Nitrogen Fixation in Pines

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Knowledge around the application and understanding of the role nitrogen fixing plants play in the natural environment has not changed a lot in the past 30 years in forest gardening and permaculture communities. Advice written in the 1990s is largely the same as what is given now. The following information summarises both older and current-day knowledge of nitrogen cycling in a forest garden context.

There are two main groups of nitrogen-fixing plants that are commonly discussed – leguminous plants that form symbioses with *Rhizobium* bacteria and actinorhizal plants that form symbioses with *Frankia* spp. Sometimes 'outliers' such as *Gunnera* spp. which form symbioses with *Nostoc* cyanobacteria are also mentioned. Unlike the previous two overarching categories, nodules do not form on the roots of *Gunnera*, but they do have a specialised mucous-producing gland present on their stems – the *Nostoc* cyanobacteria live inside these and largely forgo their previously photosynthetic lifestyle in order to feed directly from the carbohydrate-rich mucous, in return providing nitrogen-fixing services to the plant. It took approximately 70 years from the date of the formation of the hypothesis that *Gunnera* was involved in a strong nitrogen fixing symbiosis for it to be conclusively proven – so it can take a while nitrogen cycling knowledge to evolve.

The next group of nitrogen fixing plants identified were those without obvious physical characteristics, and these also formed less-coupled symbioses – thus were much more difficult to identify. The two main zones of the plant where these nitrogen-fixing symbionts live are in the rhizosphere, or within the plant's leaves and stems (an endophyte). Nitrogen fixation research discovers new examples of these symbioses regularly, and the evidence is mounting that nitrogen fixation is much more common than previously thought (despite there being large numbers of observations and experiments originating 70 years ago that resulted in the unexplainable input of vast amounts of nitrogen into many agricultural and ecological systems).

Pines have long been known as a pioneer species, able to colonise difficult environments all over the northern hemisphere – from growing in acidic, nutrient-poor bog soils, to fire-razed ecosystems and newly exposed glacial till. The speed of growth has also been noted with many species being grown as commodity timbers.

Research with pines

The first strong hints that pines were able to form strong nitrogen-fixing relationships came from the 1950s, when nitrogen accounting processes found unexplainable increases in nitrogen in forests and ecosystems all over the world. Stevenson (1959) notes "*In New Zealand, the rapid growth of exotic pines on depleted mountain country and even on sand dunes is striking. The author measured the nitrogen in a 25-year-old Pinus radiata forest on depleted hill country near Nelson, and found the gain in nitrogen per acre to be approximately 300 lb. in the standing trees and 500 lb. in the litter, and average increment of 32 lb. N/ac/yr*." This amount (35.8 kg N/ha/year) is a significant quantity for exposed hill terrain. The same paper also refers to a site planted predominantly in *P. ponderosa* with a gain of 56 lb/ac/year (62.7 kg N/ha/year).

A series of semi-controlled pot experiments in New Zealand, again conducted by Greta Stevenson, were published in 1959. These were aimed at determining where this nitrogen fixation took place.

Pinus radiata seedlings were sterilized and inoculated with various fungi, or a sterile soil. The seedlings were then grown in perlite and fed with a nutrient mixture lacking only in N (so that nitrogen was guaranteed to be the limiting growth factor). The sterile-soil inoculated plants died quickly, *Amanita*-inoculated plants took longer to die but also perished, and all the other plants inoculated with various fungal species were thriving after a period of six months, providing evidence that mycorrhiza were indeed responsible for nitrogen fixation in these trees. Additional experiments with 15N nitrogen tracing, (using an isotope of nitrogen) suggested that *Pinus* roots could fix nitrogen, but not the leaves. Collectively, these findings suggested that the mycorrhizal fungi were responsible for the increase in nitrogen content in the surrounding soil.

In 1982, the Hubbard Brook sandbox study commenced, and was an attempt to deduce and quantify more accurately the existence of unexplained nitrogen sources in model ecosystems (mesocosms) by careful accounting of the initial nitrogen content and the outputs and inputs into each system. Box-shaped pits were dug, lined with landfill-liner plastic, and filled with glacier sand. Sixteen pines were planted in each in a grid pattern (as well as alder and black locust in other identical boxes). These were left to grow for six years. The amount of water that drained through was carefully measured (along with the nitrogen leaching losses), and at the end of the experiment, the trees were removed and sampled for nitrogen content. The initial experiment's conclusion was "*These results provide strong evidence for* N_2 *fixation in pine systems of almost-equal-to 50 kg* ha^{-1} yr^{-1} N" but due to conventional understanding, such a large amount of non-nodulated nitrogen fixation was largely dismissed as an error.

The original paper was later re-examined for sources of these errors, with the initial soil re-checked and as a result the original conclusion was strengthened: "*New estimates for accumulation of N in the entire sandbox soil (0–135 cm), based on the fixed-mass method, were also large with small confidence intervals: 70 +- 21 kg ha/y for red pine and 63 +- 16 kg ha/y for pitch pine.* This was the lower-end estimate – the maximum was 149 +- 23 kg N/ha/year and 130 +- 43 kg N/ha/year for red pine and pitch pine respectively. The same experimental conditions resulted in 175 kg N/ha/year for *Alnus glutinosa* (below its maximal rate in other experiments – perhaps suggesting conservative measurements all round).

The conclusion that nitrogen fixation rates of above 25 kg N/ha/year were possible from unknown sources of nitrogen was not accepted in the review conducted by Binkley et al. (2000), despite all other studies to the contrary being less controlled observation studies, not mesocosm studies.

A 2012 study from British Columbia found very strong indirect evidence of nitrogen fixation in wild stands of *Pinus contorta*. Trees observed growing in gravel pits had almost identical rates of growth to those growing in more fertile soil, however the foliar 15N ratio was much lower in the gravel pit trees than the trees in fertile soil. The simplest explanation for this discrepancy is that the gravel pit trees were receiving substantial amounts of biologically-fixed nitrogen. The total nitrogen content of plant tissues were virtually identical between the two sites. The gravel also had a higher nitrate content than the fertile soil, while the fertile soil was much higher in ammonium. This is perhaps due to accumulated biological nitrification inhibition (BNI) effects.

The authors concluded "*Based on our previous work, which demonstrates high levels of acetylene reduction in tuberculate mycorrhizae in P. contorta, we conclude that the simplest and most likely explanation for comparable growth and nitrogen levels between trees growing on intact soil and those growing on very low nitrogen gravel is that this pine species, in conjunction with certain symbionts, is capable of fixing biologically significant quantities of nitrogen.*"

Direct evidence for nitrogen fixation was presented in a 2013 study, also from British Columbia. *P. contorta* var. *latifolia* seed was first sterilised, then inoculated with *Paenibacillus polymyxa* (a bacteria isolated from within the pine tissues, that has been determined to fix nitrogen) and grown on a sand-clay mixture (fertilized once with 15N-tagged fertiliser) for 13 months. The innoculated pines grew approximately twice as large as the non-inoculated seedlings and had a much higher survival rate. What was interesting about this study was that there was no mycorrhizal development noted in the roots, but there were large amounts of *P. polymyxa* present in the root tissue. This suggests that bacterial endophytes are strong nitrogen-fixing partners of pines.

A 2019 study of post-fire recovery rates of *P. contorta* var. *latifolia* in Yellowstone National Park, USA found that nitrogen content increased in all measured components (foliage and soil horizons) countering the expectation that tree recovery would remove soil nitrogen. For live biomass, an increase of 7.2 kg N/ha/year was calculated, with N accumulation in the soil (at 0-15 cm depth) measured at 34.3 kg N/ha/year. The O-horizon (the uppermost layer, consisting of humus/organic matter) accumulated approximately 1.1 kg N/ha/year, for a total accumulation of 42.6 kg N/ha/year. From this, the authors concluded "*The large increases in N pools cannot be explained by atmospheric N deposition or presence of known N fixers. These results suggest an unmeasured N source and are consistent with recent reports of N fixation in young lodgepole pine.*"

Conclusion

Based on these results, there seems to be strong evidence that nitrogen fixation takes place in pines, likely through a combination of both endophytes and mycorrhiza in the roots (which may in turn be utilizing the same endophytes), and, depending on the species, inoculation conditions and climate. The amount fixed seems to be approximately 50 kg N/ha/year as a conservative estimate, for faster growing pioneer pines on lower elevation sites.

There is limited evidence in regards to whether pines are facultative (fixing nitrogen only to meet their needs) versus obligate (always fixing nitrogen at the same rate) nitrogen fixers, but there is evidence from studies carried out using 15N that indicate that pines with sufficient soil nitrogen still obtain significant proportions of their nitrogen needs from biologically-fixed nitrogen, with further evidence indicating some obligate tendencies.

How can this information influence the utilisation of pines in agroforestry systems?

I will refrain from making sweeping statements, but we can however deduce with confidence that:

Pines can be expected to fix at least 50 kg N/ha/year (for known pioneer species) and perhaps up to twice that amount at lower elevation sites (although it is difficult to estimate the potential amount of biologically-fixed nitrogen if there is sufficient soil nitrogen present).

Unlike other N-fixing species, the C:N ratio is much higher in pines (because the total biomass of pines is higher – they are also 'carbon fixers'), and the foliage is much longer-lived too. To release this nitrogen into the system would require the tree to be felled and used for hugelkultur, allowing for a temporary nitrogen deficit and decomposition of the lignin to be spread out over time, with the nitrogen from the pine slowly re-entering the soil.

https://agroforestry.co.nz/

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