

Biological Dinitrogen Fixation in Chaparral¹

Mark Poth²

Nitrogen is lost from Mediterranean-type ecosystems through many mechanisms. These include fire-induced volatilization, sediment and erosion-al losses (DeBano and Conrad 1978), nitrate-nitrogen lost in runoff and leaching waters (Riggan and Lopez 1981, Vitousek and Melillo 1979), and denitrification. Some of these processes have been studied extensively. For example, more is known of the magnitude of fire volatilization losses and the effects of variables such as fire intensity and time of year in which the burn occurs (DeBano and Conrad 1978, Christensen and Muller 1975, Hanes 1971), than of any other mechanisms of nitrogen loss, such as denitrification, which remains unquantified in the Mediterranean ecosystems. Historical records, ocean cores, and geological evidence indicate that in southern California chaparral these loss mechanisms have been operating for millennia (Byrne and others 1977, Vogl 1977). The flux of nitrogen out of chaparral ecosystems is evidently large and ongoing; yet chaparral, though low in nitrogen, is not completely impoverished, as might be expected. Nitrogen inputs into the system then must be high enough to allow for this degree of ecosystem resiliency. Consequently, nitrogen inputs are one of the important factors which determine the resiliency of Mediterranean-type ecosystems to various management manipulations.

Ecosystem nitrogen inputs fall into three general categories. The first is the industrial-commercial synthesis of nitrogen fertilizers via the Haber-Bosch process (Finneran and Czuppon 1979). This process consumes large amounts of energy, usually in the form of natural gas or electricity, which renders the widespread use of fertilizers on Mediterranean-type ecosystems economically unfeasible--both now and in the future. The second major category is aerial inputs, discussed elsewhere in this Proceedings. Lastly, there is biological dinitrogen fixation.

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²Microbiologist, Pacific Southwest Forest and Range Experiment Station, Forest Service, U.S. Department of Agriculture, Riverside, Calif.

Abstract: Presently available data indicate that biological dinitrogen fixation is responsible for the greatest proportion of nitrogen entering chaparral ecosystems. Dinitrogen fixation by the *Rhizobium*-legume symbioses is relatively unimportant in chaparral when compared to the possible substantial fixation by free-living soil bacteria and the *Frankia*-woody shrub (*Ceanothus*) symbioses. At present, estimates of these processes are scant. Site degradation is possible if management practices adversely affect biological dinitrogen fixation.

ENZYMOLGY

Only specific bacteria, blue-green algae, and actinomycetes (all procaryotic microorganisms) are able to synthesize the nitrogenase enzyme and fix dinitrogen. The active enzyme consists of two protein subunits: the smaller subunit is the iron protein, the larger is the molybdenum-iron protein. The intact enzyme catalyzes the reduction of atmospheric dinitrogen to ammonium, which can then be used in biosynthetic pathways to produce proteins, nucleic acids, and other biomolecules as required. Nitrogenase is not highly specific and will reduce other substrates, for example, hydro-nium ions to dihydrogen. This represents a net energy loss if the dihydrogen is evolved to the atmosphere. Azide, nitrous oxide, acetylene, and many other substrates may be reduced by nitrogenase (Burris 1979). The reduction of acetylene to ethylene forms the basis of the most widely used technique for the determination of nitrogenase activity (Hardy and others 1971); the reduction is quantified by standard gas chromatography. The technique is advantageous in that it is very sensitive and fairly simple to use (Sprent 1979). Acetylene reduction rates can be used as an index of dinitrogen fixation for relative comparisons.

The heavy isotope of nitrogen ¹⁵N can also be used to measure dinitrogen fixation. This technique requires much more time, technical skill, and access to a mass spectrometer. Some work has been done with the radioactive isotope of nitrogen ¹³N, but its use is severely hampered by a short (10.5 min) half-life; the laboratory must be very close to a cyclotron producing the isotope, and few laboratories are so situated (Sprent 1979).

Several factors affect nitrogenase activity and biosynthesis. The partial pressure of oxygen must be low. Oxygen may be low in the environment or may be regulated by the synthesizing organism. Oxygen diffusion into the site of nitrogenase activity may be restricted by specialized structures such as the heterocysts of blue-green algae, or it may be sequestered by specialized compounds such as leghemoglobin. Ammonium and nitrate levels must be low, since mineral nitrogen inhibits nitrogenase activity. The organism must have a readily available energy source since the reduction of dinitrogen is a highly energy-demanding

process (Gutschick 1978). Finally, moisture, pH, and temperature conditions must be favorable for the organism.

DINITROGEN FIXATION BY LEGUME-RHIZOBIUM SYMBIOSES

Legumes (members of the family Fabaceae) form symbiotic associations with bacteria of the genus Rhizobium. Root nodules are formed by a complex infection process, involving the production by Rhizobium of enzymes that degrade the plant cell wall and of plant hormones (Dart 1977). The host specificity of the rhizobia is used as a major taxonomic criterion (Vincent 1977). Once the bacteria have infected the root cells, these proliferate to form the root nodule. Many nodule cells are filled with rhizobia. Most of the bacteria within the root cell convert to the bacteroid form: enlarged, pleomorphic rhizobia which contain nitrogenase. Bacteroids have not been cultured on laboratory media. In this symbiosis, the plant supplies carbohydrate energy to the bacteria in the root nodules. The bacteria use a portion of this carbohydrate to "fix" atmospheric dinitrogen into forms which are usable by the plant. Historically, this symbiosis has been used to improve fertility through crop rotation systems (Delwiche 1978). Recently, much effort has been spent to maximize dinitrogen fixation by agronomic species as well as to encourage the use of new and different legume species as crops (Delwiche 1978, Hardy and others 1971). The most notable success in improving dinitrogen fixation of a crop has been achieved with soybeans. The annual fixation by many agronomic legume species is substantial (table 1).

Table 1--A comparison of dinitrogen fixation by agronomic and chaparral legumes.

Plant	Acetylene reduction rate ¹	Total N fixed
		kg/ha/yr
Agronomic species: ²		
<u>Glycine max</u>	7.3	103
<u>Medicago sativa</u>	15.1	54 to 463
<u>Pisum sativum</u>	17.4	52 to 77
Chaparral species: ³		
<u>Lotus scoparius</u>	0.17	9 to 40
<u>Lupinus excubidus</u>	0.66	24
<u>Lupinus longifolius</u>	0.25	--
<u>Lupinus polycarpus</u>	0.32	--

¹Expressed as micromoles/hr/g fresh weight nodule.

²From Torrey (1978).

³From Poth (1980).

Legumes are common in chaparral postfire, and extensive stands may develop (Poth 1980, Hanes

1971). The genera Lotus and Lupinus are often observed. As time progresses, these species became increasingly rare. Lupinus arboreus Sims stands in New Zealand have been shown to fix between 84 and 107 kg-N/ha/yr (Sprent and Sylvester 1973, Gadgil 1971). Lupines and other legumes in chaparral are routinely well nodulated. These nodules have the pink color of leghemoglobin and demonstrate the ability to reduce acetylene, which is indicative of nodulation with an effective strain of Rhizobium (Hardy and others 1973, Vincent 1970). In a recent study summarized in table 1, four chaparral legume species--Lupinus excubidus Jones var. Hallii, Lupinus longifolius Abrams, Lupinus polycarpus Greene, and Lotus scoparius ssp. scoparius Nutt. in T. and G.--were assayed for their ability to fix nitrogen under greenhouse conditions (Poth 1980).

The estimated total nitrogen fixed by these chaparral legume species is much lower than that of agronomic legume species. The reason may lie in the agronomic species themselves. Considerable effort by agronomists and plant geneticists has gone into artificially selecting plants and rhizobial strains for optimum dinitrogen fixation (Delwiche 1978, Gutschick 1978). Alternatively, the selective forces in the chaparral environment are such that a high dinitrogen fixation rate is not a primary selection criterion. Legumes are most abundant in chaparral immediately following a fire, and their numbers decline as the stand matures (Poth 1980, Hanes 1971). In the postfire environment, soil mineral nitrogen levels are at their highest (Christensen and Muller 1975, DeBano and Conrad 1978). Nitrogen does not limit plant growth during the first few years following fire (Hellmers and others 1955), and there is apparent luxury consumption of this nutrient (Rundel and Parsons 1980). Legumes using the readily available soil mineral nitrogen pool and not expending energy to fix nitrogen would be able to put more energy into reproduction, and hence be at a selective advantage. In addition, mineral nitrogen depresses nitrogen fixation activity (Burris 1979). This would most likely result in the selection of rhizobial strains which would be readily infective, easily forming nodules, but there would be no selection for dinitrogen fixation. Legumes are rare in chaparral stands during the period between fires. Rhizobia must then exist for extended periods as free-living soil bacteria. Selection then occurs for those organisms best able to survive as free-living soil bacteria.

The overall contribution by the prevailing legumes at a particular site depends on water availability (Sprent 1976). A dry year substantially reduces net nitrogen fixation. Soil temperature also affects net fixation. Lower soil temperatures decrease nitrogen fixation. With all environmental factors considered, chaparral legumes are able to fix only a small proportion of the nitrogen that is lost in fire, erosion, denitrification, runoff, and leaching.

DINITROGEN FIXATION BY ANGIOSPERM-FRANKIA SYMBIOSES

Symbiotic associations are found between soil bacteria belonging to the family Actinomycetes (*Frankia* sp.) and a large and diverse group of woody dicotyledonous plants (Torrey 1978). Some of the plants involved and their distribution are summarized in table 2. *Ceanothus*, a common genus throughout western North America, forms symbioses with *Frankia* sp. In forests of the Pacific Northwest, *Ceanothus velutinus* Dougl. has been shown to fix 56 kg-N/ha/yr (Youngberg and Wollum 1976). Kummerow and others (1978) investigated dinitrogen fixation by *Ceanothus greggii* var. *perplexans* and *Ceanothus tomentosus* in southern California chaparral. First-year seedlings of *C. tomentosus* were found to be infrequently and poorly nodulated with no dinitrogen fixation capacity. This is most likely due to the elevated soil ammonium and nitrate levels after fire (Christensen 1973, Christensen and Muller 1975, Hellmers and others 1955). Both ammonium and nitrate are inhibitors of nodulation and dinitrogen fixation (Burris 1979). These elevated levels of available mineral nitrogen have been shown to promote luxury consumption of nitrogen in *Adenostoma fasciculatum* H. & A. and *Ceanothus leucodermis* Greene (Rundel and Parsons 1980). A 25-year-old stand of *C. greggii* was shown to fix but 0.1 kg-N/ha/yr, an amount comparable to the leaching loss (Kummerow and others 1978). This low level may be caused by the general loss of vigor in these older plants.

Excavations of *Ceanothus crassifolius* Torr. seedlings have been made every year since 1976 in the area burned by a November 1975 wildfire. In 1976, 1977, and 1978, few nodulated plants were found. Those plants that were nodulated typically had but one nodule, and these were very small, in all cases being less than 0.5 g fresh weight. This situation changed dramatically in spring 1979. Excavations at that time revealed that nearly all seedlings were nodulated. The plants had from two to five nodule clusters each. The fresh weight of each cluster was in the 2 to 5 g range; the largest nodules approaching 10 g fresh weight. Field acetylene reduction assays showed these nodules to be actively fixing dinitrogen.

The point at which dinitrogen fixation drops to 0.1 kg-N/ha/yr observed by Kummerow and others (1978) is crucial to any overall estimates of dinitrogen fixation by *Ceanothus* spp. in chaparral. At present, this remains to be investigated. *Ceanothus* fixation may prove to be substantial. An estimation of the magnitude of *Ceanothus* fixation in chaparral can be gained from the data of Zinke (1969) (table 3). These data indicate a net fixation of 49 kg-N/ha/yr for lysimeter-grown *C. crassifolius* over a 13-year period. As is characteristic of plants that form nitrogen-fixing symbioses, the tissue levels of nitrogen in *C. crassifolius* are high. This is reflected in the larger amounts of nitrogen sequestered in the stems and leaves of *C. crassifolius* than in the leaves and stems of other

Table 2--Plants that form root nodules following actinomycete infection, with their relationships and distribution (From Torrey 1978).

Genus (family)	Species nodulated/ total species in genus	Geographical distribution	Ecological sites
<i>Alnus</i> (Betulaceae)	33/35	Europe, Siberia, N. America, Japan, Andes	Poor soils, sand hills/dunes, glacial till, wet bogs, mine dumps, gravel, waste-land, volcanic ash
<i>Casuarina</i> (Casuarinaceae)	24/45	Australia, tropical Asia, Pacific Islands, widely introduced elsewhere	Sand dunes, salt marshes, tropical forests, desert areas
<i>Ceanothus</i> (Rhamnaceae)	31/55	N. America especially western U.S.	Dry forest and chaparral, sub-alpine zones
<i>Cercocarpus</i> (Rosaceae)	4/20	Western U.S. and Mexico	High altitudes poor soils
<i>Colletia</i> (Rhamnaceae)	1/17	S. America (cf. Bond 1976)	
<i>Comptonia</i> (Myricaceae)	1/1	N. America	Disturbed sandy or gravelly areas
<i>Coriaria</i> (Coriariaceae)	13/15	Mediterranean to Japan, New Zealand, Chile, to Mexico	Lowlands and subalpine, sandy, or gravelly soils or clay
<i>Discaria</i> (Rhamnaceae)	2/10	Andes, Brazil, New Zealand, Australia	gravelly soils, arid zones
<i>Dryas</i> (Rosaceae)	3/4	Alaska, Canada, circumpolar	Postglacial areas, sandy, gravelly soils
<i>Elaeagnus</i> (Elaeagnaceae)	16/45	Asia, Europe, N. America	Disturbed areas, sand dunes, poor soils
<i>Hippophae</i> (Elaeagnaceae)	1/3	Asia, Europe, from Himalayas to Arctic Circle	Sand dunes, coastal areas
<i>Myrica</i> (Myricaceae)	26/35	Many tropical, subtropical, and temperate regions, extending to Arctic Circle	Acidic bogs, sand dunes, mine wastes
<i>Purshia</i> (Rosaceae)	2/2	Western N. America	Dry sandy soils, as understory plant in <i>Pinus</i> forest

species (table 3). Should fixation occur in natural stands at a rate of 49 kg-N/ha/yr, fire losses would be alleviated by Ceanothus fixation gains.

DINITROGEN FIXATION BY FREE-LIVING MICROORGANISMS

Bacteria and blue-green algae (Cyanobacteria) are able to fix dinitrogen. For fixation by these organisms to occur, the following conditions must be met: there must be a sufficient supply of available carbohydrate, or light to produce carbohydrate as the case of Cyanobacteria; levels of ammonium and nitrate must be low; oxygen must be at low partial pressures; and moisture, temperature, and pH must fall within acceptable ranges (Mulder 1979, Granhall 1979, Gutschick 1978, Peters 1978). These conditions might be met in chaparral with the first rains of the winter. Carbon availability would be at its peak after the spring and summer litter fall. The water of the first rains would immediately stimulate rapid growth of the soil microflora (Winn 1977). With this growth, much of the available ammonium and nitrate would be immobilized. Oxygen tension would become reduced as a result of rapid respiration. These factors could combine to promote transient free-living dinitrogen fixation in chaparral. This is one possible explanation for the nitrogen gains in lysimeter studies with Quercus dumosa Nutt. and Eriogonum fasciculatum Benth. (Zinke 1969) (table 3). The nitrogen accumulations seen in chaparral soils under E. fasciculatum and Q. dumosa support the hypothesis that free-living soil bacteria fix substantial

amounts of dinitrogen in chaparral. Zinke's data indicate free-living bacterial dinitrogen fixation rates of 38 kg-N/ha/yr in soil under Q. dumosa, and 32 kg-N/ha/yr in soil under E. fasciculatum.

CONCLUSION

Nitrogen is one of many factors which will determine the resiliency of chaparral to various management practices. Nitrogen management must be considered in prescribed burning plans. Should ecosystem nitrogen levels be allowed to drop, water quality (Riggan and Lopez 1981), erosion, and browse quality could all be adversely affected. If nitrogen inputs can be managed in such a fashion that they equal or exceed losses, then the ecosystem should be highly resilient, allowing land managers greater flexibility in proposed management programs.

The data I have presented seem to support the following hypotheses concerning dinitrogen fixation in chaparral:

1. Symbiotic dinitrogen fixation by chaparral legumes is insignificant in relation to ecosystem losses, because chaparral legumes are inherently poor dinitrogen fixers and their presence is short lived. (Lotus scoparius may be an exception since it is present for up to 10 years.)

2. Nodulation and dinitrogen fixation by Ceanothus spp. in the first few years following fire is repressed to very low levels by elevated soil mineral nitrogen. Once soil mineral nitrogen

Table 3--Nitrogen balance of chaparral stands in San Dimas lysimeters after 13 years (From Zinke 1969).

Part of system	Nitrogen balance, by cover type					
	Original soil	Barren	<u>Adenostoma fasciculatum</u>	<u>Eriogonum fasciculatum</u>	<u>Quercus dumosa</u>	<u>Ceanothus crassifolius</u>
	<u>Grams-N/m²(1)</u>					
Vegetation:						
Leaves	0	0	2.4	1.6	1.9	11.5
Stems	0	0	2.8	9.0	4.2	18.1
Total	0	0	5.2	10.6	6.1	29.6
Leaf litter	0	0	3.2	30.0	17.9	32.4
Mineral soil to 122 an	318.0	283.8	278.5	318.8	343.6	320.1
Total	318.0	283.8	286.9	359.4	367.6	382.1
Change from original condition		-34.2	-31.1	+41.4	+49.6	+64.1

¹To convert to kg/ha, multiply by 10; to lb/acre, multiply by 11.2.

levels have dropped, symbiotic dinitrogen fixation by Ceanothus is substantial.

3. Dinitrogen fixation by free-living soil and litter bacteria associated with scrub oak (Quercus dumosa) and California buckwheat (Eriogonum fasciculatum) is substantial.

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