed biologically. We'll come to that in a moment.

Which microbes are involved?

It is important to recognise that the ability to fix nitrogen is not limited to bacteria associated with legumes. If this was the case, how would non-legume communities thrive?

Unlike rhizobial bacteria, most nitrogen-fixing microbes are not able to be cultured in the laboratory. This has presented technical challenges to assessing their ecological function. Bio-molecular methods for determining the presence of *nifH*, the gene for nitrogenase reductase, has revealed a dizzying array of free-living and associative nitrogen-fixing bacteria and archaea across a wide range of environments.

Although procedures for quantifying the amount of nitrogen fixed by many of these groups are lacking, what we do know is that the diversity and abundance of nitrogen fixing microbes are much greater where there is living groundcover (particularly plants in the grass family) throughout the year, compared to soils that have been bare fallowed.

In addition to nitrogen-fixing bacteria and archaea, mycorrhizal fungi are vitally important to the N-fixing process. Although mycorrhizal fungi do not fix nitrogen, they transfer energy, in the form of liquid carbon (Jones 2008) to associative nitrogen fixers. They also transport biologically fixed nitrogen to plants in organic form, for example, as amino acids, including glycine, arginine, chitosan and glutamine (Leake *et al.* 2004, Whiteside *et al.* 2009).

The acquisition and transfer of organic nitrogen by mycorrhizal fungi is highly energy efficient. It closes the nitrogen loop, reducing nitrification, denitrification, volatilisation and leaching. Additionally, the storage of nitrogen in the organic form prevents soil acidification.

The liquid carbon pathway

Despite its abundance in the atmosphere, nitrogen is frequently the most limiting element for plants. There is a reason for this. Carbon, essential to photosynthesis and soil function, occurs as a trace gas, carbon dioxide, currently comprising 0.04% of the atmosphere. The most efficient way to transform CO₂ to stable organic soil complexes (containing both C and

N) is via the liquid carbon pathway. The requirement for biologically-fixed nitrogen drives this process.

If plants were able to access nitrogen directly from the atmosphere, their growth would be impeded by the absence of carbon-rich topsoil. We are witnessing an analogous situation in agriculture today. When inorganic nitrogen is provided, the supply of carbon to associative nitrogen fixing microbes is inhibited, resulting in carbon-depleted soils.

Reduced carbon flows impact a vast network of microbial communities, restricting the availability of essential minerals, trace elements, vitamins and hormones required for plant tolerance to environmental stresses such as frost and drought and resistance to insects and disease. Lowered micronutrient densities in plants also translate to reduced nutritional value of food.

Above ground, plant growth often appears 'normal', hence the connection to failing soil function may not be immediately obvious. But underneath, our soils are being destroyed.

Ideally, amendments used in agriculture should enhance photosynthetic rate and increase the flow of carbon to soil, by supporting plant-associated microbial communities (Fig.1).

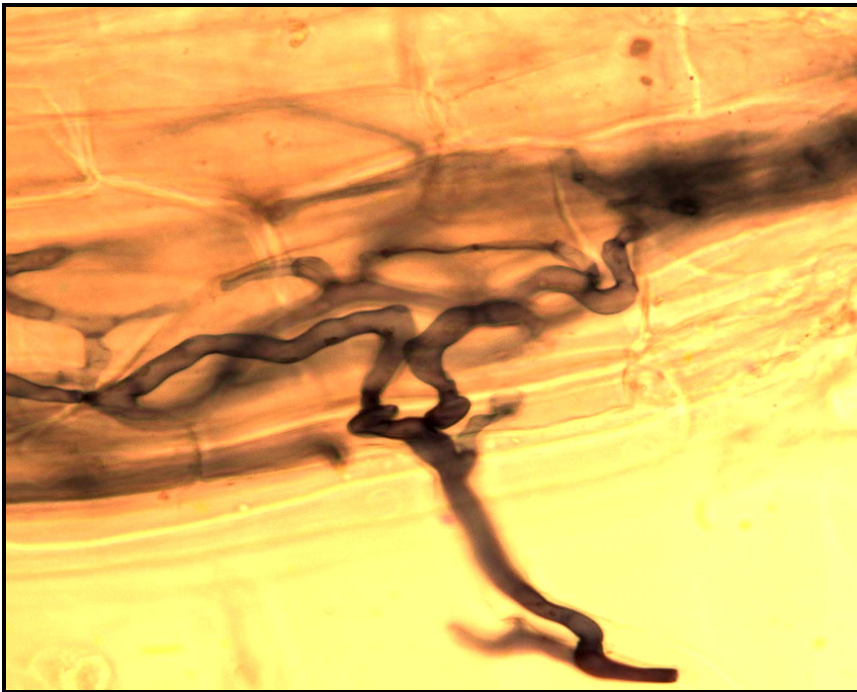


Fig.1. Cross section of a plant root showing the thread-like hyphae of mycorrhizal fungi. Mycorrhiza deliver sunlight energy packaged as liquid carbon to a vast array of soil microbes involved in plant nutrition and disease suppression. Organic nitrogen, phosphorus, sulphur, potassium, calcium, zinc and essential trace elements such as copper are returned to plant hosts in exchange for the carbon received. Nutrient transfers are inhibited when high rates of inorganic nitrogen and/or inorganic phosphorus are applied. Photo Jill Clapperton.

Determining brix levels with a refractometer is an easy way to assess the rate at which green leaves are photosynthesising and hence supporting associative soil microbes. Anything that reduces the photosynthetic capacity of land or the photosynthetic rate of vegetation is NOT sustainable.

How can we utilise our understanding of the liquid carbon pathway to restore natural fertility to agricultural land?

Aggregation is the key

Aggregates are the small 'lumps' in soil that provide tilth, porosity and water-holding capacity. Unless soils are actively aggregating, they will not be fixing significant amounts of atmospheric N or sequestering stable forms of carbon. All three functions (aggregation, biological N-fixation and stable C-sequestration) are inter-dependent.

The microbes involved in the formation of soil aggregates require an energy source. This energy initially comes from the sun. In the miracle of photosynthesis, green plants transform light energy, water and carbon dioxide into biochemical energy, which is transferred to soil as liquid carbon via an intricate network of mycorrhizal fungi and associated bacteria.

What do soil aggregates look like?



Fig.2. The two wheat plants on the left were grown with perennial grasses in a Pasture Crop treatment while the wheat plant on the right was grown in adjacent bare soil, amended with 100kg/ha DAP.

Note the little lumps adhering to the roots of the Pasture Cropped wheat (Fig.2). These clusters are formed by microbes utilising liquid carbon from the roots. Microaggregates, too

small to be seen with the naked eye, are bound together by microbial glues and gums and the hyphae of mycorrhizal fungi (also using liquid carbon), to form bigger lumps called macroaggregates, generally 2-5mm in size (around 1/8th of an inch in non-metric terms).

Macroaggregates are essential to soil tilth, structure, aeration, infiltration, water-holding capacity, biological nitrogen fixation and carbon sequestration. In short, it is not possible to maintain healthy soils without them.

Let's take a look inside a macroaggregate, courtesy of this fabulous illustration (Fig. 3) by Rudy Garcia, State Agronomist with the USDA Natural Resources Conservation Service in New Mexico.

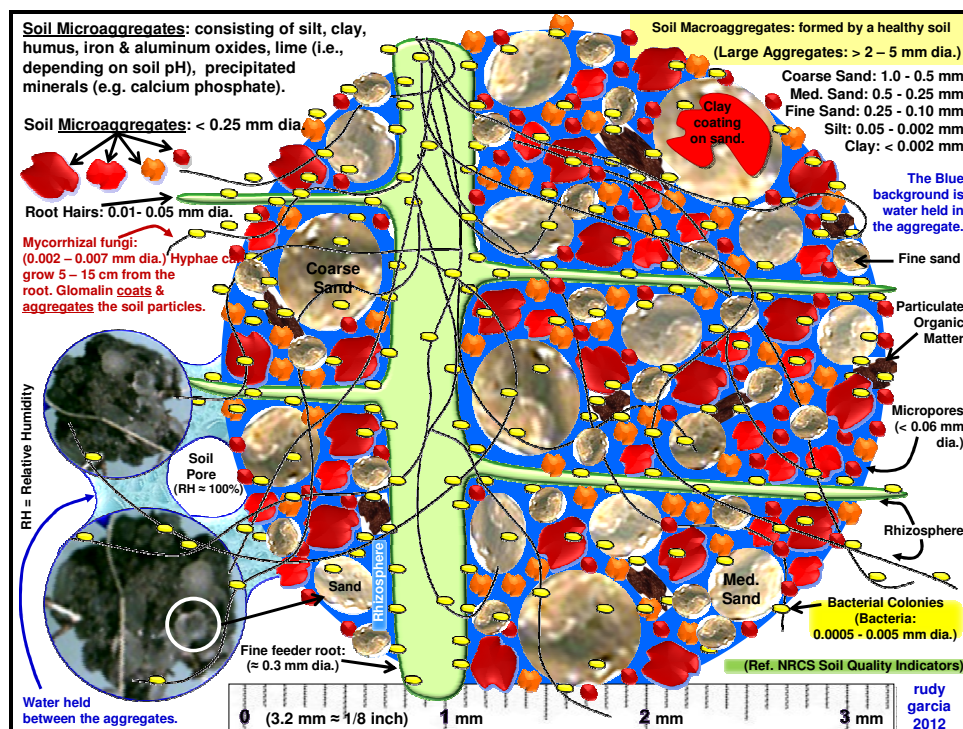


Fig.3. Diagrammatic representation of a soil macroaggregate. The green vertical line is a fine feeder root and the green horizontal lines are root hairs. The assortment of red and orange particles are microaggregates while the scattered brown shapes represent particulate organic matter. Light coloured spheres are sand grains of various sizes, often coated with oxides of iron and aluminium, while the small yellow ellipses are bacterial colonies, including nitrogen-fixing and phosphorus solubilising species. The fine strands running in all directions are the hyphae of mycorrhizal fungi, essential to the enmeshing of the soil particles and the supply of carbon to microbial communities within the aggregate. Depending on soil pH, there will also be precipitated minerals such as iron phosphate or calcium phosphate. The blue background is water held within the aggregate. Illustration courtesy Rudy Garcia, USDA-NRCS

A key feature is that moisture and liquid carbon levels are *higher* within root-supported aggregates than in the surrounding soil, while the partial pressure of oxygen is *lower* within root-supported aggregates than in the surrounding soil. These conditions are essential for the functioning of the nitrogenase enzyme utilised for biological nitrogen fixation and also to the formation of humus.

Within root-supported aggregates, liquid carbon is transferred from fine root hairs to the hyphae of mycorrhizal fungi, thence to highly complex microbial communities. The microbes receiving this carbon - and their metabolites - are instrumental in the transformation of simple sugars to highly stable humic polymers, a portion of which comprises biologically fixed nitrogen and bacterially-solubilised phosphorus. Iron and aluminium, which occur as oxides in the mineral matrix, are important catalysts.

It is now recognised that plant root exudates make a greater contribution to stable forms of soil carbon (that is, to organo-mineral complexes containing organic carbon and organic nitrogen) than does the above-ground biomass (Schmidt *et al.* 2011)

But here's the rub. Mycorrhizal colonisation is low when large quantities of inorganic N are applied ... and mycorrhiza are inactive when plants are absent. Hence biological nitrogen fixation and humification are rare in agricultural systems where heavily N-fertilised crops are rotated with bare fallows. Further, it has been shown that up to 80kgN/ha can be volatilised from bare summer fallows due to denitrification. If green plants are present, this N can be taken up and recycled, preventing irretrievable loss.

When soil is bare there is no photosynthesis and very little biological activity. Bare soils lose carbon and nitrogen, nutrient cycles become dysfunctional, aggregates deteriorate, structure declines and water-holding capacity is reduced. Bare fallows, designed to store moisture and retain nutrients, become self-defeating.

The maintenance of bare fallows - or the use of high rates of inorganic N - or worse, both together - results in the uncoupling of the nitrogen and carbon cycles that have functioned synergistically for millennia. Photosynthesis is the most important process underpinning life on earth. Non-legume biological nitrogen fixation is the second.

Enhancing the liquid carbon pathway

There is increasing recognition of the fundamental importance of soil microbial communities to plant productivity. Unfortunately, many biological functions are compromised by commonly used agricultural practices.

Redesign of farming practice is not difficult. The first step is recognition of the importance of the year-round presence of green plants and the microbial populations they support.

Redesign has the potential to significantly reduce the impact of many 'problems' associated with chemical farming, including loss of soil C, reduced soil N, soil compaction, declining pH, low nutrient availability, herbicide resistance and impaired water-holding capacity.

There are four basic principles for regenerative agriculture, proven to restore soil health and increase levels of organic carbon and nitrogen. From these, landholders can build an integrated land management package that suits their individual property and paddock needs.

1) The **first principle** is the maintenance of year-round living cover, via perennial pastures on grazed land and/or multi-species cover crops on farmed land. Almost every living thing in and on the soil depends on green plants (or what was once a green plant) for its existence. *The more green plants, the more life.*

It's well accepted that groundcover buffers soil temperatures and reduces erosion, but it is perhaps less recognised that actively growing green groundcover also fuels the liquid carbon pathway which in turn supports, among other things, mycorrhizal fungi, associative N-fixing bacteria and phosphorus solubilising bacteria - all of which are essential to both crop nutrition and the formation of stable humified carbon.

2) The **second principle** is to provide support for the microbial bridge, to enhance the flow of carbon from plants to soil. This requires reducing inputs of high analysis N & P fertilisers that suppress root formation and the complex biochemical signalling between plants and microbes.

3) The **third principle** is to promote plant and microbial diversity. The greater the diversity of plants the more checks and balances for pests and diseases and the broader the range of microhabitats for the soil organisms involved in nutrient acquisition, nutrient cycling and soil building.

4) The **fourth principle** is that land responds positively to the presence of animals provided management is appropriate. As well as the benefits arising from the addition of manure and urine to soils, rest-rotation grazing increases root exudation and stimulates the number and activity of associative N-fixing bacteria in the rhizosphere, which fire up in response to defoliation and provide the extra N required by the plant for the production of new growth.

Weaning off nitrogen fertiliser

The activities of both symbiotic and associative N-fixing bacteria are inhibited by high levels of inorganic N. In other words, the more nitrogen fertiliser we apply, the less N is fixed by natural processes.

Hence it is important to wean your soils off inorganic N - but please do it S.L.O.W.L.Y.

Microbial communities take time to adjust. Soil function cannot return overnight. The transition generally requires around three years.

Nitrogen inputs can be reduced to 80% (of previous rates) in the first year, 50% in the second year and 20% in the third year. In fourth and subsequent years, the application of a very small amount of N (around 1kg/ha) will help to prime natural nitrogen-fixing processes.

While weaning off high rates of inorganic N you should also aim to maintain as much diverse year-round living groundcover in crops and pastures as possible.

Conclusion

Biological nitrogen fixation is the key driver of the nitrogen and carbon cycles in all natural ecosystems, both on land and in water. When managed appropriately, biological nitrogen fixation can also be the major determinant of the productivity of agricultural land.

Farmers around the world - including Western Australia - are discovering first-hand how a change to year-long green, accompanied by reduced applications of inorganic nitrogen, can restore natural topsoil fertility. Improving soil function delivers benefits both on-farm and to the wider environment.

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