

Examining root nodule activity on legumes

In brief

The symbiotic partnership between bacteria and legumes is vital for the supply of biologically fixed nitrogen to soil and plants in organic cropping systems. Perennial clovers in a dense grass clover ley may contribute more than 200 kg nitrogen (N) per ha and year to the cropping system from the second year after sowing.

This technical note aims at encouraging farmers to take a closer look at the roots of legumes and become more familiar with the process of nitrogen fixation and its relevance for organic farming. The note provides some key information on biological nitrogen fixation and describes a simple method for examining nitrogen-fixing root nodules.



Biological nitrogen fixation

Symbiosis of plant roots and bacteria

In most European soils, clover, alfalfa, beans, peas and vetch form a partnership (symbiosis) with native *Rhizobium* bacteria strains. Soybean and lupine form a symbiosis with *Bradyrhizobium* bacteria, which are often missing in Europe soils. If the bacteria strains are not native in the actual soil, inoculation with the right bacteria (on seeds) is required prior to sowing.

An intricate communication between the plant roots and the free-living stage of the bacterium initiates a nodulation on the roots, in which the bacteria multiply.

The bacteria in the nodules on legume roots biologically convert nitrogen from inert atmospheric N₂ gas to ammonia and amino acids within the nodules. The process requires energy, which the bacteria receive from the plant. Thus, factors hampering the photosynthesis in plants, as water stress and lack of other nutrients than nitrogen, will also reduce the biological fixation of nitrogen. As a total, the symbiosis between the plant in good growth and the bacteria enhances the nitrogen content in the whole plant-soil system.

Nodule size and shape can differ from one plant species to another ⁽¹⁾. Large, round nodules are usually found on soybean (*Glycine max*) and faba bean (*Vicia faba*). Smaller, more elongated nodules are present on clover species (*Trifolium* spp.) and alfalfa (*Medicago*). Some plant species have more asymmetrically shaped nodules.

Active nodules are red coloured inside

Actively N-fixing nodules contain a pigmented protein called leghaemoglobin. Its presence results in a red colouration of the interior of nodules, indicating that the bacteria are alive and active ⁽²⁾. Dead, inactive and senescent nodules are usually greyish green or brown inside.

Maximum nitrogen fixation around flowering

Nodules appear 4 to 6 weeks after sowing and reach maximum activity around flowering. In autumn and at plant maturity (after flowering), the roots and nodules are senescent and some have started to decay. When the perennial crop, e.g. clover ley, is cut, nitrogen fixation may continue throughout the season. In perennial legumes, new nodulation starts again in spring.



Active, red coloured nodule on lupin (*Lupinus*). (Photo: R. Pommeresche, NORSØK)



Older, more senescent and inactive nodules with grey colour, on Hungarian vetch (*Vicia pannonica*). (Photo: R. Pommeresche, NORSØK)



Brown or "empty" nodules from red clover (*Trifolium pratense*). (Photo: R. Pommeresche, NORSØK).

Wide range of N fixation rates

Nitrogen fixation in a legume-rhizobia symbiosis varies widely according to climate, soil nitrogen status, cropping system and management. The amount of fixed nitrogen in above-ground plant tissues of ungrazed forage legumes in temperate/boreal areas ranges from 60 to 370 kg N per ha and year in red clover (*Trifolium pratense* L.), from 80 to 540 kg N per ha and year in white clover (*T. repens* L.) and from 100 to 350 kg N per ha and year in alfalfa (*Medicago sativa* L.)⁽³⁾.

Grasses grown in mixture with forage legumes can use up to 80 % of the nitrogen from the N fixation in the legumes. In grazed white clover pasture, a fixation rate of 55 to 295 kg N per ha and year was recorded above ground⁽⁴⁾.



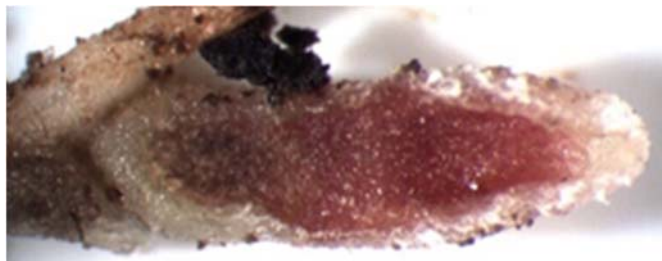
Nodules on soybeans with active (red colour) bacteria (*Bradyrhizobium*) (Photo: Monika Messmer, FiBL).

In soybeans under irrigation, average N fixation is about 175 kg N per ha and year, measured in aboveground plant-tissue nitrogen (250 kg N if roots were included), and about 100 kg N without irrigation⁽⁵⁾. Lupines fix about 165 kg N in aboveground tissue, and faba beans about 90 kg per ha⁽⁵⁾ (about 130 kg per ha and year, if roots were included). As nitrogen fixation is sensitive to water stress, it will be lower during summertime in dry areas without irrigation.

The optimal temperature for several *Rhizobium* bacteria strains is around 20 °C, but for many legumes and regions, fixations at much lower temperatures are recorded. Nitrogen fixation per ha and year in northern areas (around 60° N) and areas with a milder climate (around 40° N)⁽³⁾ was noticed to be almost the same. The reason may be that several environmental factors are involved in these processes.

Large proportion of nitrogen in root biomass

A high proportion of fixed nitrogen is found in the roots and nodules of the legume plants. Pot experiments indicate that 20 to 50 % of the total N in alfalfa (*Medicago* spp.), faba beans (*Vicia* spp.) and lupines (*Lupinus* spp.) is present in roots (including nodules) at the time of flowering. However, the allocation of the nitrogen can vary widely between shoots and roots within the same genus (*Medicago* spp., *Lupinus* spp.)⁽⁵⁾. In a red clover-grass ley, 60 % of the nitrogen was found in the clover stubble and 25 % in the roots after the first and second harvest, while about 60 % of the N in the grasses remained in stubble and roots after second harvest⁽⁶⁾.



Red and with clover roots with the shape and colour of nitrogen fixing nodules. Active, pink nodules on red clover roots (upper) and a close up of the interior red colour of a nodule on with clover. Both from meadows in Norway. (Photos: R. Pommeresche, NORSØK).

Different number and distribution of nodules on roots of different plant species

The location of the nodules along the roots is primarily linked to genus/species of the plants and the presence of rhizobia in the soil. At low frequency of rhizobia, the low number of nodules per plant is compensated by increased nodule size. In addition, the content of nutrients in the soil, local soil structure and access to air in the soil influence the distribution and size of the nodules. Therefore, comparisons of nodules should be done within the same plant species and plant stage.

Investigation method

The following method describes a simple procedure to become familiar with legume-rhizobia symbiosis and to get a first feedback on the activity of the symbionts in the field.

How to proceed

1. Optimally, two weeks before the legumes are flowering (or any time in the growing season) select two to four legume plants from a representative part of a field.
2. Dig out a soil cube (about 25 x 25 cm and as deep as the roots or the spade go) around a chosen legume plant.
3. Lift the clump out carefully with the spade and put it on a plastic sheet.
4. Carefully remove the soil from the roots manually and try to loosen the legume roots with a fork. On heavy soil, water might be needed to remove the soil without destroying nodules.

5. Look for nodules on the roots and examine, whether they are active or not by cutting three nodules of each plant in half immediately after uprooting.
6. Repeat the procedure with another plant of the same species, but choose another part of the field, which is either better or worse in growth.

Useful questions

- **Appearance of the plant:** Does it look lush with dark green coloured leaves, or does it rather look meagre with fewer and pale green leaves?
- **Development of the roots:** How well did the root system develop? Are the roots long and frequently branched?
- **Number, size and distribution of root nodules:** Can you find nodules on the roots? Are there only few nodules or are there many? Are the nodules of regular size or very large? Have the nodules developed on the main root (tap-root) only or also on lateral roots? Ideally, nodules are found on different roots. Are the nodules only on the upper third or also on the lower two thirds of the root system? Ideally, nodules are found on the whole root depth.
- **Colour of root nodules:** Which colour do the nodules have that have been cut in half? Red/purple/pinkish colour = active bacteria; greyish green or brown colour = old or inactive bacteria; white = young nodules or nodules in progress to become active or inactive.



Searching for root nodules in a clover ley, Norway. We found nodules and active nitrogen-fixating bacteria on some of the red clover roots (Photo: S. Hansen, NORSØK).

Required material

- A spade (to dig out the soil cubes with plant roots)
- Some plastic sheets (to put the soil and plants on)
- A pen, some paper, a fork, a ruler and a camera
- A bucket of water (to remove soil from plant roots)

Interpretation of the findings

Observations	Possible conclusions and recommendations
Colour and number of nodules	<ul style="list-style-type: none">▪ Regardless of the plant species, if about 15 nodules are found on the roots of one plant and most of them are red inside, it can be assumed that the nitrogen-fixating bacteria are performing well.▪ Generally, <i>Rhizobia</i> and <i>Bradyrhizobia</i> bacteria are found in the soil if the plant genus is found naturally in the area, or if the legume genus has successfully been grown on the field before. If the matching bacteria are missing in the soil, most legumes will show suboptimal growth. In such cases, inoculation with the matching bacteria is indispensable for optimal plant performance. Lack of the bacteria cannot be compensated by N fertilisation. N-fertilization affects nodulation negatively.▪ After successful inoculation and repeated cultivation of the same legume species, the number of nodules can exceed 100 per plant. However, the number of nodules is not equivalent to a high fixation rate, but a precondition for efficient symbiosis.
Size of the nodules	<ul style="list-style-type: none">▪ Evenly distributed nodules of regular size often indicate higher nitrogen fixation rates than the presence of a few, very large nodules.
Distribution of the nodules on the roots	<ul style="list-style-type: none">▪ Nodules in the upper root zone only, may indicate shortage of air in the lower root zone. In such cases, better soil structure may improve aeration of the lower root zone and access to nitrogen and, as a result of it, may induce development of nodules in deeper soil layers.
Nodules only on the tap/main root	<ul style="list-style-type: none">▪ Nodules on the tap or main root only, may indicate that the bacteria try to compensate low fixation by increasing their number inside the nodules.

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Soybean roots with nitrogen fixing bacteria in nodules. Photo: Monika Messmer (FiBL)

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About FertilCrop

Fertility Building Management Measures in Organic Cropping Systems – FertilCrop is a project funded by the CORE Organic Plus funding bodies, being partners of the FP7 ERA-Net project CORE Organic Plus. The overall aim of FertilCrop is to develop efficient and sustainable management techniques aimed at increasing crop productivity in organic farming systems. More information about FertilCrop is available at www.fertilcrop.net.

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