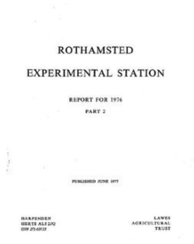
Rothamsted Experimental Station Report for 1976, Part 2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PART 2

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The Nitrogen Economy of the Broadbalk Experiments
II. Biological Nitrogen Fixation

J. F. WITTY, J. M. DAY and P. J. DART

Introduction

Nitrogen fixing microorganisms were first noted in the Broadbalk experiment by Ashby (1907) who identified *Azotobacter chroococcum* in both Wilderness soil and in the drains of the arable areas. Ziemiecka (1932) found *Azotobacter* most numerous on plots receiving only minerals but even here numbers were low. Brown, Burlingham and Jackson (1962) estimated the numbers of *Azotobacter chroococcum* at 9400 cells g^{-1} dry soil but these would be too few to affect nitrogen input significantly (Meiklejohn, 1969). *Clostridium pasteurianum* is much more numerous over all of Broadbalk, and populations of this nitrogen-fixing anaerobe can reach 100 000 cells g^{-1} dry soil and commonly exceed 10 000 (Meiklejohn, 1956). Barrow and Jenkinson (1962) demonstrated nitrogen fixation in soil from unfertilised plots incubated anaerobically with straw, but in general, except in the plant rhizosphere, lack of a suitable energy source mitigates against a significant nitrogen contribution from free-living heterotrophs, be they aerobes or anaerobes.

Bristol-Roach (1927) counted algae on the arable plots but not blue-greens because her counting procedure failed to separate these filamentous N-fixing autotrophs. She however noted that in certain areas of the low N plots blue-green algae appeared as an almost continuous film on the soil surface.

The measurement of nitrogen fixation in field soils has long been a problem. The classical method involves the determination of changes in total N within the soil and plant system by the Kjeldahl procedure. The sensitivity of this method is extremely low because small changes in soil N must be measured against a very large total quantity of nitrogen. For example, the Broadbalk plot that has not been manured for 140 years now contains about 3 t N ha^{-1} in the top 23 cm (Jenkinson, 1971).

The development of techniques using the stable heavy isotope ^{15}N have greatly lowered the threshold for the detection of nitrogen fixation and the more recently developed acetylene reduction technique, which estimates nitrogen fixation indirectly, is even more sensitive. The latter method is based on the observation that the nitrogen fixing enzyme, nitrogenase, which in nature reduces gaseous N_2 to NH_3, will also reduce acetylene to ethylene (Schöllhorn & Burris, 1966; Dilworth, 1966). The ethylene produced can be rapidly determined by gas chromatography at concentrations as low as 0·1 ppm (Dart, Day & Harris, 1972).

Broadbalk Continuous Wheat

Bacterial fixation in the soil and rhizosphere. Soil cores 17 cm deep, with the top 1 cm removed to exclude blue-green algae, were assayed for nitrogenase activity on three occasions in June and July 1973. The final sampling included cores taken from both planted and fallowed areas.

Activities were low, ranging in the first two samplings when the soil was dry (15–18% H_2O) from 5·7 g N ha^{-1} day^{-1} on plots receiving no fertiliser to 28 g ha^{-1} day^{-1} on plot 06 receiving low levels of N with minerals (see Table 1 for details of fertiliser treatments). At the final sampling soil moisture was greater (17–24% H_2O) and nitrogenase activities

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equivalent to 45 g N ha^{-1} day^{-1} were recorded on the planted areas of plot 05 receiving only added minerals. Apart from the unplanted area of this plot which fixed only 18·7 g N ha^{-1} day^{-1} there was little difference between planted and unplanted parts of the plots.

Algal fixation

Algal cover and species composition. Table 1 shows the percentage cover of green and blue-green algae on particular plots. Estimates were made in September 1971 after the wheat had been harvested; it was not practicable to estimate cover under the standing crop.

TABLE 1

Percentage of plot area with visible algal cover

Plot No.	Designation in text	Fertiliser treatment	Green algae	Blue-green algae
22	FYM	Farm yard manure	0·37	0·58
09	High N+minerals	192 kg N ha^{-1}, P, K, Na, Mg	9·4	1·5
06	Low N+minerals	48 kg N ha^{-1}, P, K, Na, Mg	0·29	17·5
05	Minerals	P, K, Na, Mg	0·09	21·4
03	Nil	—	0·03	6·5

Each value is derived from 32 quadrat estimates each covering 100 cm^2

Bristol-Roach (1927) recorded 12 species of blue-green algae on Broadbalk of which *Nostoc muscorum* and *Cylindrospermum licheniforme* are now known to fix N_2. Species isolated on nitrogen-free agar during the present study were identified by Mr. A. E. George, Curator of the Culture Centre of Algae and Protozoa (Cambridge). Although the proportion of blue-green algae decreased with increasing nitrogen application the species composition was littled changed. The most prominent forms were *Cylindrospermum licheniforme*, *Nostoc ellipsosporum*, *N. muscorum* and an *Anabaena* species, possibly *A. cylindrica*.

Nitrogen fixation by algae was monitored by acetylene reduction using small soil surface cores (0·84 cm diam × 2·5 mm) during the years 1971 and 1972. Six samples were taken from each plot and incubated in the field under ambient conditions of temperature and light intensity.

Estimates of daily fixation were based on the assumption that fixation continues at a uniform rate throughout a 16 h day. Assays over a 24 h period showed that fixation rates reached a maximum towards midday and continued throughout the night at 20–30% of the midday rate (Witty, Keay, Froggatt & Dart, 1977). The error incurred by assuming that fixation stops for 8 h at night is largely offset by the decrease in fixation rate at dawn and dusk.

Fertiliser and herbicide treatment. The plots were treated in April 1971 with 5·6 litre ha^{-1} of 'Banlene Plus' (Fisons Ltd.), a mixture of MCPA, mecoprop and dicamba. The effect of fertiliser and herbicide treatment on N fixation is shown in Fig. 1 and Table 2.

High levels of N (196 kg N ha^{-1}) applied in the spring suppressed fixation entirely until August, but low levels (48 kg N ha^{-1}) stimulated fixation, particularly on plots not treated with herbicide. Fixation on unfertilised plots was low at the beginning of the season but increased in the absence of herbicide, until it exceeded that of all other plots.

Herbicide treatment reduced fixation until the end of July but increased fixation in August. This increase failed to compensate for the lower rates in June and July and Table 2 shows that the estimated seasonal fixation is reduced on all herbicide treated plots.

The fixation rates over the season (Fig. 1) are related to both rainfall and fertiliser and

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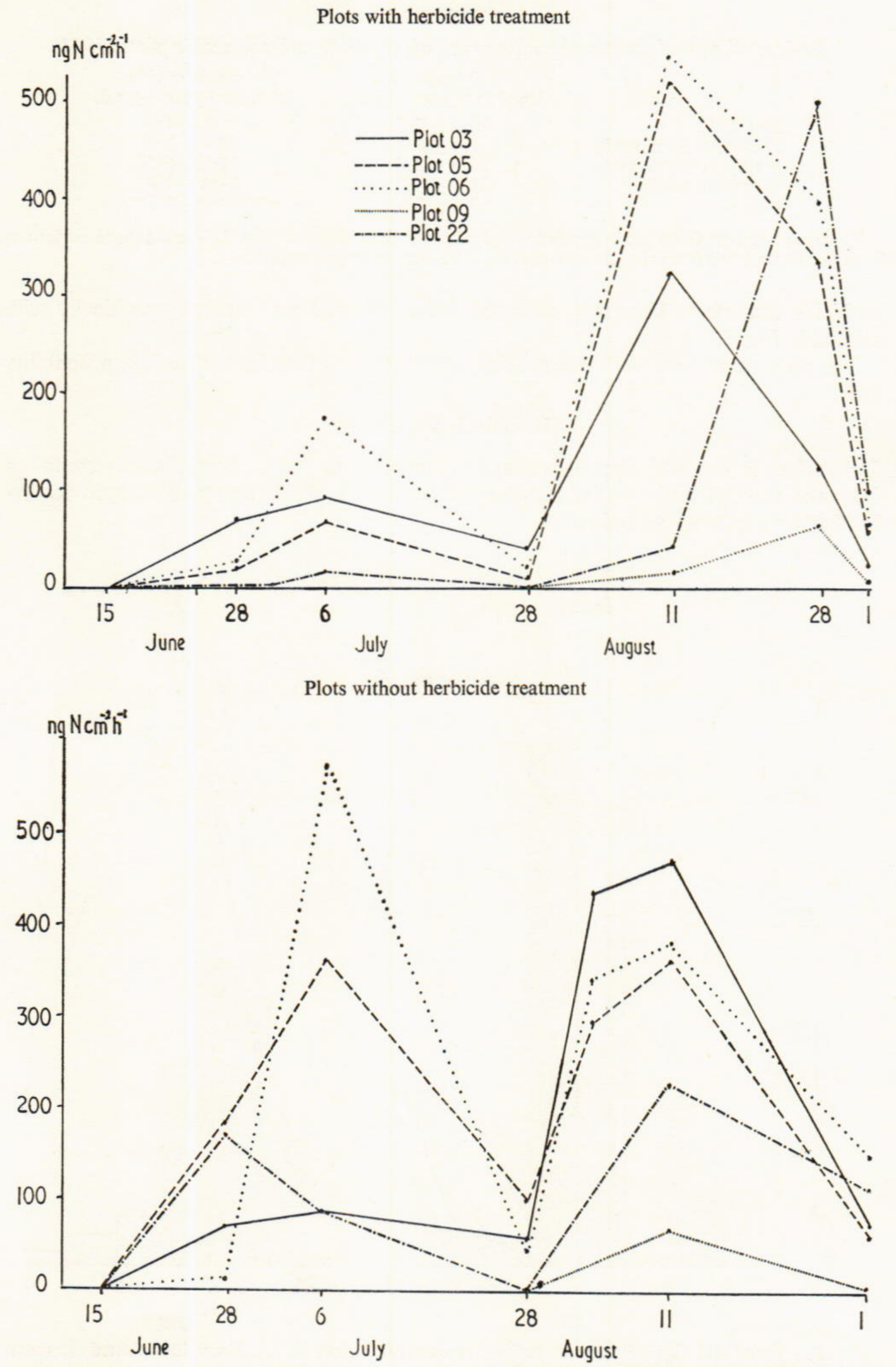

FIG. 1. Algal N fixation on Broadbalk Wheat plots (1971). The values plotted are the mean of six replicate surface samples assayed by acetylene reduction.

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TABLE 2

Estimated annual fixation by blue-green algae on Broadbalk arable plots, 1971

Plot	With herbicide kg N ha^{-1} year^{-1} $\pm$ s.d.	Without herbicide kg N ha^{-1} year^{-1} $\pm$ s.d.
22 (FYM)	$10 \cdot 7 \pm 5 \cdot 2$	$11 \cdot 2 \pm 4 \cdot 0$
09 (high N+ minerals)	$1 \cdot 4 \pm 3 \cdot 8$	$2 \cdot 4 \pm 3 \cdot 4$
06 (low N+ minerals)	$24 \cdot 6 \pm 2 \cdot 4$	$28 \cdot 2 + 6 \cdot 5$
05 (minerals only)	$22 \cdot 9 \pm 6 \cdot 0$	$23 \cdot 4 + 4 \cdot 8$
03 (nil)	$13 \cdot 4 \pm 2 \cdot 4$	$18 \cdot 9 \pm 4 \cdot 3$

Values are derived from the integrated weekly fixation data shown in Fig. 1. The standard deviations have been calculated from the summed variances on each sampling occasion

herbicide treatment. The rates, averaged for all plots, are compared graphically with rainfall in Fig. 2.

The rates were correlated with rainfall in the previous fortnight at the 2% probability level.

Broadbalk Wilderness

Soil surface layers, leaf litter, leaves and soil cores with and without plants were taken from the stubbed and wooded sections of Broadbalk Wilderness and assayed for N-fixation by acetylene reduction.

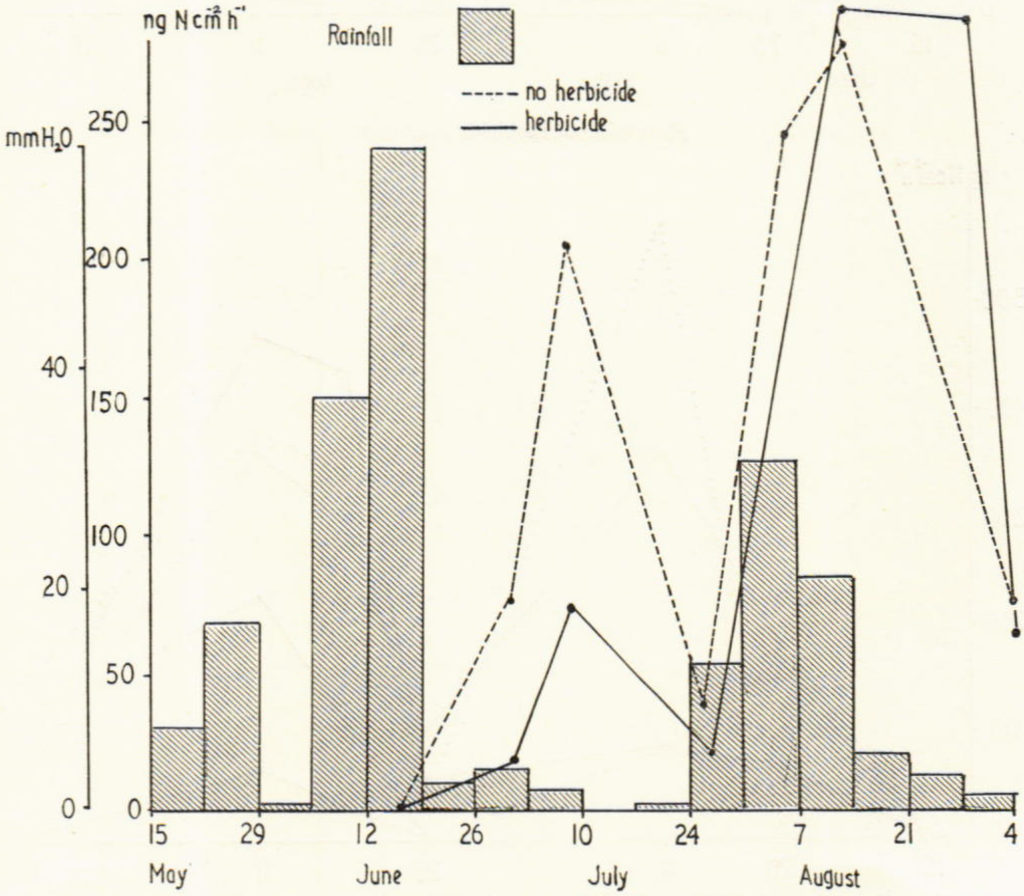

FIG. 2. Rainfall and algal nitrogenase activity in Broadbalk plots (1971). The values plotted are mean of all plots irrespective of fertiliser treatment.

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Cores were obtained by driving steel tubes (16 cm deep × 11 cm diam.) into the ground and removing them with the enclosed soil so as to disturb as little as possible the soil–bacteria–root relationship.

Almost no nitrogenase activity was detected in samples of the soil surface, leaf litter, leaves or soil cores not containing plants. Activity was associated with the roots of the following species of perennial weeds; *Heracleum sphondylium* (hogweed), *Stachys sylvatica* (hedge woundwort), *Hedera helix* (ivy), *Mercurialis perennis* (dog's mercury), and *Nepita glechoma* (ground ivy). Table 3 shows fixation rates derived from assays of core samples in June and July 1973.

TABLE 3

Nitrogen fixation in soil cores from Broadbalk Wilderness

Vegetation	June: Estimated fixation (g N ha^{-1} day^{-1})*	June: % soil moisture	July: Estimated fixation (g N ha^{-1} day^{-1})*	July: % soil moisture
	Stubbed area pH 6·8–7·2			
Hogweed	122 ± 12	28·0	106 ± 10	31·6
Hedge woundwort	112 ± 6	30·4	98 ± 8	29·7
Mixed	238 ± 24	31·7	52 ± 9	25·8
	Wooded area pH 6·9–7·2			
Hogweed	42 ± 3	21·4	32 ± 5·2	21·4
Ivy	39 ± 8	21·3	11·3 ± 0·6	19·5

* Means of four replicates

To avoid large-scale destructive samplings on the classical plots we assayed a nearby site, Manor Wood, which has a similar soil type and flora. The rates of N fixation at all sites tested was highly correlated with soil moisture (Fig. 3).

The variation in nitrogenase activity with depth was investigated at Manor Wood by taking cores in 50 cm deep × 11 cm diam. steel tubes and cutting tube and soil into 16 cm lengths.

TABLE 4

Variation in nitrogenase activity with depth at Manor Wood

Depth (cm)	Hogweed g N ha^{-1} day^{-1}	Ground ivy g N ha^{-1} day^{-1}	Hedge woundwort g N ha^{-1} day^{-1}
0–16	154	145	170
16–33	175	167	173
33–50	109	120	94
Total	438	432	437

The soil was uniformly wet at this site and the total activity for each plant species was similar with a maximum in the 16–33 cm layer. Activity in samples taken from Broadbalk Wilderness after heavy rainfall was high in the top 7 cm (equivalent to 136 g N ha^{-1} day^{-1}); rates were much lower (13–33 g N ha^{-1} day^{-1}) in the subsequent 7 cm layers. Since the top 7 cm layer was much wetter (34% H_2O top 7 cm, cf. 18–27% in lower layers) this difference probably reflects differences in soil moisture rather than differences in distribution of N fixing bacteria.

Discussion

The long-term N balance studies on Broadbalk described in part I of this paper (Jenkinson, 1977, Table 3, p. 107) suggest that there is a net annual fixation of 28 kg N ha^{-1} on

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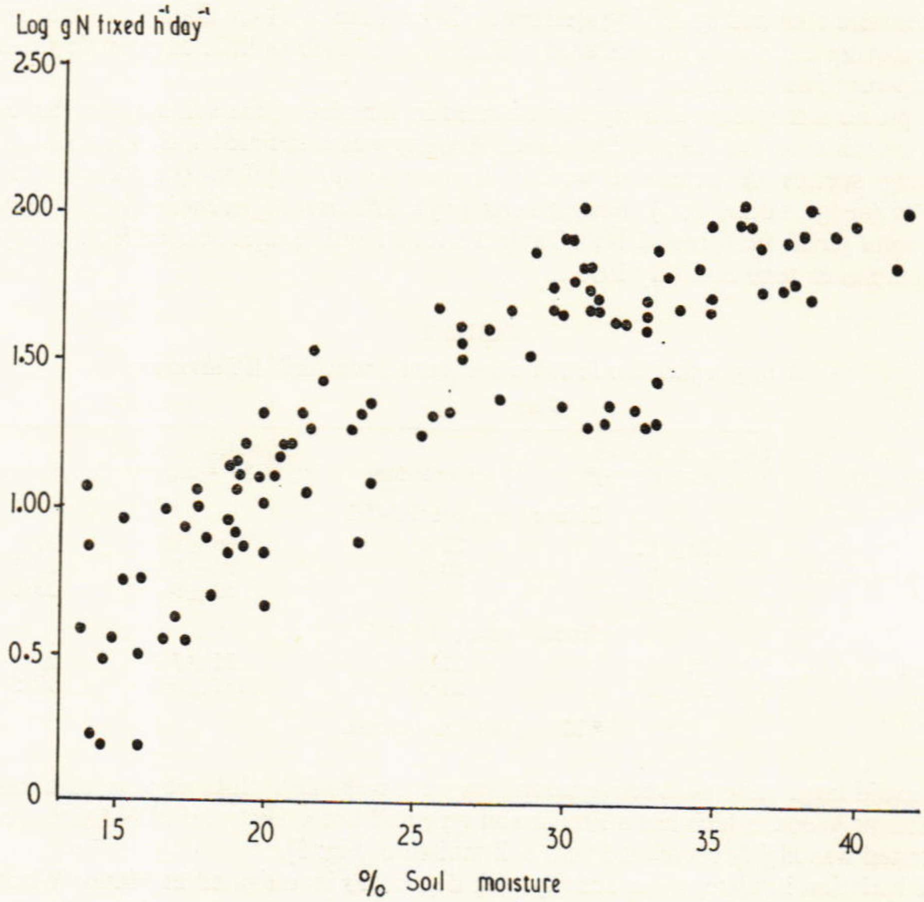

FIG. 3. Effect of soil moisture on nitrogen fixation by soil cores with plants, showing a highly significant positive correlation. From: Day, Harris, Dart and van Berkum (1975).

plot 03 (nil fertiliser) and 33 kg N ha^{-1} on plot 05 (minerals only). The most active fixation recorded in the wheat rhizosphere and soil was 45 g N ha^{-1} day^{-1}; even if this rate was continued for 100 days it could account for only 5 kg N ha^{-1} $year^{-1}$. A more significant input in these plots comes from blue-green algae which were estimated to fix from 19 kg N ha^{-1} $year^{-1}$ (plot 03 without herbicide) to 23 kg N ha^{-1} $year^{-1}$ (plot 05 without herbicide).

Algal fixation over the season is related to rainfall (Fig. 2) which during the period of monitoring was close to average (232 mm, cf. mean 234 mm).

Large amounts of N applied in the spring suppressed algal fixation until August (Fig. 1) when NO_3^--N at a depth of 5 cm had decreased from 600 to 6 ppm (Nair & Talibudeen, 1973). Apart from this evident fertiliser response it is difficult to separate the direct effect of fertiliser and herbicide treatment from indirect effects operating via the standing crop. The highest rate of fixation (28 kg N ha^{-1} $year^{-1}$) was recorded on plot 06 (low N and minerals). This plot had a sufficiently dense wheat stand to shelter the soil surface from desiccation but at the same time did not contain enough soluble N to suppress fixation. The rate of fixation in plot 03 (nil fertiliser) was also directly related to development of the plant canopy and reached a maximum in August when the canopy had closed (Fig. 1). The highest rate recorded was from the non-herbicide treated section of this plot indicating that the low nutrient status *per se* does not limit algal fixation.

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Lunkvist (1970) noted that the herbicides diuron and 2,4-D suppressed algal fixation at concentrations normally applied in the field but stimulated fixation at lower concentrations. The observed decrease in fixation on herbicide-treated plots early in the season may be a direct effect on the algae. The decrease is greater however on plots 05 and 06 which support more weeds, than it is on plot 03 (nil fertiliser) where weeds are scarce. This suggests that indirect effects via the weed canopy are involved.

Assays during periods of relatively warm weather in the winter failed to detect algal nitrogenase activity, although the soil was almost continuously moist. The development of algal N fixation has been recorded during the Antarctic summer in the South Orkney Islands at temperatures ranging from 4 to 10°C (Fogg & Stewart, 1968). The lack of fixation during periods of similar temperature in Britain may be attributable to either reduced photosynthesis in the shorter days or the lack of suitable algal species.

In contrast to the continuous wheat experiment N fixation in Broadbalk Wilderness was almost exclusively associated with the rhizosphere of non-leguminous weeds; legumes disappeared from this area in 1915 (Brenchley & Adam, 1915) and algal fixation on the soil surface was lacking, probably because of total shading by grass, weeds and leaf litter.

As with algal fixation on the arable plots, the limiting factor, overriding even inter-specific variations, was soil moisture (Fig. 3). Vlassak, Paul and Harris (1973) reported a correlation between soil moisture and nitrogen fixation in soil cores from grasslands. As soil becomes wetter the soil crumbs and rhizosphere become more anaerobic causing an increase in nitrogenase activity. Provided that sufficient water is available to support microbial activity, oxygen tension rather than soil moisture *per se* is more likely to affect the rate of fixation (Knowles, 1975). Broadbalk arable field had very little bacterial nitrogen fixation, probably because under normal agricultural practice such soils are rarely wet enough to support significant rhizosphere fixation. Activity would perhaps be greater in heavy soils, or under non-tillage wheat or in subsoils under agricultural crops.

The wooded sections of Broadbalk were rarely wet enough to support much rhizosphere fixation (Table 3). Fixation on leaves and leaf litter was negligible and the origin of the 49 kg N ha^{-1} $year^{-1}$ accumulated in the wooded section since 1882 (Jenkinson, 1973) is still uncertain.

At the first sampling, fixation in the top 16 cm of soil beneath mixed weeds on the stubbed section of the Wilderness was equivalent to 238 g N ha^{-1} day^{-1} (Table 3). This estimate must be low since samples from the Manor Wood site showed considerable activity below this depth (Table 4). Several other woodside and hedgerow sites in North Hertfordshire containing similar species to Broadbalk Wilderness were sampled and all had similar levels of activity. Rhizosphere fixation in the stubbed area of the Wilderness probably largely accounts for the accumulation of 34 kg N ha^{-1} $year^{-1}$ estimated in the long-term studies described in Part I of this paper.

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