

Relationships between microbes, legumes, and chemicals demand astute agricultural practices

By Prof Charlie Reinhardt

Over the ages, virtually all microbial species have had the opportunity to be distributed worldwide in surface soils. However, regional (large scale) and site (small scale) differences in type and population size of micro-organisms show that they respond to specific environmental control factors and substrates.

The composition of soil microbial communities, which can consist of thousands of species per gram of soil, is the result of specific differences in their environment. Soil microbial species respond differently to factors such as crop rotation, soil tillage, type of crop, nutrition of the host crop, temperature, soil moisture content, soil pH, and substrate concentration (NH_4^+ [ammonium ion] concentration in the case of the nitrifier bacteria responsible for conversion of NH_4^+ to NO_3^- [nitrate]).

The rhizosphere

A multitude of phenomena occur at or near the root-soil interface, and the sum total of their effects has a great impact on both plants and soil biota. Hiltner, in 1904, coined the term 'rhizosphere' for the area of bacterial growth around the roots of legume plants. Later the rhizosphere was generally considered to be that zone of the soil in which plant roots occur and in which there is proliferation of micro-organisms due to the influence of plant roots.

Organic substances found in, on or in the vicinity of roots reveal a vast array of chemical compounds varying from simple molecules to complex, high molecular mass substances. Included among these

compounds are microbial attractants and repellents, which are species (plant)-specific. Generally, plants contribute 2 to 16% of their photosynthate (carbohydrates formed during the photosynthesis process) to the soil carbon (C) and microbial biomass – for example, soya bean reportedly contributes 2% of net photosynthate production.

The majority of soil-living organisms are 'organotrophs' that obtain their C input from plant roots and organic residues supplied by living and dead plants; this and other essential elements for growth are obtained from the host in the case of a symbiotic relationship between microbe and living plant host, or are acquired by free-living (asymbiotic) microbes.

In addition to supplying soil microbes with C for growth, plant roots have numerous effects on soil biota – by far the largest effect is the lowering of soil moisture content due to water uptake, which in turn reduces leaching of nitrate (a positive effect).

Another important role of the plant root is mineralisation of soil organic matter (SOM) – moisture removal would decrease SOM mineralisation under dry conditions. Natural grassland with high root biomass is associated with humus-rich, or humic, soils with the highest SOM.

The humic soil group is typified by marked accumulation of humus, high degree of weathering, well-drained landscapes of high rainfall and cool temperatures, and dense grassland or forest cover. Such soils are easy to work, drain well despite high clay content, and possess a useful organic reserve of mineralisable nutrients.

Biological nitrogen fixation

Nitrogen (N) exists in solid, dissolved and gaseous forms. In rocks, i.e. the

lithosphere, N occurs primarily as the ammonium ion (NH_4^+); the gas N_2 makes up approximately 80% of the earth's atmosphere; and the hydrosphere pool is represented by N_2 dissolved in water.

The content of N_2 in both the atmosphere and hydrosphere is around 10^6 (one million) times greater than in the biosphere (total complement of organisms). Therefore, from an evolutionary point of view, it makes sense that certain organisms have evolved specialised mechanisms for harnessing or harvesting the abundant supply of N in their surroundings. Even more telling is that organisms at the lower end of the food chain, i.e. micro-organisms and plants, has this ability.

Nitrogen fixed by microbes has the advantage over mineralised N in that the former process is less susceptible to N losses, at least until the microbe or host plant dies and decomposition releases fixed N. Symbiotic fixation of atmospheric nitrogen, the gas N_2 , includes that which occurs in nodules on the roots of legume plants.

Beneficial effects of leguminous plants (legumes) on cultivated plants (crops) had been recorded in Roman times (approximately 2 000 years ago), and Chinese farmers knew that the water fern (*Azolla* sp.) in paddy rice boosted the crop's productivity. Today, we know that this true fern, *Azolla*, has a symbiotic relationship with the blue-green algae *Anabaena azollae* that occurs in the terminal lobe of each leaf and fixes N in the form of atmospheric N_2 . From ancient times to the present, *Azolla* has been tolerated in paddy rice because, as 'green manure', it supplies N to the crop, and it is used as protein-rich fodder for pigs and ducks.

Even though the mechanisms behind those beneficial effects of legumes may not have been fully understood in ancient times, the knowledge grew to such an extent that in the nineteenth century “legumes used in rotation (with other crops) provided the agricultural stability that allowed the industrialization of western Europe”. In 1813, Sir Humphrey Davey was the first to suggest that legumes had the ability to obtain N from the atmosphere, and moreover, that this characteristic benefited subsequent non-legume crops.

Bacterial infection in plants

Rhizobia is the collective name given to a group of soil bacteria that is responsible for symbiotic N fixation in legumes. The genera *Rhizobium* spp. and *Bradyrhizobium* spp. is best known for this characteristic. N fixation is under the control of the nitrogenase enzyme that occurs in nodules that form on root hairs as a result of infection by these bacteria.

Infection occurs in response to chemical signalling that reportedly involves flavonoids (secondary plant metabolites) secreted by the root hairs of legumes, and lipopolysaccharides released by the bacteria. The latter attaches to root hairs by secreting recadhesin, a bacterial protein, which interacts with plant-secreted lectins. Infection of root hairs progresses to curling of root hairs, and infection thread formation that releases bacteria into the cytoplasm of plant cells, which prompts the formation

of specialised symbiotic cells that develop into nodules in which N fixation occurs.

Fixed N in the form of ammonia (NH_3) is supplied to the plant symbiont, and the bacterial partner acquires certain energy-rich carbohydrates and inorganic elements, which are required in high concentration, from the host plant.

Effects of chemicals

Much research has been done to assess whether pesticides applied directly to soil can be harmful to soil micro-organisms. Less research has been focused on the impacts of pesticides targeting the foliage of plants, i.e. post-emergence applied herbicides in the case of weeds, and insecticides, fungicides and bactericides in the case of crops.

The majority of research findings point to non-harmful effects on soil microbes, or impacts of transient nature, provided that pesticides are used at standard (registered) field rates. The nitrification process in soil, i.e. the bacterial-driven process of oxidative transformation of NH_4^+ to NO_3^- , appears to be particularly sensitive to certain pesticides, obviously those toxic to the bacteria involved, namely *Nitrosomonas* and *Nitrobacter*.

In cases where pesticides have direct harmful effects on legume crops, as occasionally happens where herbicides are applied to a legume crop, or when a susceptible legume crop is exposed to

herbicide spray drift, injury of the crop is likely to negatively affect nodulation, nodule numbers and N_2 fixation. Because the harmful effects of all herbicides involve disruption of physiological processes in plants, the type and amount of root exudates will be affected, which in turn affect microbial populations in the rhizosphere.

Much greater disruption of soil biota is caused by soil-applied fungicides, bactericides and especially the drastic practice of soil fumigation (chemical sterilisation), but again, adherence to registered products, dosages and frequency of application ought to prevent long-term harmful effects.

Almost any fertiliser, especially when applied in a concentrated band, can cause local inhibition of microbial activity due to osmotic stress, i.e. the lowering of water potential that prevents water uptake by the organism, and damage caused to cell membranes. Injections of ammonia can produce biocidal concentrations in the centre of the injection zone, and band-placed urea can have a similar effect.

Such effects of fertilisers are transient in nature, and if label recommendations for products are followed and registered rates applied, any negative effect should be short-lived. Fortunately, soil biota are extremely resilient and can recover rapidly from negative impacts, irrespective of whether they are chemical (e.g. pesticides and fertilisers), physical (e.g. soil tillage, extremes in temperature and moisture), or biological (e.g. monocultures) in nature.

However, we must be ever alert and show responsibility by embracing astute agricultural practices. This endeavour is dependent on awareness and knowledge of the Big Truth, i.e. that for every action exerted by humans in nature, the latter will respond in the form of several reactions – most of those reactions will initially be surreptitious and will only fully exert itself in the long run when it might be too late to reverse the damage done. 🌱

Prof Charlie Reinhardt is lecturer and researcher in the Faculty of Agriculture at North-West University, Potchefstroom campus, and research leader in the South African Herbicide Resistance Initiative (SAHRI) at the University of Pretoria. For more information and references, send an email to dr.charlie.reinhardt@gmail.com.

Figure 1: Summarised model for symbiotic nitrogen fixation in legumes by rhizobia.
(Source: Microbial Biotechnology, December 2019)

