Investigations into chemical aspects of peatland ecology with special reference to nitrogen fixation

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INVESTIGATIONS INTO CHEMICAL ASPECTS OF PEATLAND ECOLOGY, WITH SPECIAL REFERENCE TO NITROGEN FIXATION

A thesis submitted to the University of Durham for the Degree of Doctor of Philosophy.

By

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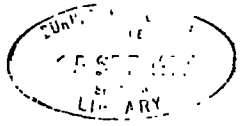

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Stimulus for the research reported here came primarily from Bill Williams, whose counsel helped me to more fully appreciate the art of scientific investigation; and David Bellamy, with whom I have spent many happy weeks on the peatlands of Southern Europe. My understanding of statistics has been greatly helped by many discussions with Jack Beggs. To these persons, for their advice and friendship, many thanks.

ABSTRACT

The first part of this thesis examines nitrogenase activity in Alnus, Myrica, Anabaena, Plectonema, and peat, with particular attention to field use of the acetylene reduction assay. The rate of nitrogenase activity was found to be very sensitive to temperature change, except for blue-green algae in reduced light. Low pO_2 values reduce the rate of nitrogenase activity by nodules of Alnus and Myrica. Nitrogenase activity in Anabaena occurs at 40%°C in condition of low pO_2. Characteristics of acetylene reduction by peat indicate that the reaction is caused by a living enzyme. A mixture of aerobic and anaerobic conditions stimulates acetylene reduction by peat, therefore it is suggested that microbial associations may be important for heterotrophic nitrogen fixation in peat. Theoretical and practical aspects of the relationship between nitrogenase activity and temperature are discussed.

The second part of the thesis is concerned with the distribution of certain elements, and heterotrophic nitrogenase activity in relation to peatland ecology. The nutrient ecocline in mires is defined by a floristic gradient, in relation to which the above mentioned factors are examined. Heterotrophic nitrogenase activity was estimated in peat collected from eleven different mire complexes; a trend of increasing activity was observed from bogs to rich fens, with a significant decline in extreme rich fens. The amounts of Ca, Mg, K, Na, Fe, Al, Mn and Zn, in peat and mire vegetation in relation to the ecocline are described, with nitrogen and phosphorus being discussed in detail. In rich mires, P, N and K are the elements most highly concentrated into the mire vegetation, in poor mires the most concentrated elements are N and Mn. With regard to cycling K, Mn, Zn and Na appear to be more mobile than other elements. The amounts of soluble N and soluble P are greatest in the peat from ombrotrophic sites, and in both cases becomes lower along the gradient in the direction of rich fens.

CHAPTER ONE :

INTRODUCTION TO PART ONE

1(a) Brief history of nitrogen fixation studies

The ability to incorporate atmospheric molecular nitrogen into organic compounds has been discovered only in certain micro-organisms. Most ecosystems depend very much upon these organisms because this element is required by all living things in large quantities, and atmospheric nitrogen is virtually the only source.

The micro-organisms which perform biological nitrogen fixation are members of the blue-green algae, the actinomycetes, the bacteria, and the yeasts. Most of the species are free-living, but a few form symbiotic relationships with higher plants; in these cases the micro-organism involved usually grows in root or leaf nodules of its symbiotic partner. From an agricultural point of view the legumes are of outstanding importance, this is the only group of plants used in agriculture where the symbiotic relationship is, as yet, known to exist. The micro-organism involved in this relationship belongs to the bacterial genus Rhizobium. Fourteen genera and 118 species of non-legumes are known to possess nitrogen fixing nodules, of which all but one are angiosperms; the other, Podocarpus, is a gymnosperm (Rodriquez-Barrueco, 1969). In most of these non-legume species the micro-organism involved is believed to be an actinomycete (Plotho, 1940).

The value of legumes was well known to the ancient world (Fred, et al 1932), but the first experimental evidence that these plants could utilise atmospheric nitrogen is attributed to Boussingault in 1837 (Wilson, 1957). However, it was not until 1888 that Beijerinck isolated the bacterium responsible for nodule formation, he called it Bacillus radicicola

(Beijerinck, 1888), which was later changed to Rhizobium leguminosarum. The earliest suggestion that non-leguminous plants might have the ability to fix nitrogen came in 1892 when Nobbe and others noted that nodulated specimens of Elaeagnus grew more vigorously than non-nodulated plants. In 1897 Hiltner reported a similar observation for Alnus and in 1904 he provided more definite evidence by demonstrating that this species would not grow successfully in nitrogen deficient soils if the root nodules were excised (Bond, 1963). In spite of these early discoveries it was not until 1932 that direct proof of non-leguminous fixation was obtained by Aldrich-Blake who measured the actual nitrogen gains of Casuarina equisetifolia plants growing in nitrogen-free media (Aldrich-Blake, 1932).

At the turn of the century the free-living anaerobe Clostridium paseurianum (Winogradsky, 1893), and the aerobic Azotobacter (Beijerinck, 1901) were isolated from the soil. It should be mentioned that throughout the preceding half century, the existence of these organisms had been postulated by many workers (e.g. Davy, Jodin, Berthelot), but none had managed to isolate and culture them. It was not until 1928 that Drews reported definite evidence of nitrogen fixation in blue-green algae (Drews, 1928).

Almost a century of slow progress is testimony to one simple fact: that the gas nitrogen is very inert, and therefore difficult to measure. No such problems have bedevilled progress in photosynthesis because carbon dioxide, by contrast, is very easy to quantify. The only methods which were available for the study of nitrogen fixation until the early 1950's involved growing organisms on nitrogen-free media, and measuring the nitrogen gain using Kjeldahl digestion followed by distillation. Attempts have been made to estimate the loss of nitrogen from closed containers (Umbreit et al., 1951; De and Mandal, 1956), but these

manometric methods were never extensively used outside of the laboratory.

In 1941 a report appeared in the magazine 'Science' suggesting that the heavy isotope of nitrogen might be useful in biological research (Burris and Miller, 1941). So there was, at last, a method whereby nitrogen fixation could be measured directly. After the isotope became widely available in the late 1940's nitrogen fixation was confirmed in many species, the first being the pigmented bacterium Rhodospirillum rubrum (Kamen and Gest, 1949). In the context of this investigation the work of Bond in the early 1950's is noteworthy in that he confirmed nitrogen fixation in nodules of various non-legume species using ^{15}N (Bond, 1955).

During this period there were many attempts to obtain a cell free extract of active nitrogenase, this was finally achieved in 1960 by a group who successfully extracted the active enzyme from Clostridium pasteurianum (Carnahan et al., 1960). Once a method of obtaining the extract was established, a variety of alternative substrates to N_2 were identified using these in vitro systems. It was discovered that CN^-, N_2O, N_3^- and CH_3NC could all serve as alternative substrates; but it was the substrate acetylene that was to have the greatest impact on research into nitrogen fixation.

Although the application of ^{15}N to nitrogen fixation studies had been a major development, there were, and still are, problems associated with its use. Estimation of ^{15}N in protein requires Kjeldahl digestion and distillation followed by gas analysis on a mass-spectrometer. This instrument is expensive and requires considerable expertise on the part of the operator. Secondly, the isotope is also very expensive, and must be used at high concentration in order to achieve maximum sensitivity with the method. These problems are disinsentives for carrying out

many replicate tests, and were probably the reason for Bond concluding at first, on the basis of ^{15}N studies, that nitrogen fixation in Myrica nodules was unaffected by reductions in the partial pressure of oxygen (Bond, 1957), but later deciding the opposite (Bond, 1961).

No such problems were associated with acetylene reduction: gas chromatographic apparatus is relatively inexpensive and easy to use, acetylene is cheap, and the method is simple. Another major advantage is that the technique can be 100 time more sensitive than the isotope method. In 1967 Stewart et al., indicated the potential of the method by easily demonstrating nitrogenase activity in legumes, non-legumes, soil, and blue-green algae (Stewart, et al., 1967).

Unencumbered by the problems associated with the isotope technique the acetylene method soon found great favour, stimulating both research and publications on nitrogen fixation. One of the pioneer groups carried out about 40,000 tests in 4 years (Hardy et al., 1973). Using ^{15}N, the cost of the isotope alone would have been more than US $250,000.

Important developments were soon made; substituting vanadium in the prosthetic group of the enzyme (Burns, et al., 1971); development of functioning chemical models of nitrogenase (Schrauzer and Doemeny, 1971); in vitro symbiosis between Rhizobium and soybean cells (Holsten et al., 1971); and recently non-symbiotic cultures of Rhizobium have been persuaded to reduce acetylene by manipulation of their growth medium. This development was reported simultaneously by three research groups in the magazine 'Nature' (see Nature 256, 1975), and it is significant that not one of the three independent teams that made this important discovery felt it necessary to confirm their findings with ^{15}N. The ease with which nitrogen fixation could now be detected also gave rise to reports of the process in unexpected locations such as the Camel's rumen (Elleway et al., 1971) and human faeces (Bergersen and Hipsley, 1970).

1(b) Problems of the acetylene reduction method

During the late 1960's and early 1970's, ecology entered into unholy wedlock with systems analysis, and it has become very fashionable to try and measure the amounts of various elements which are moving through ecosystems; actual figures are quoted where generalities once held sway. A flush of ecology papers have reported amounts of nitrogen fixed based upon acetylene reduction, and utilising knowledge of the theoretical electron requirements, which indicate that acetylene reduction should proceed three times faster than nitrogen fixation; such a ratio has been well established in laboratory conditions (Hardy et al., 1973). But any who believed that acetylene reduction was to be a universal panicea for field measurements of nitrogen fixation were soon to be disabused by reports that the ratio varied with environmental conditions (Bergersen, 1970), and could be as high as 15-1 in some circumstances (Rice and Paul, 1971). However, such problems do not detract from the potential of acetylene reduction as a tool to assist investigations designed to gain understanding of the ecology of nitrogen fixation, in contrast to actually measuring it.

There are many parameters which affect the acetylene reduction tests, including aeration and temperature, both of which are subject to considerable fluctuations in the soil, and changes due to the actual assay procedure. There is a considerable body of information relating to the effect of oxygen on both nitrogen fixation and acetylene reduction, however this is not so with temperature. Therefore, although both of these factors are discussed in the first half of this thesis, special emphasis is placed upon temperature.

Field assays are carried out at many different temperatures, but when this work was started it was difficult to ascertain from the literature just how important temperature fluctuations might be. In his

1966 book Stewart gave equal weight to reports that temperatures did, and did not affect nitrogenase activity, but more recent reviews of nitrogen fixation generally conclude that the process is temperature sensitive. (Mishustin and Shil'nikova, 1971; Hardy et al., 1973).

However, the extent and pattern, if any, of this sensitivity is by no means apparent; indeed many research reports have reached contradictory conclusions on the matter of temperature. Growth experiments led to the conclusion that temperatures above about 20°C were inhibitory to nitrogen fixation in Trifolium subterraneum (Mes, 1959; Meyer and Anderson, 1959). Furthermore, support for the contention that temperature was directly affecting the fixation process was obtained from the fact that the nitrogen content of plants grown with an inorganic nitrogen source was unaffected by temperature (Possingham, et al., 1964). The advent of the acetylene reduction assay permitted a more direct approach to the investigation of temperature, and using this technique Roughley and Dart (1969) concluded that temperature did not affect nitrogenase activity per se in Trifolium subterraneum. However, later reports indicated not only that acetylene reduction in this species was slightly sensitive to temperature below 20°C, but also that a rise from 20°C to 30°C was not directly inhibitory (Dart and Day, 1971; Gibson, 1971). It remains to be established whether or not these contradictions are merely the result of different experimental procedures.

Acetylene reduction by cell free extracts of Azotobacter vinlandii is very temperature sensitive (Hardy et al., 1968; Burns et al., 1971), and considerable sensitivity to temperature change has also been established for acetylene reduction by whole cells of Clostridium pasteurianum (Hardy et al., 1968).

There are less data available on the relationship between temperature and nitrogenase activity in other groups of plants. However,

the few acetylene reduction assays of detached nodules of Alnus carried out by Wheeler (1971); and the ^{15}N tests in natural populations of blue-green algae (Stewart, 1970) suggested that nitrogenase activity in both these groups may be very temperature sensitive.

One of the main objectives of the investigations reported in the first half of this dissertation has been to gain information on the way in which the rate of acetylene reduction is affected by temperature change. Any overall pattern of temperature response or even patterns within the various groups might be both of theoretical and practical interest. To this end, results obtained in the experiments described here are discussed in the light of those obtained by other workers, in order to gain a better overall understanding of the relationship between nitrogenase activity and temperature.

1(c) Nitrogen fixation in peatland

Two members of the mire flora have long been established as nitrogen fixing species: Alnus spp. which grow in rich fen situations, and Myrica gale which is more common on poor fens. Pollen analysis indicates that both Myrica and Alnus were once far more widespread than now (Tansley, 1939); thus the possibility exists that, having improved the nitrogen status of their respective communities, they were unable to compete in the later stages of succession when nitrogen may no longer have been a limiting factor. More evidence of this possibility is found in recent North American studies, where it has been demonstrated that alder dominated the vegetation during the phase of nitrogen accumulation on the till in Glacier Bay, Alaska (Crocker and Major, 1955; Lawrence et al., 1967). Pollen analysis indicates that throughout the whole of N.E. America, alder initially colonised the wetlands on retreat of the Wisconsin ice sheet, to be succeeded in many regions by poplar and pine.

Where Alnus and Myrica occur they undoubtedly make a contribution to the pool of biologically fixed nitrogen, although exactly how much remains to be determined. However, these species are of restricted distribution in comparison with the vast areas of peatland which exist in the northern hemisphere. Although a considerable amount of ecological work has been carried out on peat-producing ecosystems, especially regarding the relationships between the constituent mire flora and the mineral content of the mire waters (see Chapter 6), little is known about the nitrogen balance of these systems. Holden (1966) states that the input of nitrogen by precipitation in the Scottish Highlands (much of which is covered by peat), is between 0.1 and 1.8 g/m^2/year, whereas a figure of $4g/m^2$/year is quoted for nitrogen uptake by tundra mire forests (Rodin and Basilevich, 1967). P'yavchenko (1960) concluded that nitrogen fixation could contribute to the enrichment of sphagnum peat but offered no direct evidence. More recently Sonesson (1970b) concluded that there was a considerable deficit between the nitrogen accumulated in growing peat on some poor mires in Sweden, and the input by precipitation.

This lack of information represents a serious gap in our knowledge of the biosphere, especially when the following facts are taken into consideration: (a) mires cover more than 150 million hectares of the earth's surface (Tibbets, 1969); (b) they include ecosystems, the entire nutrient supply of which is contained in the rain falling directly upon them; (c) many, including the latter type, are being ameliorated for forestry and agriculture.

During the past 50 years there have been numerous reports of nitrogen-fixing bacteria being isolated from peat soils. (e.g. Snyder and Wyant, 1932; Wilson and Wilson, 1933; Vavulo, 1958; Popova, 1961; Boyd and Boyd, 1962). However, most of these investigations have been

based upon traditional bacteriological methods of isolation and estimation, with the inherent problems of interpreting the findings in terms of the field situation.

The acetylene reduction assay permits a more direct approach to the study of nitrogen fixation than traditional bacteriological methods; unfortunately, conditions of the assay are not the same as in the undisturbed peat, and in order to obtain maximum value from the results it is necessary to know how the assay is affected by different environmental conditions. Furthermore, combining this information with the accumulated knowledge of the reaction in pure cultures can afford us insight into physiology of the peat microflora with respect to nitrogen fixation, in addition to providing estimates of the level of activity.

The use of acetylene reduction on peat does present an additional problem: the possibility exists that some of the numerous non-living products of decay might catalyse the conversion of acetylene to ethylene. Therefore various tests were designed to substantiate the validity of acetylene reduction results obtained for peat, these are described in Chapter 4.

The experiments reported in this first part were intended to obtain information regarding the way in which acetylene reduction by some of the nitrogen fixing components in mire ecosystems is affected by different conditions of light, oxygen and temperature. It is hoped, not only that the results might enable the method to be used with greater confidence in this sphere, but also that some of them might be of theoretical interest in their own right.

A full discussion of nitrogen fixation in mire ecosystems is presented in part two, where results of the field assays on peatland are presented in detail.

CHAPTER TWO :

INVESTIGATION OF NITROGENASE ACTIVITY IN DETACHED NODULES OF NON-LEGUMES

2(a) Plant material

The species studied were Myrica gale L., and Alnus glutinosa (L) Gaert. Whole plants of Myrica were dug from Filingdales Moor, Yorkshire, and then taken to the laboratory in plastic bags with the nodules still attached to the roots. Nodulated roots of Alnus were dug out of the river bank at Waldridge Fell, Co. Durham; these were also embedded in peat and taken to the laboratory in plastic bags.

In the laboratory the root systems were cleaned with tap water, the nodules removed, and the experiments started as soon as possible.

2(b) Experiments with temperature : methods

Detached nodules of the species under investigation were bulked, and sub-sampled into replicate aliquots of about 3-5 g fresh weight; dry weights of each sub-sample were determined after completion of the experiments. The sub-samples, together with a 1 cm diameter disc of moist filter paper, were placed in 30 ml containers fitted with screw tops and rubber liners.

The ambient gas phases were exchanged for a mixture containing 78% Ar and 22% O_2. This exchange was achieved by flushing with the aid of a hand pump. The tubes were allowed to equilibrate for 30 min in water baths stabilised at various temperatures, before starting the assays by injecting C_2H_2 calculated to give 10%. The ambient gas phases in the chambers at the start of the temperature experiments contained 70% Ar, 20% O_2, and 10% C_2H_2.

Ten minutes separated the start of each temperature set of 5

(Myrica) or 7 (Alnus) replicate assays, this allowed time for the ethylene produced to be determined at the end of 40-50 minutes incubation, by injecting directly into the gas chromatographic apparatus; the exact incubation time of each replicate was recorded to enable rates per unit time to be estimated. Linearity of the time courses was established prior to starting the experiments.

Previous experiments had shown that the rate of activity was affected by nodule age, therefore in order to reduce variability only the more active young clusters were used.

Ethylene in all investigations was analysed on a Varian Aerograph 1200 gas chromatographic apparatus fitted with a hydrogen flame detector. The 183 cm x 6.5 mm stainless steel column was filled with Poropak T and operated at 80°C, the detector temperature was 110°C.

The ethylene contamination of the acetylene was checked and subtracted in the final claculations. The possibility of ethylene from biological activity other than nitrogenase was monitored in all experiments.

2(c) Experiments with oxygen : methods

These were performed in 110 ml flasks fitted with Suba Seal closures. The larger volume was used in order to reduce the effect of gas changes resulting from respiratory activities of the nodules during the assay.

About 3-5 g fresh weight of nodules was placed in each of 3 bottles. The atmosphere in these bottles was replaced with argon by flushing with the aid of a hand pump. Volumes of oxygen were then injected into each bottle, these volumes were calculated to give 20% in the ambient gas phase after the assays had been started by injecting C_2H_2 to give 0.1 atm. After an incubation period of 45 minutes the ethylene produced was

estimated by injecting a 1 ml sample directly into the gas chromatographic apparatus.

All three bottles were then completely reflushed with argon and the process repeated with an injection of oxygen to give 15% O_2 at the start. Altogether this process was repeated 6 times with starting oxygen concentrations of 20%, 15%, 10%, 3% and 1%, before finally repeating at 20% to ensure that the specific reaction rate had not changed. This non-destructive method was used in order to circumvent the problems of high variability that had been experienced in the earlier experiments, the technique was made possible by virtue of the fact that the time courses for both species were linear for at least 5 hours (Silver and Mague, 1970; Waughman, 1972).

2(d) Seasonal fluctuations : methods

Due to the distance of the Myrica location these tests were performed only with Alnus.

The assays were set up as described for the temperature experiments, except that 9 replicates were used. These assays were carried out in the field on young nodules washed in stream water. The method of performing the assay in the field has been previously described (Waughman, 1971). The temperature and leaf state were noted on each occasion that field tests were carried out.

2(e) Effect of oxygen : results and discussion

Results of the experiments with oxygen are presented in Figure 3. In both species the rate of acetylene reduction increased with increase in the oxygen concentration from 0-15%, further increase in the oxygen concentration to 20% had no additional effect on activity in the detached Alnus nodules, whereas a similar increase in oxygen concentration con-

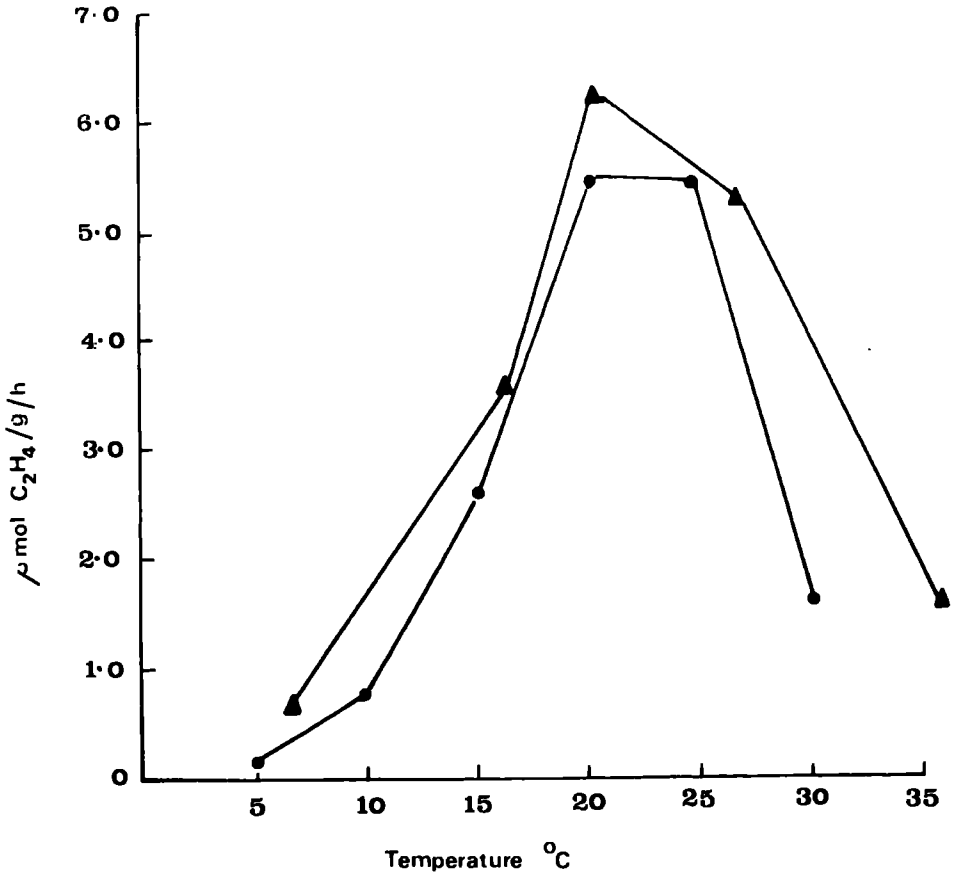

FIG. 1 EFFECT OF TEMPERATURE ON ACETYLENE REDUCTION BY DETACHED NODULES OF ALNUS (●), AND MYRICA ÷ 3 (▲)

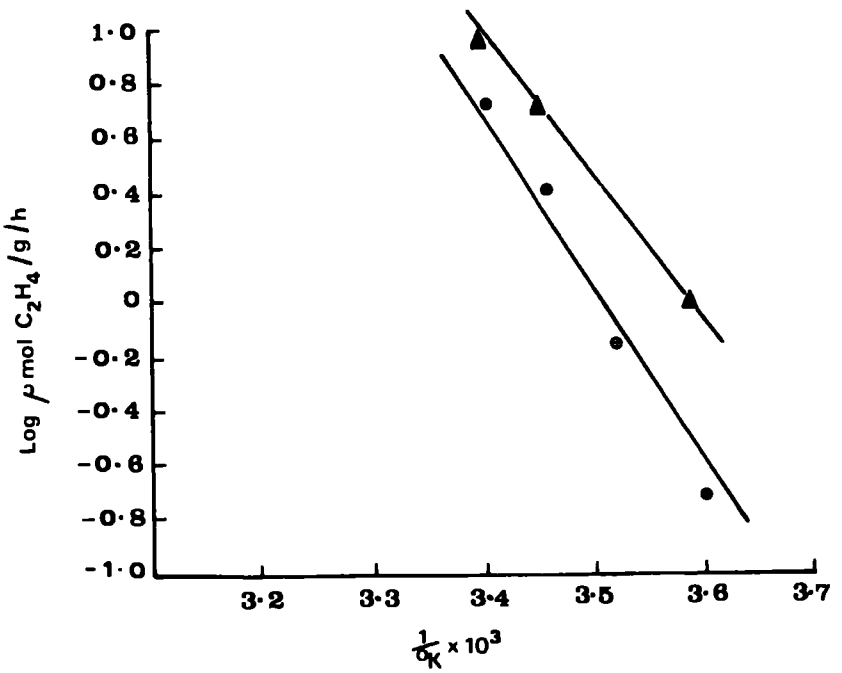

FIG. 2 ARRHENIUS PLOTS OF DATA IN FIG 1 (MYRICA ÷ 2).

tinued to stimulate activity in the Myrica nodules to rates higher than those obtained at 15%. Results for both these species confirm the later work of Bond (1961) who used ^{15}N; in his earlier studies Bond had concluded that nitrogen fixation by Myrica nodules was not oxygen sensitive (Bond, 1957). Sloger (1968), who carried out acetylene reduction tests at only two different pO_2 values, also concluded that the process in Myrica nodules was oxygen sensitive.

The environment of both these species is fairly anaerobic, therefor unless some oxygenating mechanism exists it would appear that the nitrogenase in both species normally operates well below maximum efficiency. It has been suggested that the long nodule rootlets of Myrica and the conical out-growths which sometimes occur on Alnus nodules, may have an aerating function (Bond, 1952; Fletcher, 1955).

The sensitivity to oxygen is not sufficiently great to complicate the actual assay, provided the incubation times are kept short enough to avoid drastic reduction of oxygen in the ambient gas phase; it was mentioned above that linear time courses of several hours were confirmed in both species for the experimental conditions used. The major question associated with oxygen and the assay of nodules in these two non-legumes is whether or not they are active in the anaerobic conditions of the soil. Testing of nodules embedded in fairly large volumes of peat might provide some clue to the answer.

2(f) The effect of temperature : results and discussion

Acetylene reduction in both species was very temperature sensitive, with optima of about 20°C (Figure 1). These results agree with those obtained in experiments with ^{15}N for Alnus viridis (Beneck, 1970) and Myrica cerifera (Sloger, 1968). The decline in activity above the optimum was very rapid (Figure 1). There is no evidence in the literature

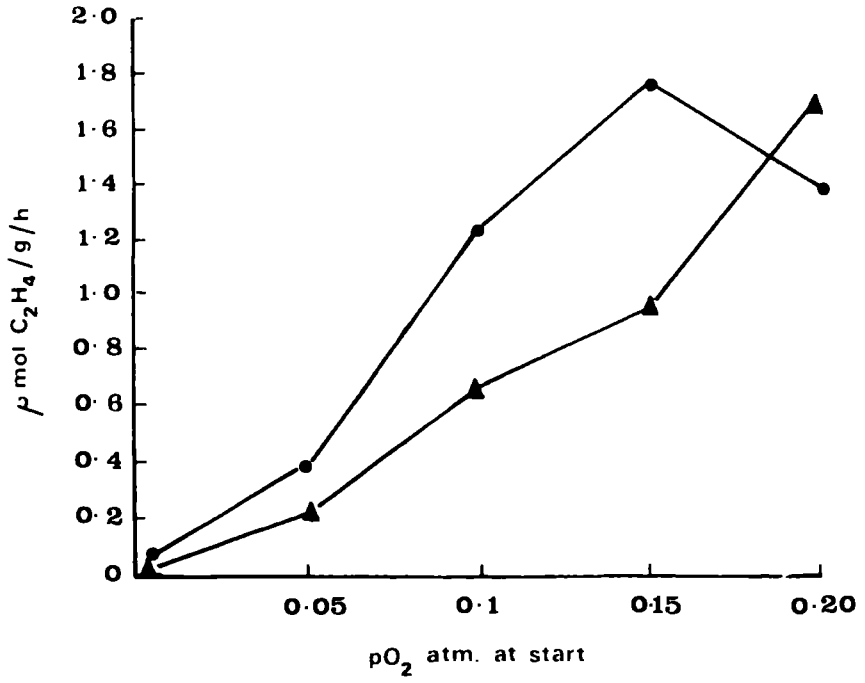

FIG. 3 EFFECT OF OXYGEN ON ACETYLENE REDUCTION BY DETACHED NODULES OF ALNUS (●). and MYRICA ÷ 10 (▲).

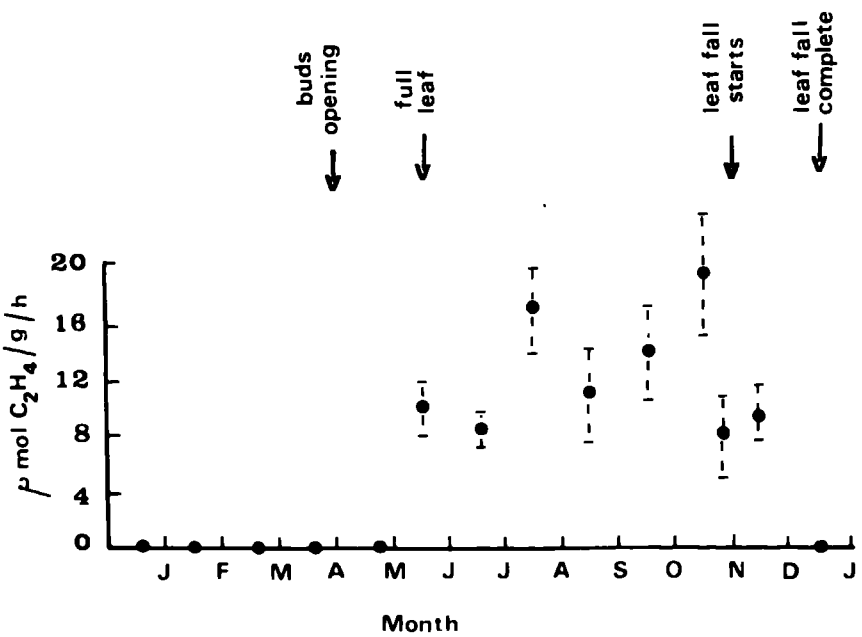

FIG. 4 SEASONAL NITROGENASE ACTIVITY IN ALNUS. BARS INDICATE p = 0.05.

that nitrogenase in any species is thermolabile at 20°C, therefore it is possible that the rapid decline above the optimum may have resulted from increased rates of oxygen diffusion at the higher temperatures: high pO_2 values are inhibitory (Bond, 1961), and whatever mechanism protects the enzyme from oxygen, its capacity to do so must be limited.

The energy of activation for both these species (and all other species used in this investigation) was obtained by plotting a graph of $\log_{10}$ rate vs $^1/o_K$ (an Arrhenius plot). When this relationship is linear, the slope b is related to the activation energy E by:

$$E = -4.576b \text{ cal/mol}$$

An activation energy of 38 Kcal/mol was calculated for Alnus and 30 Kcal/mol for Myrica (Figure 2 and Table 4).

Arrhenius plots of response to temperature change have certain advantages with regard to identifying changes of rate and limiting reaction, as well as simplifying temperature corrections. Arrhenius plots and activation energy are more fully explained in Chapter 5, together with a full discussion of the temperature response of acetylene reduction by various species.

2(g) Seasonal fluctuations : results and discussion

The labour involved in finding nodules restricted the number of assays that could be performed during each field visit. This fact, combined with the great variation in specific activity of different nodules, prevents the formulation of detailed conclusions relating to any monthly variation. However, the results displayed in Figure 4 show that there is no activity in the winter; that the start of activity in the spring is coincident with the trees coming into leaf; and that ceasation in the autumn is associated with leaf fall. Such a relationship between foliation and nitrogen fixation also exists in legumes

(Hardy et al., 1968; Moustafa, 1969; Gibson, 1971) and in the non-legume Hippophae (Waughman, unpublished results).

Daily cycles of nitrogen fixation have been detected in both Alnus and Myrica, and these fluctuations have been shown to be associated with the flow of photosynthate from the leaves (Wheeler, 1971); no autonomous rhythms have been detected. Dullart (1970) found considerable quantities of I.A.A. in root nodules of Alnus glutinosa, but did not find any seasonal variation in the auxin content. These reports suggest that the seasonal cycle of nitrogenase activity in Alnus is simply a manifestation of the seasonal energy supply from the leaves.

CHAPTER THREE :

EFFECT OF TEMPERATURE ON NITROGENASE ACTIVITY IN BLUE-GREEN ALGAE

3(a) Choice and culture of material

Anabaena was selected for study because of its occurrence in mire systems (Flensburg, 1967). Plectonema has not been mentioned in lists of algae recorded in mires, however the fact that it fixes nitrogen only in anaerobic conditions is of special interest, particularly when the anaerobic conditions in mires are considered. It is possible that other species of blue-green algae may also be obligate anaerobes with respect to nitrogenase activity. For example Lyngbia is a species which occurs in mires, and appears to have similar nitrogen fixing characteristics to Plectonema (author unpublished); unfortunately a supply of Lyngbia suitable for experimental purposes was not available.

Anabaena cylindrica Lemm. from the Cambridge University collection was cultured from stock in 25 ml aliquots of nitrogen free ASM (Gorham et al., 1964) sparged with air; NaCl replaced $NaNO_3$ in the growth medium. The cultures were grown in 100 ml medical flats under a light intensity of about 3000 lux. Plectonema boryanum strain 594 from the Indiana University collection was cultured in the same manner as Anabaena, except that the medical flats were sparged with a gas mixture containing 99.96% Ar and 0.04% CO_2 in order to obtain material containing nitrogenase (Stewart and Lex, 1970).

3(b) Temperature experiments : methods

Algal material was harvested by slow speed centrifugation; after discarding the supernatent, 1 ml aliquots of the algal suspension were placed into 7 ml serum bottles together with 0.1 ml of 0.5 m molar

$NaHCO_3$ in order to maintain an appropriate CO_2 level in the ambient gas phase. The effect of temperature change on nitrogenase activity in Anabaena was assayed under conditions of light saturation (Cox and Fay, 1969) and a starting oxygen concentration of 20%; saturated light and reduced pO_2; and reduced light with 20% O_2. The effect of temperature change on nitrogenase in Plectonema was tested under anaerobic conditions with both bright light and reduced light.

In normal aerobic tests the ambient gas phases were exchanged for a mixture containing 77.96% Ar; 22% O_2; and 0.04% CO_2. In the experiment with Anabaena using reduced pO_2 the mixture was 97.76% Ar; 2.2% O_2; and 0.04% CO_2. A mixture of 99.96% Ar and 0.04% CO_2 was used for the anaerobic tests with Plectonema.

Triplicate sets of assays were set up at each temperature. The tubes were pre-incubated at the appropriate temperature for 15 minutes, then the assays were started by injecting C_2H_2 to give 10%. All incubations and pre-incubations were carried out in water baths set at various temperatures.

Incubations were terminated after 30 minutes by injecting 0.5 ml of trichloroacetic acid (50% w/v) into each serum bottle, after which ethylene was estimated on the gas chromatograph.

3(c) Plectonema : results

The two species used in this study were chosen because they have contrasting physiology with regard to nitrogenase activity, in which respect Plectonema is an obligate anaerobe, whereas Anabaena is not.

Plectonema, assayed under anaerobic conditions at a light intensity of about 6000 lux had an exponential response to temperature between 5°C and 20°C; the reaction was also very temperature sensitive from 20°C to the optimum of about 30°C. In conditions of reduced light, activity

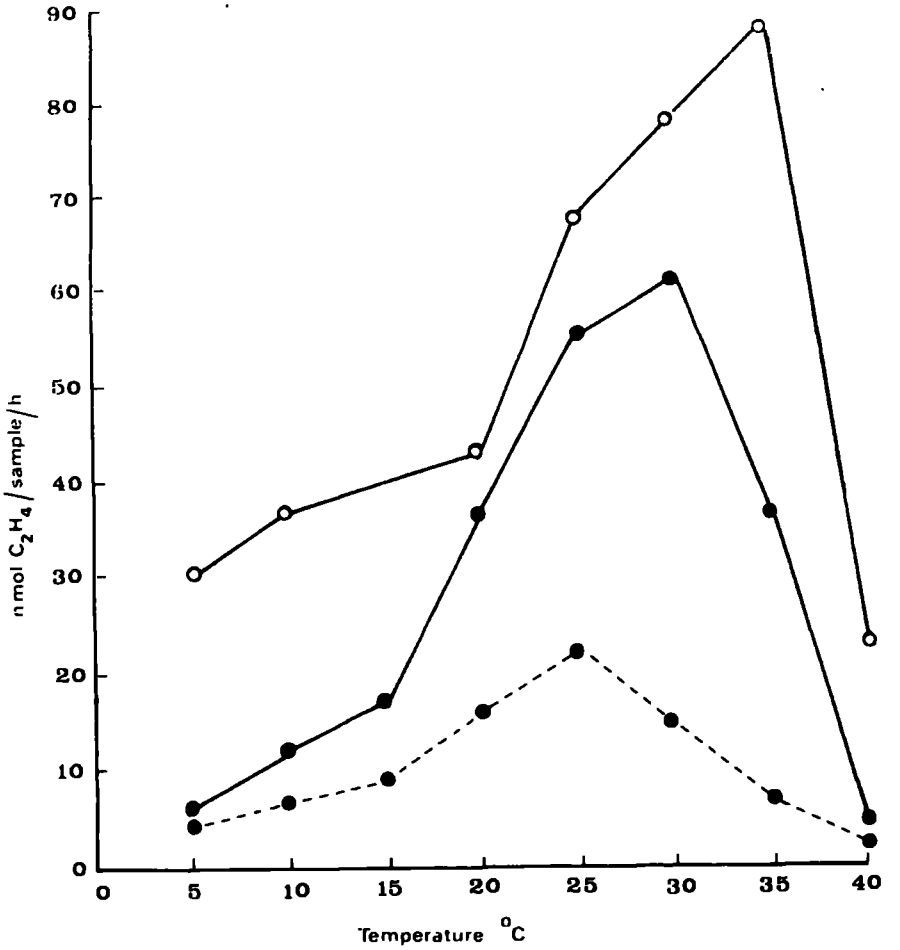

FIG 5 EFFECT OF TEMPERATURE ON ACETYLENE REDUCTION BY ANABAENA:
AEROBIC ●——● : LOW LIGHT ●- - -●: LOW OXYGEN CONCENTRATION ○——○

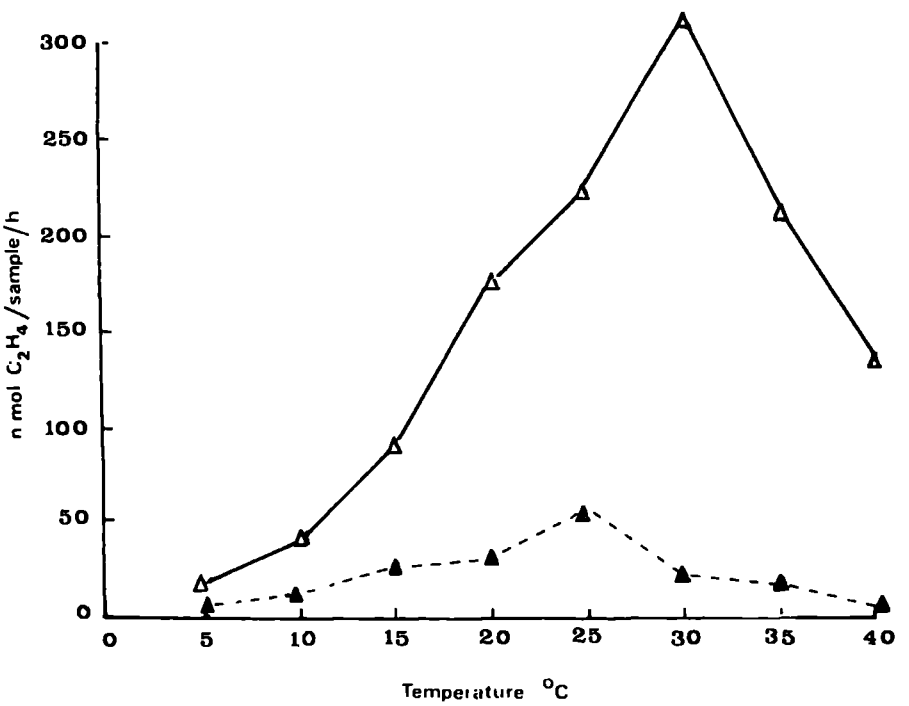

FIG.6 EFFECT OF TEMPERATURE ON ACETYLENE REDUCTION BY PLECTONEMA;
FULL LIGHT △——△ : LOW LIGHT ▲- - -▲ .

increased with increasing temperature between 20°C and 25°C, but was very insensitive to temperature below 20°C; the optimum was also lower at 25°C (Figure 6). An Arrhenius plot of the results obtained under full illumination gave two discontinuous slopes; the discontinuity occurring at 20°C (Figure 7). Activation energies estimated from these slopes were 30 Kcal/mol below, and 12 Kcal/mol above the discontinuity.

3(d) Anabaena : results

In conditions of full light intensity and a pO_2 of 0.2 atm., the pattern of temperature response of nitrogenase activity in Anabaena was similar to that of Plectonema (Figure 5). The activation energy below 20°C was about 27 Kcal/mol (Figure 7), however, the Arrhenius plot did not give a straight line above this temperature. In reduced light the reaction was unaffected by temperature change between 5°C and 15°C, but responded to increasing temperature above 15°C.

The optimum oxygen concentration for nitrogenase activity in Anabaena is about 10% (Stewart and Pearson, 1970), and concentrations lower than 20% are generally favourable to nitrogenase activity in this species. Reduction of the oxygen tension in the ambient gas phase to 2% generally reduced the sensitivity to temperature change in this species, as compared with the response in 20% O_2. Under conditions of reduced oxygen concentration the optimum was also higher at 35°C, and even at 40°C the reaction was still proceeding at a considerable rate (Figure 5).

3(e) Implications for field assays

Only two projects have so far specifically investigated acetylene reduction by blue-green algae in mire systems; these two reports, and others relating to blue-green algae in mires are discussed in Chapter 4.

The gas phase used during a field assay must be either aerobic or

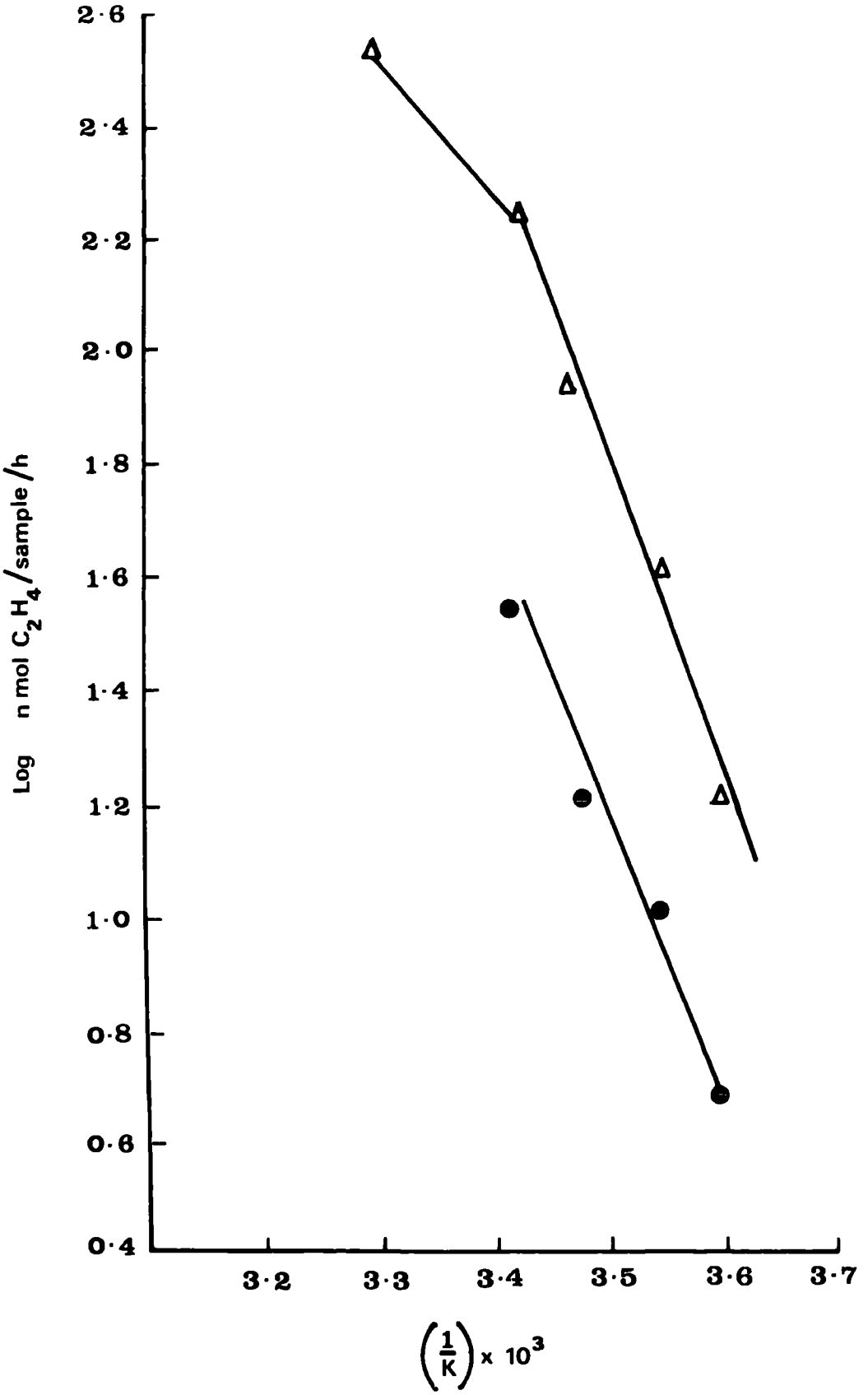

FIG.7 ARRHENIUS PLOTS OF DATA IN FIGS. 5 AND 6 (ANABAENA x 20).

anaerobic according to the type of blue-green algae under investigation; the results for Anabaena (Figure 5) confirms that this aerobic species has a higher rate of activity under reduced oxygen tensions. However the difference resulting from reduced oxygen tension is not great, and it might be expected that a cell, which generates oxygen as part of its physiology, could have evolved some protection mechanism for the oxygen sensitive nitrogenase (Kelly, 1969). There is evidence that heterocysts are the source of this protection (Van Gorkam, 1971), and it is noteworthy that Plectonema is non-heterocystous.

Different oxygen tensions do not appear to seriously complicate the assay regarding response to temperature change, because although a lowering of the pO_2 causes a reduction of sensitivity in Anabaena, this difference is not excessive.

In the experiments with both Plectonema and Anabaena, the response to temperature under conditions which were light saturated with respect to nitrogenase was very great. But with the light reduced to a level of 25% saturation there was almost zero response to temperature between $5^{o}C$ and $15^{o}C$ (Figures 5 and 6). Such a great difference of temperature response over such a narrow range of light intensities greatly complicates the interpretation of field data obtained for blue-green algae.

In Chapter 5 a method of correcting assays for temperature difference is suggested, this method makes use of the slope b, obtained in the Arrhenius plots. If the change of response varies directly with the change of light intensity, then the change of slope in the Arrhenius plots will also have a simple relationship to the light intensity. Even if this relationship is only approximately linear then an adequate correction of b could be achieved by (1):

$$B = b \frac{I_o - I_z}{I_s - I_z} \qquad \ldots\ldots (1)$$

where B is the value of b corrected for sub-saturation light intensity, I_o is the light intensity at which B is required, I_s is the light intensity which gives the value b, and I_z is the highest light intensity giving zero response to temperature. As $I_o \longrightarrow I_s$ then $B \longrightarrow b$, and as $I_o \longrightarrow I_z$, $B \longrightarrow 0$. With $I_o = I_s$ no correction is made.

These are only tentative suggestions, but it is apparent that the problem of interpreting field assays of blue-green algae in mire or any other system , is considerable. Unless efforts are made to make correction for light and temperature, the conclusions based on comparisons will, in many instances, be of very limited value.

The method of temperature correction, together with theoretical implications of the temperature response are fully discussed in Chapter 5.

CHAPTER 4 :

INVESTIGATION OF NITROGENASE ACTIVITY IN DEVELOPING PEAT

4(a) Location and collection of peat : methods

The material used in all but one of the experiments (4(c)), was rheotrophic peat collected from Tarn Moor, Nr. Orton, Westmorland, England. ($54^{o}28'N$, $2^{o}30'W$; British National Grid Reference NY6707). Unless otherwise stated blocks of peat about 10 cm sq. and 20 cm deep were dug with a trowel, these were immediately sealed into plastic bags and returned to the laboratory, where the experiments were started at once. On each occasion six cores were removed from different locations in the area, these were bulked and sub-sampled in the laboratory.

4(b) The assay for nitrogenase : methods

About 15 ml aliquots of peat material were put into 110 ml medical flats fitted with perforated screw tops and rubber liners in order to facilitate gas exchange; the exception to this was the experiment designed to investigate light and anaerobic conditions (4(e) and 4(f)), in this case 30 ml universal bottles each containing 10 ml of fresh peat were used. At the end of the assays, or at appropriate time intervals, the ethylene content of the ambient gas phases was estimated by withdrawing 1 ml samples with a plastic syringe, and then injecting these samples directly into the gas chromatographic apparatus. The ethylene produced has been expressed in terms of fresh volume of peat, therefore the volume of peat and ambient gas phase in each chamber was estimated at the end of the experiments. In all cases ethylene resulting from contamination of the acetylene, and from non-nitrogenase activity, was estimated and allowed for in the final calculations.

4(c) Time courses of acetylene reduction : methods

It was necessary to gain information regarding linearity of the reaction with peat soils, therefore experiments were set up in triplicate using different types of peat. 1 ml of gas was withdrawn at intervals and the ethylene content estimated by gas chromatography. The gas phase was maintained at atmospheric pressure by injecting 1 ml of water into the incubating chamber each time that a 1 ml gas sample was withdrawn.

4(d) Effect of high pO_2, glucose and Carbon monoxide : methods

Time courses were set up as described in Section 4(c), except that after 20 h incubation modifications were made to three sets of triplicate assays as follows: Set 1: carbon monoxide injected to give approximately 10%. Set 2: completely reflushed with a gas mixture containing 50% O_2. Set 3: 10 ml of 0.01 M glucose injected. A control set was left unaltered.

4(e) Effect of anaerobic conditions : methods

Sixteen 30 ml Universal bottles fitted with perforated screw tops and rubber liners were completely filled with distilled water before leaving the laboratory. On reaching the field site each bottle in turn was pushed to a depth of about 30 cm below the surface of the peat mass, where the tops were unscrewed and a small volume of peat pushed inside, replacing some of the water. The top was then screwed tightly down before the bottle was pulled up to the surface. This somewhat messy procedure ensured minimum, if not zero contact of the peat sample with air. The procedure was repeated at eight different randomly selected locations within an area of about 150 m^2; two bottles were prepared in this manner at each location. Two additional peat samples were collected from each location, these were taken back to the laboratory in unclosed 30 ml

containers.

On returning to the laboratory the remaining water in the bottles containing anaerobic samples was displaced with argon by venting through the rubber seal via a hypodermic needle. The tops of the bottles containing samples which had been exposed to air were also screwed down, and the atmosphere replaced with a gas mixture containing 20% O_2 and 80% Ar. Acetylene was then injected into all bottles to give 10%.

Eight aerobic and eight anaerobic samples were incubated in the dark at 25°C for 48 hours, after which time the ethylene content of each bottle was estimated.

4(f) Effect of light : methods

The remaining eight aerobic and eight anaerobic samples, prepared as described in the above section, were incubated for 48 hours at 25°C under a light intensity of about 6000 lux.

4(g) Effect of temperature : methods

The thermal denaturation of nitrogenase in the peat microflora was examined by pre-incubating one set of 20 replicate peat samples in air for 24 hours at each of the following temperatures: 5°C, 12°C, 23°C, 32°C, 37°C and 59°C. The samples were then assayed for nitrogenase activity by incubating in the dark for 24 hours at 25°C.

The effect of temperature on the rate of acetylene reduction was studied by incubating sets of 20 replicate peat samples in the dark at each of the following temperatures: 7°C, 16°C, 25°C, 33°C, 37°C, 45°C. Each sample was allowed to equilibrate aerobically for 4 hours at the appropriate temperature prior to starting the assays.

4(h) Effect of different initial oxygen tensions : methods

The ambient gas phases in medical flats containing peat samples were replaced with argon by venting through the rubber seals via a hypodermic needle. Volumes of oxygen were then introduced to give starting oxygen concentrations of 0, 4%, 16%, and 40%, after the addition of C_2H_2 to 10%. Triplicate assays were set up at each oxygen concentration.

In this experiment the rate of ethylene production was followed by sampling at various time intervals during a time course of 47 hours.

4(i) ^{15}N tests : method

Twenty-four 2 ml samples of rheotrophic peat were placed into 7 ml serum bottles, which were then evacuated with a hand pump. A premixed gas phase containing 20% O_2 and 80% N was then introduced; the N was enriched with 90% ^{15}N. ^{15}N is very expensive, however this level of enrichment had to be used because the acetylene reduction results had indicated a very low rate of nitrogenase activity.

Activity was terminated after 7 days incubation at ambient temperature by injecting 65% ethanol. After Kjeldahl digestion and distillation ^{15}N uptake was estimated using an A.E.I. MS.20 mass spectrometer, and expressed in terms of atom % excess ^{15}N over ^{14}N (Burris & Wilson, 1957).

4(j) Plate counts and acetylene tests of bacterial isolates : methods

Ombrotrophic and rheotrophic peat samples weighing 30 g were used in attempts to count aerobic N_2-fixing bacteria by the pour plate method. The 30 g of peat were shaken with 970 ml of sterilised water, then triplicate counts of each serial dilution were made using standard plate count technique. The medium used for Azotobacter spp. was the glucose medium

described by Brown et al., (1962). Attempts to plate anaerobic N_2-fixing bacteria were made using the potato extract medium described by Jurgensen and Davey (1971). The anaerobic incubations were carried out in anaerobic jars under argon.

In the case of both the anaerobic and aerobic plates some colonies were isolated into liquid versions of the above media. These isolates were cultured for 24 hours in 110 ml medical flats, either aerobically or anaerobically as appropriate, and then tested for acetylene reduction in the same medical flats.

4(k) Time courses : results and discussion

Experiments with soil indicated that the rate of acetylene reduction might be low, requiring a long period of incubation in order to obtain a measurable amount of ethylene (Stewart, 1967). Inhibition of nitrogenase activity in legume nodules can occur after only 7 h incubation (Sprent 1969), however results of a time course experiment (Figure 8) show that the reaction in peat is still active after 70 h under conditions of the field assay; in fact acetylene reduction was still taking pace 5 days after the incubations had commenced. During the 5 days incubation the ambient oxygen concentration had been reduced from 20 per cent to less than 2 per cent by metabolism of the peat microflora (Table 1).

Table 1

pO_2 values at end of the first time course experiment. Measured with mass spectrometer.

Peat type	pO_2 atm.
Rheotrophic	0.007
Transitional	0.012
Ombrotrophic	0.013
Blanket	0.14

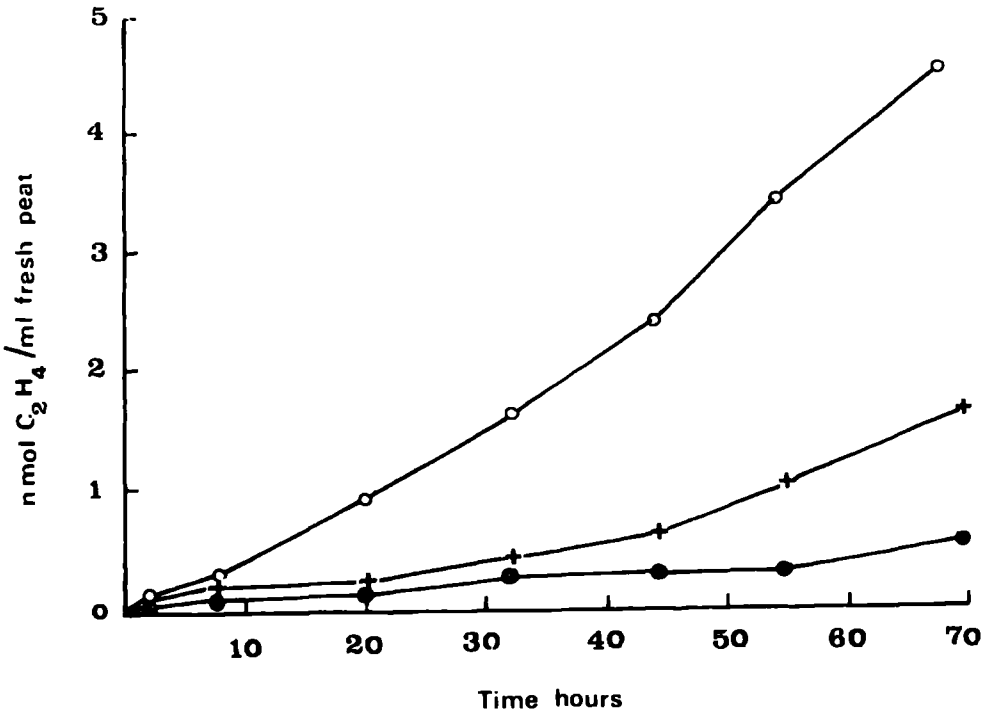

FIG.8 TIME COURSE OF ACETYLENE REDUCTION BY FRESH PEAT
RHEOTROPHIC o—o ; TRANSITIONAL +—+;
OMBROTROPHIC •—• .

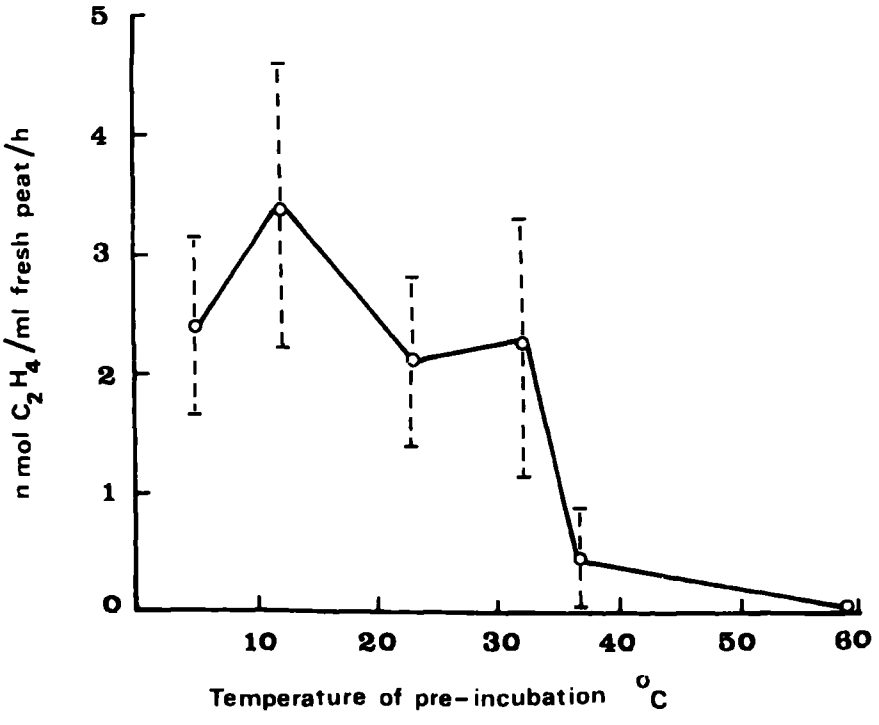

FIG.9 EFFECT OF DIFFERENT PRE-INCUBATION TEMPERATURES ON ACETYLENE REDUCTION BY RHEOTROPHIC PEAT. BARS INDICATE p = 0.05.

4(1) Confirmation of living nitrogenase activity

The possibility that the acetylene reduction was due to non-living organic catalysts had to be investigated, because this reaction had been catalysed *in vitro* by organic complexes of molybdenum with ligands containing sulphur (Schrauzer and Doemeny, 1971). Such compounds might be generated during the decomposition processes in peat. The experiments with high pO_2, glucose, carbon monoxide, thermal stability, and ^{15}N, were all performed in order to test the contention that the observed acetylene reduction by rheotrophic peat did in fact result from living nitrogenase activity. Schrauzer and Doemeny (1971) reported that carbon monoxide had little effect on non-living organic catalysts of acetylene reduction, however living nitrogenase is completely inhibited by this gas (Hardy and Knight, 1967). In Figure 12 it can be seen that the rate of reaction changed soon after the introduction of carbon monoxide, followed in due course by complete inhibition (Curve I). The apparent delay in complete inhibition may have resulted from delay of penetration by carbon monoxide to sites of activity inside the peat mass, also possibly from delay in equilibration of previously formed ethylene with the ambient gas phase. Curve II (Figure 12) shows that an ambient gas phase containing 50 per cent oxygen inhibited the reaction almost completely for about 10 hr; Drozd and Postgate (1970) reported a similar inhibition of acetylene reduction by *Azotobacter chroococcum* under high oxygen levels. The subsequent renewal of activity was probably due to lowering of the ambient oxygen concentration by metabolic activities of the peat microflora as mentioned above. Glucose had a stimulating effect on the reaction (Figure 12, Curve III), causing an exponential increase in the rate of ethylene production after a lag of about 5 hr.

Results of the pre-incubation experiment indicate that the chemical responsible for the reduction is unstable above about 37°C (Figure 9).

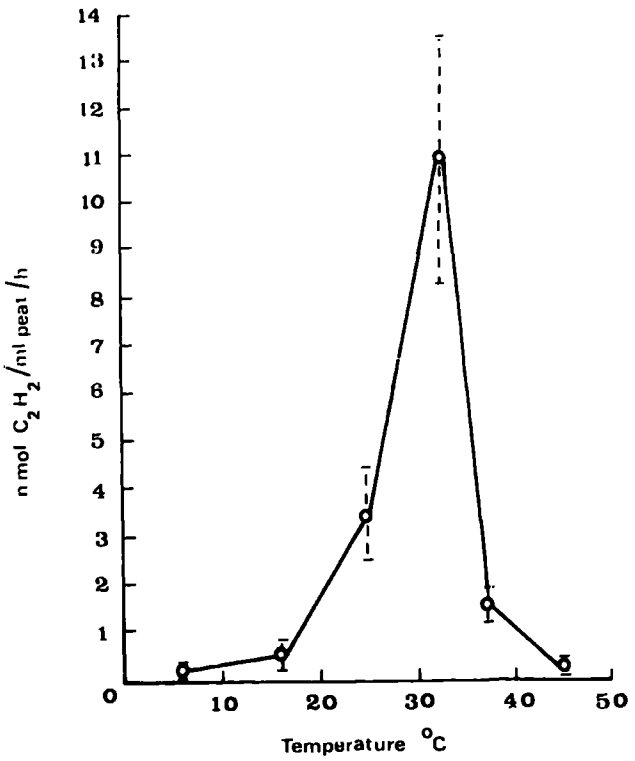

FIG. 10 EFFECT OF TEMPERATURE ON ACETYLENE REDUCTION BY MICRO-ORGANISMS IN RHEOTROPHIC PEAT. BARS INDICATE p = 0.05.

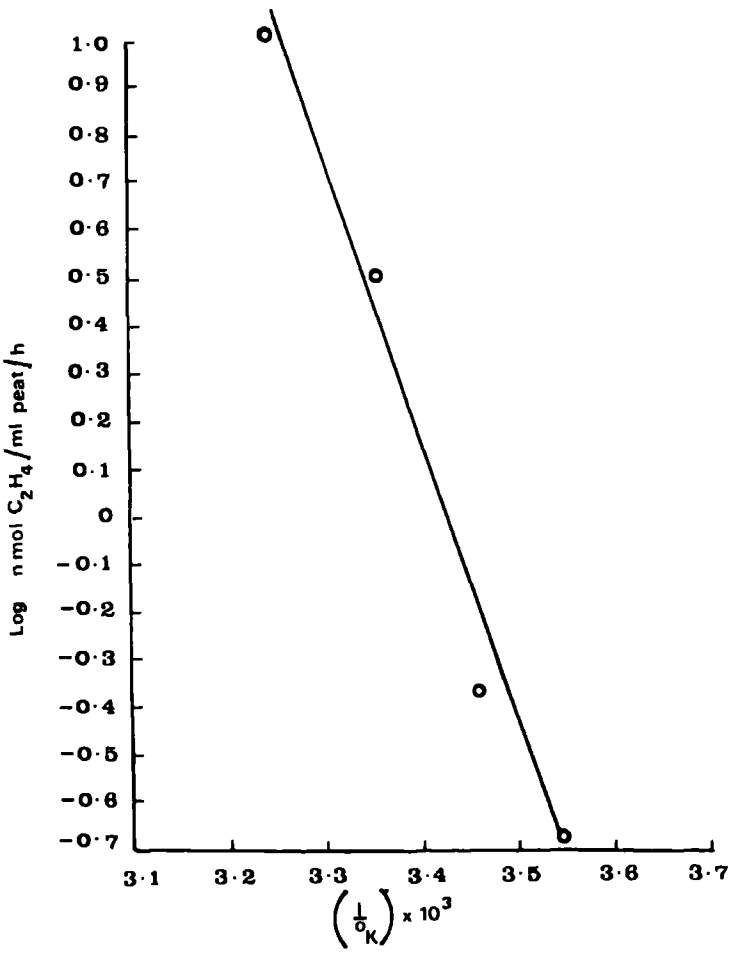

FIG. 11 ARRHENIUS PLOT OF DATA IN FIG. 10.

This again is more characteristic of a living enzyme than of a non-living catalyst. Furthermore, the response of acetylene reduction by peat to temperature change (Figure 10) is very similar to the response of heterotrophic bacteria (Hardy et al., 1968).

Eighteen of 24 peat samples tested became enriched with ^{15}N, and in 11 of these the enrichment was statistically significant at $p = 0.1$ or less (Table 2).

Table 2

Fixation of ^{15}N by developing rheotrophic peat. Exposure to ^{15}N for 7 days at 20°C. Average for 6 replicate controls not exposed to ^{15}N was 0.373 atom % with a standard deviation of 0.006. Enrichment significant at $p = 0.1$*; at $p = 0.05$**; at $p = 0.01$***; N = not significant.

Sample No.	^{15}N atom % excess	Sample No.	^{15}N atom % excess
1	0.013 *	10	0.006 N
2	0.150 ***	11	0.001 N
3	0.211 ***	12	0.024 **
4	0.001 N	13	0.013 *
5	0.015 **	14	0.010 N
6	0.006 N	15	0.043 ***
7	0.013 *	16	0.018 **
8	0.009 N	17	0.022 **
9	0.006 N	18	0.028 **

6 other samples had no detectable enrichment

All these results serve to confirm that the phenomenon of acetylene reduction by developing peat is in fact the result of living nitrogenase activity, and that the technique might be used with confidence in peat studies.

Although the results of the ^{15}N tests were positive, the levels of enrichment were low when compared with enrichment by other nitrogen-fixing systems. The largely anaerobic incubation was probably one cause of these very low enrichments (see below); these values are too near the limit of sensitivity to allow any experimental manipulation, or to be useful in any wide ranging field survey, this is not the case with the acetylene reduction assay.

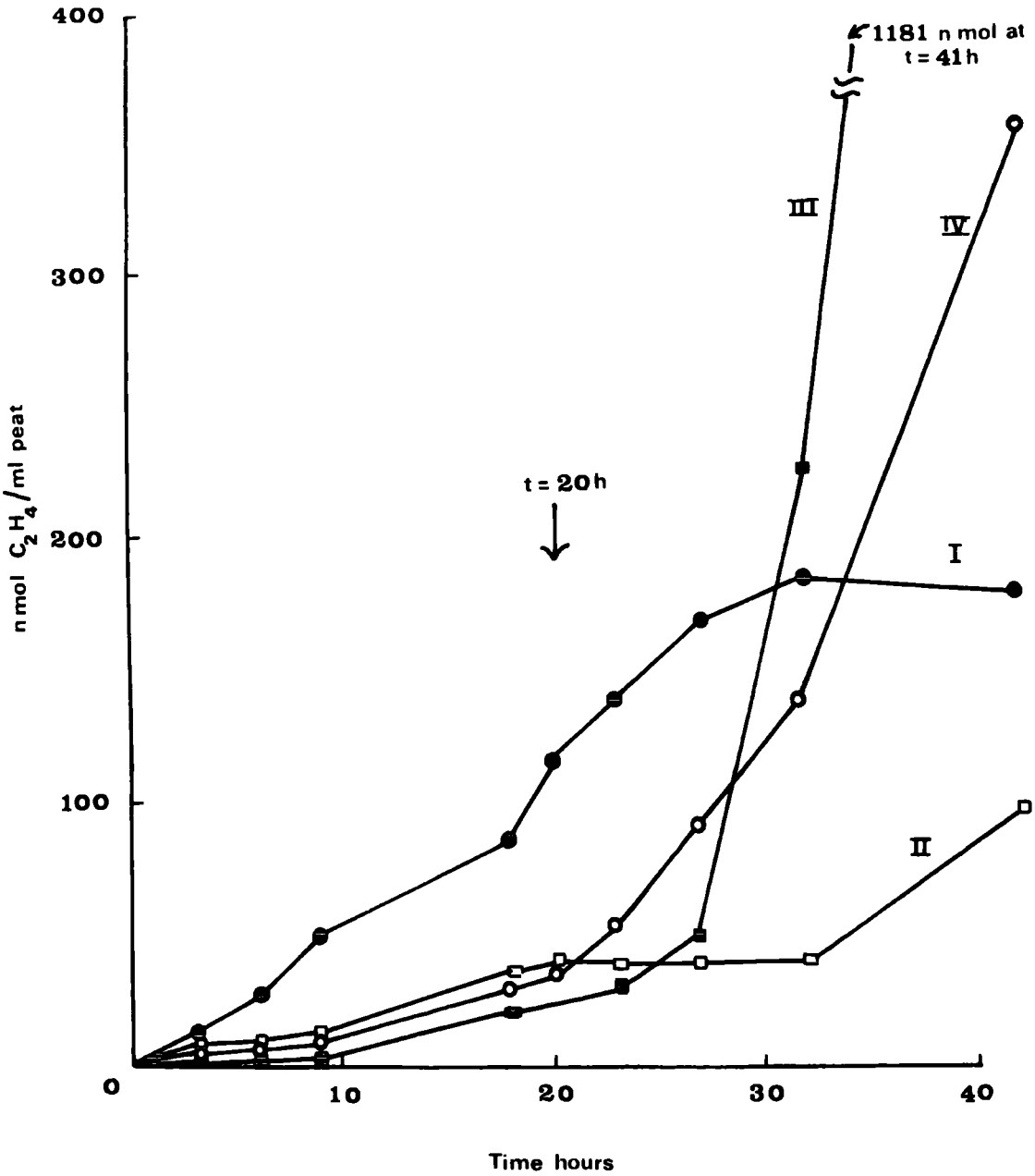

FIG. 12 EFFECT OF CARBON MONOXIDE (I), HIGH AMBIENT OXYGEN CONCENTRATION (II), AND GLUCOSE (III) ON ACETYLENE REDUCTION BY RHEOTROPHIC PEAT. CONTROL (IV). CONDITIONS OF I, II AND III CHANGED AT t = 20h.

4(m) Effect of light : results and discussion

The results presented in Table 3 show that light had no effect on nitrogenase activity. This was to be expected as most of the peat samples were collected from below the surface of the peat mass where little or no light penetrates. Some blue-green algae can grow in the dark provided a source of chemical energy is available (Tretyakova, 1966). Microflora surveys of Swedish mires by Flensburg (1967), and Flensburg and Malmer (1970) record the presence of blue-green algae in these ecosystems. However, whilst Granhall and Selander (1973) found that autotrophic fixation was locally significant in a sub-arctic mire in the Tornetrask region of Sweden, a recent investigation of Irish peats resulted in the conclusion that blue-green algae did not make a significant contribution to biologically fixed nitrogen (Dooley & Houghton, 1973). At present there is insufficient evidence to indicate that autotrophic fixation is of widespread importance in the nitrogen balance of mire ecosystems.

Table 3

Summary of effect of different light and oxygen conditions on acetylene reduction by rheotrophic peat. (Details in Table A13).

	Conditions			
	light aerobic	light anaerobic	dark aerobic	dark anaerobic
nmolC_2H_4/ml/48h	40	26	48	25

L.S.D. between any two means 26 nmolC_2H_4/ml/48h for p = 0.05, and 21 for p = 0.1 (A13).

4(n) Effect of temperature : results and discussion

Nitrogenase activity was unaffected by prolonged exposure to temperatures up to about 35°C, but inactivation occurred above this temperature (Figure 9). The rate of reaction was very sensitive to temperature change with the optimum between 30°C and 35°C for a 24 hour

incubation period (Figure 10). Although these data are consistent with known facts concerning temperature and nitrogen fixation by free living heterotrophic bacteria (Mishustin and Shil'nikova, 1971), they do contradict the field results of Granhall and Selander (1973), who found no correlation between acetylene reduction and temperature in peat. Granhall and Selander came to their conclusions on the basis of field assays carried out at 4 temperatures on two separate occasions, and as the material assayed at each of these 4 temperatures came from 4 different field sites, it is possible that their failure to detect any temperature response was a result of their experimental methods.

The rate of acetylene reduction by the peat microflora is considerably less sensitive to temperature change than laboratory cultures of free living heterotrophs for which 20 fold increases between $10^{o}C$ and $20^{o}C$ have been reported. (Hardy et al., 1968; Burns et al., 1971). The approximately 6 fold increase obtained in this investigation is closer to the early results of Koch (1907) who found that the nitrogen fixed by free living bacteria was 5 times greater at $24^{o}C$ than at $7^{o}C$.

This estimate of temperature response may be of value when acetylene reduction by the peat microflora obtained at one temperature must be converted to another for purposes of field comparison and evaluation. All field estimates of peat nitrogenase activity obtained in this investigation have been standardised with respect to temperature using the constant derived from the Arrhenius plot of temperature response shown in Figure 11. (Full details of themethod of temperature correction are described and discussed in Chapter 5). Further research is required in order to establish whether the constant shown in Table 4 (-6200) is valid over a wide range of conditions; it is this constant which is used in the suggested temperature correction equation.

4(o) Effect of oxygen : results and discussion

It was mentioned above that a high level of oxygen in the ambient gas phase inhibited nitrogenase activity in the peat (Figure 12). However, other experiments suggested that although the peat microflora was capable of reducing acetylene in strictly anaerobic conditions, the rate in such conditions was much reduced (Table 3). In order to gain further insight into the effect of oxygen a series of time courses were run with different levels of oxygen in the ambient gas phase at the start; the results of this experiment are presented in Figure 13. Figure 14 shows the rate of reaction at different times after the start, these were estimated by using the central difference between the various time intervals as an estimate of $\Delta Y/\Delta X$.

For about the first eight hours the anaerobic assays had the highest rate of activity, those started with an O_2 concentration of 40% the lowest; however during this early period the former were deceleracting, whilst the latter were accelerating. After about 25 hours the anaerobic assays had stabilised at about 0.4 nmolC_2H_4/ml peat/h. This is close to the figure obtained in the assays where precautions were taken to exclude air at all times from collection onwards (Table 3).

The assays which had been started with an oxygen concentration of 4% had a greater rate of activity over the first few hours than those started with higher levels of oxygen. This initial pattern suggests that at least one factor involved was the reduction of the oxygen concentration by respiratory activities to a level suitable for nitrogenase activity. Even the aerobe Azotobacter functions more effectively at low oxygen levels (Parker and Scutt, 1960; Dalton and Postgate, 1969). Drozd and Postgate (1970) demonstrated that the site of nitrogenase activity in Azotobacter chroococcum could be protected from oxygen inhibition by its own respiratory activities. Azotobacter spp. have been isolated from the

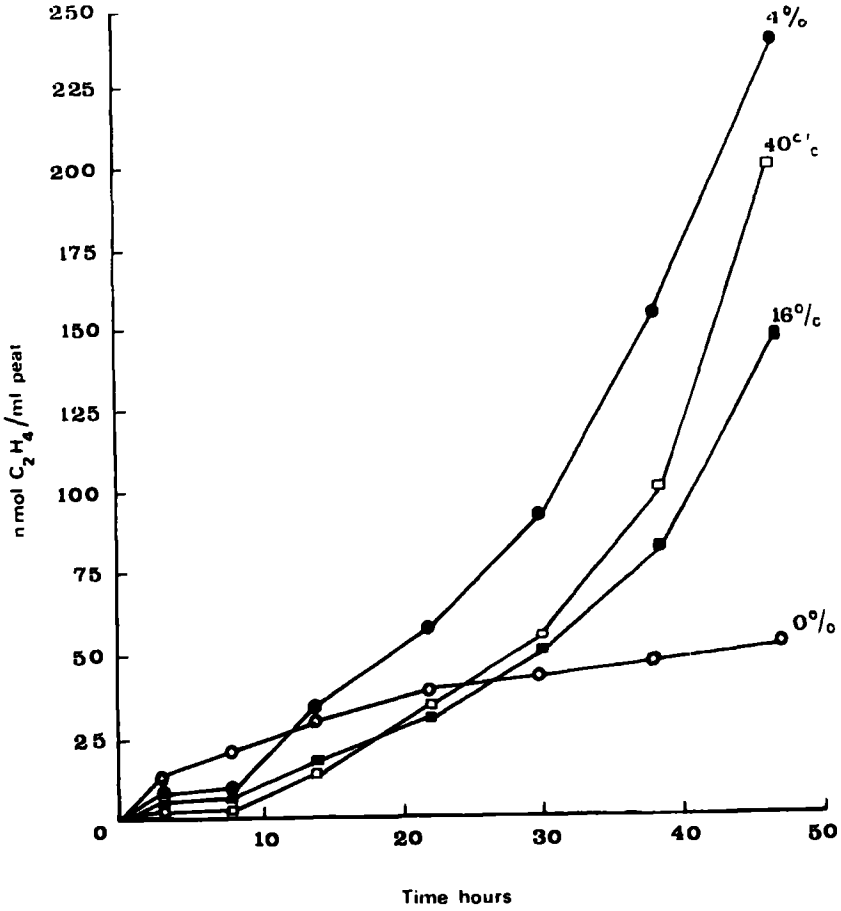

FIG. 13 EFFECT OF DIFFERENT INITIAL OXYGEN CONCENTRATIONS ON ACETYLENE REDUCTION BY RHEOTROPHIC PEAT. INITIAL CONCENTRATION OF OXYGEN AS INDICATED.

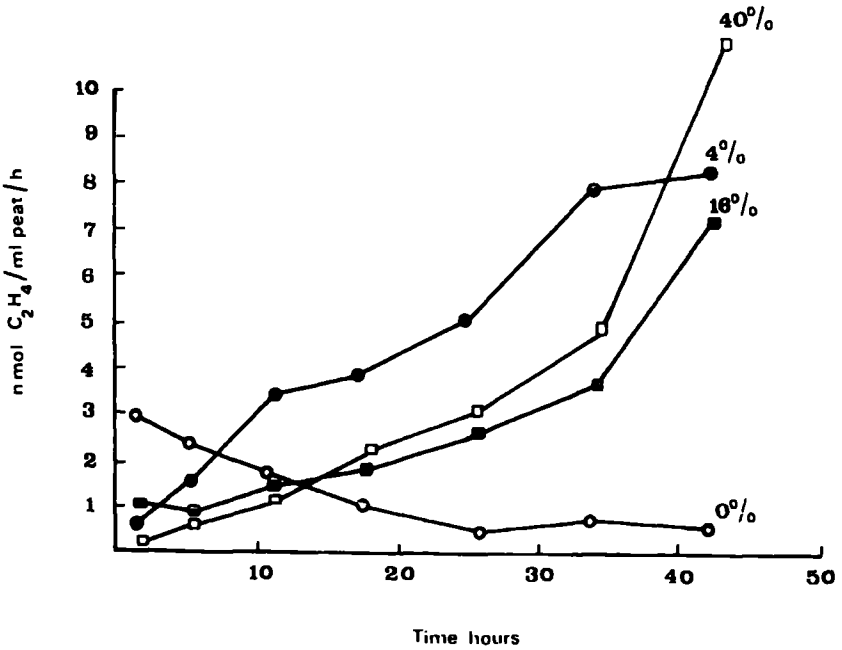

FIG. 14 RATE AT DIFFERENT TIMES AFTER START: OBTAINED FROM FIG. 13 USING CENTRAL DIFFERENCE TO ESTIMATE $\frac{\Delta Y}{\Delta X}$.

peat (Table A17), and it is possible that such a mechanism may have been operating in conjunction with the background respiratory activities of the non-nitrogen fixing microflora. Facilities for the accurate monitoring of the oxygen level during the time course were not available, but when measured at the end of the first time course experiment there was only 0.7% O_2 in the ambiant gas phase of the rheotrophic peat assays (Table 1).

No diagram of the second derivative is shown, however the slope of the curves at 40 hours in Figure 14 indicates that the assays started with a 40% O_2 were accelerating most rapidly, followed by those started with 16%, those with 4% O_2 were accelerating the slowest, and the anaerobic assays were fairly steady. Thus after 45 hours the rate of acetylene reduction by the peat microflora was related to the initial oxygen tension, and this relationship was the reverse of that during the first few hours of the time course. These results demonstrate that nitrogenase activity in peat is stimulated by exposure to air. The gradual decline of the rate from 3.0 to 0.4 C_2H_4/ml peat/h in the assays started anaerobically could be explained by the fact that in this experiment the peat was exposed to the air for a time whilst the assays were being set up. (Unlike the previous experiment, described above, where every effort was made to exclude air from collection onwards).

An abundance of colonies was obtained in plating experiments using both Azotobacter and Clostridium media (Tables A16, A17). However, in the context of this discussion it may be significant that when individual colonies were isolated into liquid media for acetylene reduction tests, all the aerobic assays gave negative results whereas 4 out of 9 anaerobic tests were positive (Table A19). Similarly Line and Loutit (1973) were unable to obtain any acetylene reduction of aerobically incubated putative nitrogen fixing species of Pseudomonas; but when their cultures

became contaminated with a strain of Clostridium butyricum, they obtained positive results in aerobic incubation with Pseudomonas as well as with other oligonitrophilic species. These results support the view that mixtures of aerobic and anaerobic organisms may be significant in nitrogen fixation.

In laboratory experiments with model soils Magdoff and Bouldin (1970) found that the aerobic-anaerobic interface was significant in nitrogen fixation; the mixture of aerobic and anaerobic conditions stimulating activity. They suggested that products of anaerobic fermentation of cellulose might serve as an energy source for aerobic nitrogen fixers. In contrast Rice et al., (1967), working with straw ammended soil, concluded that the products of aerobic metabolism might be used for anaerobic fixation. Whatever the precise explanation, these three earlier reports, together with the results presented here, show that a mixture of aerobic and anaerobic conditions can stimulate nitrogenase activity; the fluctuating water table in many mire ecosystems will frequently create such conditions.

Various workers have suggested an interaction between soil aerobes and soil anaerobes in order to explain their results (e.g. Pringsheim, 1909; Shklyar, 1956). Associations of nitrogen fixing bacteria with fungi have also been proposed (Krishna, 1928; Vartiovaara, 1938) whilst Jensen (1942) found evidence of symbiosis between Corynebacterium sp. and nitrogen fixing bacteria. Andriyuk (1967) reported associations of Azotobacter and actinomycetes in plate cultures. Specific reference to microbial associations in peat was made by Makrinov and Stepanova (1930) who suggested a relationship between cellulytic and nitrogen fixing species; also by Kaila (1954) who found considerable nitrogen gains in sedge peat samples incubated aerobically, and yet failed to isolate any Azotobacter species from these same samples.

Kalininskaya, who used the term 'symbiotrophic' to describe these relationships, attempted to isolate specific microbial combinations from the soil, and considered Bacillus polymyxa to be an important partner in numerous associations (Kalininskaya, 1967). This facultative anaerobe was once thought to fix nitrogen only in anaerobic conditions (Hino and Wilson, 1958), but Moore and Becking (1963) later showed that it was capable of this activity under both aerobic and anaerobic conditions. It is also pertinent in the present context, to note that Bacillus polymyxa has been isolated from peat (Popova, 1961). These facts, together with the results of oxygen studies presented above, indicate that a rewarding approach to future microbiological investigations of peat might be one which concentrates on a search for nitrogen fixing associations, with particular attention being paid to any involving Bacillus polymyxa.

4(p) Conclusions

The results of various tests confirm that acetylene reduction by developing rheotrophic peat is a manifestation of living nitrogenase activity, thus the acetylene reduction assay provides a valuable tool for the investigation of nitrogen fixation in peatlands.

Acetylene reduction, and therefore presumably nitrogen fixation, occurs in the complete absence of oxygen; the rate of activity in rheotrophic sedge peat is of the order of 0.5 nmolC_2H_4/ml/h at 20°C. Exposure to air stimulates activity, although results of time courses indicate that the reaction is faster after microbial respiration has lowered the oxygen tension in the ambient gas phase. This stimulation of activity after exposure to air occurs even if the assay is carried out under anaerobic conditions. All these responses to oxygen are probably a manifestation of the interrelationships and associations which exist within the peat microflora; however they also have implications regarding

use of the assay in peatland studies. If the peat is disturbed prior to the assay (c.f. some literally in situ method), then every effort should be made to ensure little or no contact between the peat and the atmosphere; errors will be greater with anaerobic assays of short duration, e.g. less than about five hours, during which time stimulated activity resulting from contact with the atmosphere is considerable. This stimulation caused by exposure to air adds to the difficulty of interpreting results of aerobic assays.

The theoretical electron requirements indicate that C_2H_2 will be reduced three time faster than N_2, and in many investigations this ratio has been approximated. However in certain instances, especially under anerobic conditions larger ratios have been reported. This could result from the anaerobic conditions affecting a part of the biochemistry of N_2 fixation that is not involved in C_2H_2 reduction. Rice and Paul (1971) have reported that waterlogging may also affect the ratio, due to the lower solubility of N_2 as compared with C_2H_2. Solubility is one of the factors that determines the diffusion rate of a gas in water, therefore Rice and Paul suggest that in waterlogged conditions the concentration of nitrogen at the site of nitrogenase activity could be insufficient to saturate the enzyme.

All of the above considerations indicate that the use of acetylene reduction for estimating actual amounts of biologically fixed nitrogen in peat is unreliable with our present state of knowledge. However, providing correction is made for temperature differences, results of the assays can give valuable information regarding potential nitrogen fixation in peatlands, for purposes of comparison.

CHAPTER FIVE :

THE RELATIONSHIP BETWEEN ACETYLENE REDUCTION AND TEMPERATURE

The discussion which follows considers both the practical and theoretical implications of the way in which nitrogenase activity responds to temperature change. Although the effect of temperature on acetylene reduction by legume nodules has been well studied (e.g. Hardy et al., 1968; Gibson, 1971; Dart and Day, 1971), this is not the case with other types of nitrogenase: in order to construct the fullest possible picture additional data will be discussed which relates to species not associated with mires.

5(a) Two main categories of temperature response

If the temperature response results presented in Figures 1, 5 and 10, together with the data summarised in Table 4 are contrasted with those for the 8 species of legumes quoted in the references mentioned above, one very obvious distinction emerges: acetylene reduction has an exponential response to temperature change between about 5°C and 20°C in all the non-legume material investigated to date. This gives a linear 'Arrhenius plot' between these temperatures with a marked discontinuity of slope at 20°C, such a pattern does not appear to be characteristic of acetylene reduction by legume nodules. The results for Azotobacter nitrogenase (Burns et al., 1971) and for Clostridium whole cells (Hardy et al., 1968), are consistent with this pattern, as are the results obtained for detached nodules of the non-legumes Hippophae rhamnoides and Casuarina equisetifolia shown in Table 4. Temperature responses of the lichens Stereocaulon paschale (kallio, 1973), Nephroma arcticum and Solorina crocea (Kallio et al., 1972) also follow this pattern: all these lichens contain symbiotic blue-green algae. Thus an exponential

pattern of temperature response by acetylene reduction is found in all the 11 non-legume species for which data are now available. (Nitrogenase activity in neither of the two blue-green algae that are reported here, nor in the lichens mentioned above show this response to temperature when the light intensity is reduced to below the saturation level.)

5(b) Legume and non-legume nodules

The results obtained for acetylene reduction by detached nodules of Glycine in three separate investigations are all very similar (Hardy et al., 1968; Dart and Day, 1971; Waughman, 1977). The most interesting feature is a marked inflexion in the temperature response curve at about 25°C, which appears to be characteristic of this species. The temperature response curves for detached nodules of 8 legume species presented by Dart and Day (1971), may be contrasted with curves for non-legumes (Figure 1). In addition to the facts already mentioned the main points which emerge are as follows: (1) acetylene reduction in legumes does not appear to have any distinctive pattern of response to temperature change between 5°C and 20°C; (2) the process in legumes is generally less sensitive to temperature than in non-legumes; (3) in some legumes there is sensitivity to temperature change, but this follows no consistent pattern and gives rise to various inflexions of the temperature response curve.

It is possible that the differences in temperature response between nitrogenase activity in detached nodules of legumes as compared with responses of nitrogenase in detached non-legume nodules are a manifestation of fundamental differences in the physiology of the symbiosis in the two groups. In this respect it is perhaps pertinent that the nitrogenase enzyme is very sensitive to oxygen (Kelly, 1969), and that the effect of oxygen on acetylene reduction by nodules is very different in legumes and non-legumes; the optimum oxygen concentration for nitrogenase

activity in nodules of non-legumes studied to date is 15-20% (Figure 3 and Bond, 1961), whereas there is evidence that in legume nodules the optimum oxygen concentration may be closer to 50%. (Bergersen, 1962). Bergersen observed that the rate of respiration in detached nodules of Glycine increased in two steps as the oxygen concentration increased; the first maximum, which occurred at 50% O_2, also corresponded to the oxygen concentration at which nitrogen fixation was the greatest. In order to explain these results Bergerson suggested that oxygen permeability barriers may exist between the bacteroids and the nodule tissue. Later, Bergersen suggested that flow of oxygen to the site of nitrogen fixation in the bacteroid might be controlled by leghaemoglobin (Bergersen, 1969). Temperature would affect permeability of oxygen through any such barriers, therefore these may also be responsible for the various inflexions which occur in the temperature response curve for acetylene reduction by Glycine and other legumes. No equivalent to leghaemoglobin has been found non-legumes (Becking, 1970), and it is possible that the lack of any such barriers may account for the simple response to temperature of nitrogenase in the nodules of non-legumes.

No activation energies have been calculated for the legume nitrogenase because Arrhenius plots of the available data do not give straight lines; however, acetylene reduction by legume nodules appears to be generally less sensitive to temperature change than nitrogenase of non-legumes, and this does indicate lower activation energies in the former group. This difference between activation energies suggests that specific rates of acetylene reduction in legumes might be higher than in non-legumes. Results obtained so far do support this view: the activity at 15°C in detached nodules of the 4 non-legumes for which data are available is about 1 $\mu mol C_2H_2$/g dry wt/h, compared with an average of about 70 $\mu mol C_2H_2$/g dry wt/h for the legumes studied by Dart and Day

(1971); the average for field tested material in the data summarised by Hardy et al., (Table 3, 1973) is 14 μmolC_2H_2/g fresh wt/h for legumes and 2.4 umolC_2H_2/g fresh wt/h for non-legumes (no temperatures stated). All the differences described above suggest that the nitrogen fixing symbiosis in non-legume nodules may be less efficient than the relationship between the symbionts in legume nodules.

Nitrogenase activity in detached nodules of the tropical non-legume Casuarina (Table 4) is considerably more tolerant of higher temperatures than in nodules of the 3 temperate non-legume species (Table 4). This may also be associated with oxygen tolerance because the temperate species for which data are available are considerably less tolerant of oxygen than Casuarina (Bond, 1961). Uemura (1964) concluded that the actinomycetes associated with Alnus and Casuarina were of the same type. However, it is not yet possible to say whether the tolerances exhibited by Casuarina are properties of the micro-organism or of the higher plant, or both.

5(c) Temperature response of acetylene reduction by blue-green algae

Lowering of the oxygen concentration caused the optimum temperature for acetylene reduction by Anabeana to be raised from 25°C to about 35°C, and in conditions of reduced oxygen tension activity at 40°C was still about 38% of maximum rate, whereas in 20% O_2 the rate at 40°C was only about 9% of the maximum (Figure 5). An increase in tolerance of temperature above the aerobic optimum has also been observed for nitrogenase activity in Nephroma arcticum under anaerobic conditions (Kallio et al., 1972). These results suggest that nitrogenase in blue-green algae may be more tolerant of high temperatures in conditions of low oxygen tension; such conditions occur in hot springs, and may have contributed to the success of blue-green algae in these environments (Copeland, 1963).

Photosynthesis in blue-green algae is believed to be intimately

involved in nitrogenase activity; possibly by directly supplying the reductant (Haystead and Stewart, 1972) or the ATP (Cox and Fay, 1969). Physical reactions are not very sensitive to temperature, and where light limits nitrogenase activity in blue-green algae, little response to temperature would be expected. This is the situation in Anabeana below about 15°C (Figure 5), and in Plectonema below about 20°C (Figure 6). Above these temperatures acetylene reduction does respond to temperature change. A similar change of sensitivity in low light intensities has also been recorded in ^{15}N studies with Anabeana cylindrica (Fogg and Than-Tun, 1960). These facts indicate that light does not limit the reaction above about 20°C; as light apparently can be the limiting factor below this temperature the simplest explanation would be that alternative reactions exist for at least some aspects of nitrogenase activity, and that a change of mechanism occurs at about 20°C. It is perhaps noteworthy that 20°C is the approximate temperature at which a change of activation energy occurs in many species (Figures 2, 7 and Table 4).

5(d) Anaerobic nitrogenase activity

It was pointed out above that the response to temperature change of legume nitrogenase appears to be different from the response of nitrogenase derived from non-legume material, and it was suggested that such differences may be a manifestation of fundamental differences in physiology. Nitrogenase from non-legume material shows two temperature response patterns, one of which appears to be associated with the anaerobic habit. Arrhenius plots of nitrogenase activity by Plectonema (Figure 7), Clostridium cells, and anaerobically incubated extract from Azotobacter (Hardy et al., 1968), all have an exponential response to temperature above 20°C, the logarithmic plot of this response gives a second straight line discontinuous with the one below 20°C. This second slope indicates that the rate limiting

processes above 20°C have an activation energy of 12-14 Kcal/mol (Table 4). These biphasic Arrhenius plots probably result from the fact that nitrogenase activity consists of many sequential processes with different temperature coefficients (discussed below).

5(e) The significance of different activation energies

The velocity of a complex biological system in steady state operation is considered to be limited by the rate of the slowest process of the complex (Blackman, 1905), or by a small number of processes (Hearon, 1952). Activation energies determined by Arrhenius plots (Table 4) refer to the overall activation energy of the limiting reaction, or reactions. Velocities of different processes have different temperature coefficients, therefore different reactions become limiting as the temperature is changed, a break of slope in an Arrhenius plot is thus indicative of a change of limiting reaction (Dixon and Webb, 1966).

Owing to the complexity of the many reactions involved in 'nitrogenase' activity a precise explanation of the values obtained for activation energies is difficult. Hardy et al., (1968) suggested that the activation energies which they had determined were associated with the nitrogenase *per se* rather than with reactions which supply energy or reductant; however, based upon features of temperature response of ATP hydrolysis by extracted nitrogenase Burns (1969) later concluded that the activation energies referred to this process. Although an activation energy of about 30-40 Kcal/mol below 20°C, and change of temperature response at about 20°C are characteristic of all the non-legume material investigated to date, the exact significance of these facts must await more detailed *in vitro* analysis of nitrogenase activity from many species.

5(f) Temperature correction of acetylene reduction results

All the data discussed above indicate that the rate of nitrogenase activity is very sensitive to temperature change. This is a fact of some practical importance in field work where the temperature cannot be controlled. If field results are to be compared some allowances for temperature differences must be made.

The traditional way of expressing the response to temperature change of a biological activity is the Q_{10}: the quotient of two rates obtained at a temperature difference of $10^{o}C$. Q_{10} values of more than 20 have been recorded for acetylene reduction, therefore the Q_{10} value is not a particularly useful way of expressing the effect of temperature on nitrogenase activity.

The velocity of a chemical reaction is related to temperature change by the Arrhenius equation (2) (Morris, 1965)

$$V = \frac{A}{e^{E/R.T}} \qquad \ldots\ldots (2)$$

where V is the rate of reaction; A is a constant; E the overall activation energy; R is the gas constant: 1.987 cal/mol/degree; and T is the temperature in degrees Kelvin.

As A is a constant, a graph of $Log_{10}V$ *vs* $1/_{T}$ (an Arrhenius plot) is a straight line of slope b, such that $E = -4.576b$ cal/mol. However, what is more useful is the fact that the nature of the relationship is such that for any value of E different specific activities only affect the position of the Arrhenius plot in relation to the $Log_{10}V$ axis, but not the slope. Thus, provided b is known only a single specific activity is required to plot the graph of $Log_{10}V$ *vs* $1/_{T}$ which can then be used to find the rate of activity which would have occurred at any other required temperature. It is, of course, not necessary to plot a curve for every

specific activity, provided that b is known the conversion between temperatures can be made using the simple expression (3).

$$\text{Log}_{10}V_2 = \text{Log}_{10}V_1 + b(^1/_{T_2} - ^1/_{T_1}) \qquad \ldots\ldots(3)$$

where V_1 is the known rate at temperature $T_1{}^oK$ and V_2 refers to the unknown rate at temperature $T_2{}^oK$.

An investigator carrying out acetylene tests at ambient field temperatures should, ideally, determine the extent to which their experimental material is affected by temperature before the results are used for comparative purposes. However few investigators will be disposed to carry out such experiments, even if they have the facilities available, therefore the data presented in Table 4 may be of some help to field workers.

The information presently available indicates that between 5^oC and 20^oC the values of b for non-legume material can be used with some confidence. However, low light conditions greatly reduce the response of nitrogenase activity in blue-green algae, so that unless conditions are known to be light saturated, corrections cannot be made by the above method. The single value for a peat assay shown in Table 4 (Chapter 4) indicates a sensitivity slightly lower than in laboratory cultures of bacteria.

Considering the variability of field material and the present state of knowledge, in most cases only an approximate temperature correction is justified, therefore for temperatures between 1^oC and 25^oC, conversion can be adequately approximated by (4)

$$V_2 = V_1(1.27)^{T_2 - T_1} \qquad \ldots\ldots (4)$$

where the constant 1.27 is the average increment per ^{o}C below 25^{o}C for non-legume material, derived from the b values shown in Table 4.

5(g) Concluding Discussion

It has been suggested above that the responses of different types of nitrogenase to temperature change may be divided into three categories. (i) The anaerobic group, in which the response is exponential but with a slight change of sensitivity occuring between about 20 and 25^{o}C. This type of response gives a linear arrhenius plot up to about 35^{o}C, but with a break in slope corresponding to the change in sensitivity. Species reported to date: Clostridium pasteurianum, Plectonema boryanum, Azotobacter vinlandii nitrogenase (anaerobic in vitro assay), and Anabaena cylindrica low pO_2 assay. (ii) Aerobic nitrogenase activity but excluding legumes. This group exhibits an exponential response to temperature change only up to about 25^{o}C, thus the linear arrhenius plot terminates at this temperature. Species reported to date: Stereocaulon paschale, Nephroma arcticum, Solorina crocea, Anabaena cylindrica, Alnus glutinosa, Hippophae rhamnoides, Myrica gale, Casuarina equisetifolia. (iii) Legume nitrogenase activity, which appears relatively insensitive to temperature, and has no discernible pattern of response which is consistent for the various species tested. Examples: All legumes tested to date, see references. (Details of all non-legume species mentioned above are listed in Table 4).

The three types of temperature response are all manifestations of physiological differences, and it is possible that these differences may be associated with the supply of either the energy, or the reductant used in nitrogen fixation, rather than with differences in the enzyme itself. Furthermore, it is interesting to speculate on the possibility that the three groups represent stages in the evolution of the nitrogenase system.

TABLE 4. Some data relating to the effect of temperature on acetylene reduction by nitrogenase.

Species	Type of Organism	Approx. Optimum Temp. °C.	E Kcal/mol	b	Range for E & b. °C	Incubation Time (minutes)	Source
Clostridium pasteurianum	bacterium	N.S.	50	-11,000*	10-20	60	Hardy et al., (1968)
Clostridium pasteurianum	bacterium	N.S.	14	- 3,057*	20-35	60	Hardy et al., (1968)
Azotobacter vinlandii cell free extract	bacterium	N.S.	39	- 8,500*	15-21	N.S.	Burns, (1969)
Azotobacter vinlandii cell free extract	bacterium	N.S.	35	- 7,700*	10-20	10	Burns et al., (1971)
Stereocaulon paschale	lichen (blue-green symbiont)	20	46*	-10,000*	5-15	N.S.	Kallio, (1973)
Nephroma arcticum	lichen (blue-green symbiont)	20	38*	- 8,300*	5-15	60	Kallio et al., (1972)
Solorina crocea	lichen (blue-green symbiont)	15	38*	- 8,300*	0-15	60	Kallio et al., (1972)
Anabaena cylindrica	blue-green alga	30	(1) 28	- 6,100	5-20	30	This project
Plectonema boryanum	blue-green alga	30	(2) 30	- 6,600	5-20	30	This project
Plectonema boryanum	blue-green alga	30	(3) 12	- 2,612	20-30	30	This project
Alnus glutinosa	non-legume	25	31*	- 6,800*	15-25	60	Wheeler, (1971)
Alnus glutinosa	non-legume	20	38	- 8,300	5-20	45	This project
Hippophae rhamnoides	non-legume	25	35	- 7,700	5-20	45	Waughman (1977)
Casuarina equisetifolia	non-legume	35	39	- 8,500	7-20	30	Waughman (1977)
Myrica gale	non-legume	20	30	- 6,600	7-20	45	This project
(4)Sandy loam soil	heterotrophic bacteria	37	19	- 4,150*	10-25	60	Knowles et al., (1973)
(4)Sandy loam soil	heterotrophic bacteria	37	5	- 1,092*	25-35	60	Knowles et al., (1973)
(5)Peat Soil	heterotrophic bacteria	36	37	- 6,200	5-30	400	This project

(1) Average of 3 separate experiments giving 20,29 and 30 Kcal/mol. (2) Average of 3 separate experiments giving 28,30 and 31 Kcal/mol. (3) Average of 3 separate experiments giving 9,12 and 14 Kcal/mol. (4) Glucose amended anaerobic incubation. (5) Aerobic at start. N.S.-not stated. *-Values calculated from data presented in publication quoted.

It is generally accepted that during the early development of living material on this planet the atmosphere was anaerobic; thus the biphasic response to temperature may be manifestation of the most primitive stage in the evolution of nitrogen fixing systems, and the simple linear response possibly reflects some later adaptation to oxygen. In the legumes we can observe the most efficient nitrogen fixing arrangements of all, and nitrogenase activity in the group appears to be comparitively insensitive to temperature change. In other words the most efficient and, presumably, most highly evolved system behaves least of all like a simple chemical reaction.

The hypothesis that nitrogenase systems may fall into three categories is based upon the results of only about 25 species. Clearly data from many more species are required before this hypothesis can be accepted, rejected, or modified.

Finally, I would like to once more stress the importance correcting for temperature differences when results of acetylene reduction assays on non-legume material are to be used for comparative purposes. This importance is emphasised by the fact that between 5 and 20^{o}C the known data (Table 4) indicate that a rise in temperature of only 3^{o}C may cause the rate of reaction to more than double. All the field assays reported in Part Two have been standardised using the method described here.

CHAPTER SIX :

INTRODUCTION TO PART II

6(a) Nomenclature and terminology

Plant identification and naming in this thesis follows Clapham et al. (1958), with reference being made to Oberdorfer (1970) for more detailed information on the naming and distribution of species in Southern Europe.

Rheotrophic (from rheophilous, Kulczynski, 1949) and rich mires are used synonymously to describe vegetation and peat occuring in mineral rich conditions; likewise ombrotrophic and poor mires are used to describe mineral poor conditions, i.e. nutrient supply is mainly from precipitation.

Peat and vegetation are referred to one of the floristic classes described by Sjors (1950); these categories are extreme rich fen, rich fen, fen, poor fen, and bog. As used here, rich mires will include: extreme rich fen, rich fen and fen; poor mires will include poor fen and bog. It will be pointed out below that it is not always possible to ascribe vegetation to such categories with great accuracy, however the value of these terms lies in the fact that most english speaking ecologists are familiar with them.

When the term ecocline is used it refers specifically to the gradient associated with change in chemical conditions from fen to bog, unless otherwise stated. Complex gradient and coenocline refer respectively to the edaphic and vegetational gradients which comprise the above defined ecocline (Whittaker, 1967).

6(b) The nutrient ecocline

It is self evident to any person who has examined mires that an excess of water is an essential characteristic of the ecosystem, and by

the beginning of this century the importance of both dissolved calcium, and water flow were well appreciated (Ramann, 1895). It was also in the first decade of this century that Weber outlined his terrestrialisation hypothesis, in which he suggested that different types of mire comprised an ontogenetic sequence, reflecting the edaphic succession from fen to bog (Weber, 1908). The fact that different vegetation is characteristic of different types of mire had been recorded before 1850 (Witte, 1947) and by the 1930's many vegetation units in mires had been identified and named (Du Rietz, 1936). With this well established taxonomy, attempts were made to identify chemical distinctions between bogs and fens. The work of Kivinen (1935), and Thunmark (1942) indicated a figure of about 1 p.p.m. calcium in the mire water as the limit below which no types of fen would develop. This criterion has been generally confirmed in many studies since then (e.g. Sjors, 1948; Witting, 1949; Malmer, 1962b; Heinselman, 1970).

It must be emphasised that this critical level of calcium is merely a measurable chemical distinction, and not necessarily the direct or indirect causative factor; indeed the movement of water in itself has been particularly stressed by various workers (e.g. Kulizynski, 1949; Gorham, 1950; Bellamy, 1968).

Chemical and vegetation distinctions between fen, poor fen, and bog were thus accepted at the general level (Du Rietz, 1949 and 1954), but the existence of critical chemical limits and the concept of 'indicator vegetation' encountered criticisms.

It is easy to appreciate why efforts had been made to define discrete vegetation units with distinct chemical perameters when we realise that in the three or four decades preceding 1950, most European ecologists had been trained in a tradition of phytosociology which adopted the organismal concept of community almost as a sacrosanct tenet. Seen in this light,

Sjörs' conclusion that mire vegetation could not be considered as being composed of discrete taxa is a development of considerable significance (Sjörs, 1950a). Sjörs divided the mire system from fen to bog into seven types based upon floral assemblages of indicator species, but he considers these are abstractions from a continuum of vegetation change and are not finite units on the real mire surface (o.c. 248-249).

The existence of gradients in mires, whether in the form of a continuum or discrete taxa, had been implicit in most reports of mire investigations since the work of Weber. Even in Finland, where the classification is based upon tree stand (Cajander, 1913), more detailed investigations recognised, and utilised gradients (e.g. Ruuhijärvi, 1960; Heikurainen, 1972). North American mires have been described in terms of gradients (Heinselman, 1970; Pollett, 1972); and it is interesting to note that Osvald, in his postumus paper on North American peatlands, accepted the validity of the continuum concept for mire complexes in that country (Osvald, 1970, p. 94).

The concepts of indicator species, critical calcium levels, and Ca/Mg ratios (<1 in ombrotrophic peat and water) have been of only limited usefulness in defining different types of peatland, especially in N.W. Britain, and W. Scandinavia. From his detailed study of a Swedish fen, Gorham (1950) concluded that supposed tolerance ranges of species was of very limited value as a criterion of minerotrophy; and following their measurements of calcium in mire waters of Northern Britain, Gorham and Pearsall (1956) criticised the value of calcium concentration as a criterion of bog development, concluding that the amount of water in soil was equally, if not more important. In the Tornetrask area of Northern Sweden, Sonesson (1970a) was unable to distinguish different mire types on the basis of floristics: he found fen plants and minerotrophic conditions near to the surface of bog sites, and

decided that the hydrotopographic conditions in the region must have been different from those elsewhere. 'Fen plants' are also found growing on the 'bogs' of Western Ireland (Gorham, 1953a). Attempts to relate peat type and floristics with detailed analysis of peat chemistry have also produced discrepancies (Kivekäs and Kaila, 1957; Mörnjsö, 1968). Recently Schneekloth and Schneider (1972) conclude that it was not possible to infer specific growth conditions in German mires from the vegetation. The last mentioned authors wrote a statement which is apposite in the context of some of the above facts: 'There is no developmental sequence for bogs which could be extended to consistant systematics ... a bog is characterised by partly uncorrelatable criteria' (transl. p. 62).

In practice the value of floristics as a criterion of peat quality is probably a function of the detail in which the information is required, many workers have utilised vegetation cover of mires as an indication of general land-use potential (e.g. Puustjärvi, 1960; Abramova, 1965; Boch, 1965; Holmen, 1964). One possible reason for the problems that have arisen in attempts to develop a chemical background to the mire complex, is that so much attention has been paid to chemistry of the mire water. The composition of water entering a complex depends upon climate, rock type of the catchment, uptake by vegetation, etc. The daily variation in precipitation will affect the dilution, which in turn will affect the ionic concentrations. Continual monitoring of the water composition over a period of years would be required to provide an estimate upon which any confidence could be placed. Provided that the mire has developed naturally, it is possible that a more satisfactory integration of the chemical conditions might be obtained by analysis of the total quantities in peat, or in the vegetation cover; the evolved homeostasis of the mire ecosystem reduces the extent to which daily

fluctuations in water composition will be reflected in the chemical composition of either the peat or the vegetation.

There are very many reported determinations of chemicals in peat, but only a minority of these have been made with reference to the mire ecocline. There have also been numerous reports on the chemical constitution of the vegetation over the years. (e.g. Wiegmann, 1837; Zailer and Wilk, 1907; Minssen, 1913; Simonis and Hirsch, 1962), but again the number of detailed studies of the vegetation chemistry with respect to the ecocline are very few (e.g. Kivinen, 1933; Gorham, 1953; Malmer and Sjörs, 1955; Malmer, 1962b). These and others will be discussed in more detail later.

If one scrutinises the data available for the mire chemical gradient, surprisingly few facts are consistent in the different reports. It is possible to state with confidence that calcium and magnesium are present in high concentrations in fen peat and fen vegetation, but little else. There are possibly three main reasons for this situation. (1) Phytosociologists generally do not have a strong inclination towards chemistry, and peat chemists often do not have the taxonomic knowledge required to abstract detailed phytosociological gradients; apart from the fact that phytosociology itself is a somewhat esoteric subject. (2) Until recent years, instrumentation appropriate for rapid and detailed chemical analysis has not been available; this is especially true with respect to trace element and nitrogen fixation studies. (3) There are few locations where an uninterrupted chemical gradient from bog to fen can be studied without too much complication being introduced by various other gradients that exist on mires (Sjörs, 1950b).

6(c) Nitrogen, phosphorus and the mire ecocline

From the previous section it will be apparent that most of the

critical chemical investigations of the bog-fen ecocline have centred upon acid-base relationships, or concentrations of the alkali-earth cations. Calcium dominates the acid-base reaction, and although the latter is generally regarded as being one of, if not the most important causative factor of the phytososiological gradient, there is no evidence that calcium itself is directly involved; in fact Sjörs considers that calcium is probably never limiting, even on the most nutrient poor bogs (Malmer and Sjörs, 1955, p. 61). If the acid-base status is the main determinant of the gradient, it is likely much of its affect is via other chemicals. Many workers have concluded that a deficiency in phosphorus limits growth on ombrotrophic sites (e.g. Tamm, 1954; McVean, 1959; Malmer, 1962b; Holman, 1964; Watt and Heinselman, 1965); nitrogen has less frequently been proposed as a limiting chemical. In this context it is pertinent to note that Saebo (1970), in his short review of xeromorphy in bog plants, concluded that these plants are well adapted to low levels of available nitrogen.

There have been few, if any, investigations designed specifically to study phosphorus and nitrogen in mires with respect to the ecocline. No doubt one reason for this is that analytical procedures for these two elements are more difficult and time consuming than for the cations, and this is especially true for nitrogen. Regarding nitrogen fixation in peatlands, there have been few investigations of any description, and most of those with which I am aquainted have been discussed in Chapters 1 and 4. Studies of hetreotrophic nitrogen fixation with regard to the mire ecocline are, to the best of my knowledge, non-existent, although some investigations concerning the distribution of Alnus sp. and Myrica sp. have relevance to the nitrogen balance, and these will be discussed in Chapter 12. Although studies involving phosphorus and nitrogen in mires have rarely been designed specifically with reference to the ecocline,

workers often present their data in a manner which approximately corresponds to the phytosociological gradient, or they give details of pH, base saturation, ash content, or floristics which permit such an approximation to be made. Thus it appears that the total nitrogen content of peat is lower in bogs than in fens (Gorham, 1953; Sjörs, 1961; Malmer, 1962a; Sonesson, 1971b). The same appears to be true for total phosphorus although the few data available do not demonstrate such an obvious gradient (Kaila, 1956; Sjörs, 1961; Malmer, 1962a; Pollett, 1972).

Thus it is generally considered that bogs contain less phosphorus and nitrogen than fens, however, although the few data on total nitrogen and phosphorus lend some support for this contention, the results for easily soluble phosphorus (often regarded as available phosphorus) are inconclusive. Sjörs (1961) in his chemical survey of Swedish soils found the lowest values in peatland to occur in poor fens; Holman (1964) recorded an increase in lactate soluble phosphorus with increasing acidity of sites drained for forestry, and in Malmer's study of a nutrient poor mire in Götland the highest levels of available phosphorus were found on bog sites (Malmer, 1962a). These results, together with others to be discussed in Chapters 10 and 12, suggest that the higher levels of total phosphorus in fen peat may not be matched by higher levels of soluble phosphorus. The few data on soluble nitrogen known to me also show no obvious trends, these too will be fully discussed later.

The earliest foliar analysis of bog plants dates back at least 140 years (Wiegmann, 1837) and in the present context it is interesting to note that Wiegmann found more phosphorus in the leaves of the fen species Carex caespitosa than in the bog species Eriophorum vaginatum. Since then there have been investigations involving the chemical composition of mire species, but most of these have been directed towards site potential for agricultural or forestry purposes, (e.g. Watt and Heinselmen, 1965;

Holmen, 1964). Gorham (1953b) found that the nitrogen content of bog plants was lower than fen plants, this work involved the analysis of a number of species from several locations in the English Lake District. Malmer and Sjörs (1955) analysed samples of a single species (Menyanthes trifoliata) from several vegetation types at one mire, no trends in either phosphorus or nitrogen are apparent from their results.

6(d) Objectives and arrangement of Part Two

The above discussion draws attention to the dearth of information regarding nitrogen and phosphorus in relation to peatlands, especially with regard to the chemistry of the plants themselves; it was also pointed out that previously there have been no investigations into the distribution of heterotrophic nitrogen fixation in peatlands. The second half of this thesis is concerned with these aspects of mire chemistry. Many other chemical parameters have been estimated in both the peat and vegetation, these are somewhat ancillary to the main objectives, and although the data are presented in Chapter 11 they are not discussed so fully.

Chapter 7 contains details and, where appropriate, discussion concerning choice of methods. For convenience some details of the phytosociological analysis are also presented in this section. The field and preliminary laboratory procedures are described, but not details of the analytical methods utilised for estimation of different chemicals and nitrogen fixation, those being dealt with in the appropriate chapters.

Chapter 8 presents results of the nitrogen fixation field investigations, with special attention being paid to chemical factors which might affect the rate of activity.

Chapter 9 examines the distribution of N in peat and vegetation with respect to the mire ecocline. Chemical factors which may affect the

nitrogen content of mire vegetation, as well as the extent to which the different forms of inorganic nitrogen in peat may be used as an indication of available nitrogen, are considered.

Chapter 10 repeats Chapter 9 for phosphorus.

Chapter 11 deals briefly with cations in relation to the ecocline.

Chapter 12 examines interrelationships between the various parameters, and discusses some theoretical implications of the findings. A general summary of nitrogen fixation in peatlands is also presented in this chapter.

6(e) Choice of locations

In order to study mineralogical changes along a mire gradient it is a pre-requisit that a wide range of vegetation should exist in the mire complexes developing within a single water catchment. Locations with all phases of the nutrient ecocline well developed are difficult to find in England, where one type or another usually predominates. For example, most of the mires in East Anglia tend to be rheotrophic, whereas in Scotland and the North of England they are predominately ombrotrophic. Flat tertiary mires, where a wide range of types develop within a single complex, occur extensively in central Europe (Moore and Bellamy, 1974). It is for this reason that locations in Southern Germany were chosen as the main investigation sites.

Pfrule Moss, situated between Mürnau and Garmisch, is a tertiary mire of several square kilometers in area, within this mire complex many different stages in the fen-bog ecocline are very well developed; for this reason Pfrule Moss was chosen as the main investigation area. The chemical gradient is one of, and possibly the dominant gradient in mires, however, others do exist (Sjörs, 1950b). At Pfrule Moss the effect of other gradients was reduced by utilising the open mire expanse of pioneer vegetation as the main investigation area, and avoiding, as far as possible,

open pools, hummocks, trees, springs and mud bottoms. Pfrule Moss was chosen as the location for the main investigation because a wide range of vegetation types corresponding to different parts of the ecolcine are present, without extensive development of the various small scale hydrotopographic features that are very abundant in Northern mire complexes.

Some samples were also collected from three other locations in S. Germany: Wurzacher Ried, Taufach Moss; and Grundlen Ried; these tertiary mires all carry a wide range of vegetation, but not the extensive development of intermediate types found at Pfule Moss

The ecology of nitrogen fixation in developing peat had received little or no attention prior to this project, therefore this aspect was considered sufficiently important to warrent extending the investigation beyond the German sites. Acetylene reduction tests were carried out on developing peat in numerous sites both in the United Kingdom and elsewhere, although in all cases the range of mire types within any one catchment was limited. Locations, other than those in Germany and the United Kingdom, were not chosen specifically, but were surveyed when travel for other purposes made a visit to the site possible.

A full description of all the sites visited is given in Appendix A.

6(g) Vegetation chemistry

Previous attempts to study the chemistry of mire plants have utilised individual species: either one, two or several. A problem with the use of individual species is that no one species exists which grows throughout the entire mire ecocline, in fact no important species covers more than about half of it. Another problem is phenology: various species reach their vegetation peaks at different times, indeed a single species may have slightly different vegetative peak dates for each association in which it

occurs. Ideally, many if not all species should be analysed, preferably throughout the season. This would involve a sampling and analytical programme of considerable size, to the extent that it would constitute a major investigation in its own right. For this project advantage was taken of the fact that all the vegetation under investigation is herbaceous, thus enabling samples of the entire sward to be cropped. Although this method does not provide detailed information regarding the chemistry of individual species, it can provide an integrated result for the entire vegetation at approximately the seasonal peak of vegetative growth. Implicit in this work is the assumption that, in this natural system, the vegetation chemistry will have evolved to a steady state situation with the edaphic chemical template. It is this steady state situation which is being investigated.

CHAPTER SEVEN :

DISCUSSION AND DESCRIPTION OF METHODS

7(a) Field methods

At the German locations, stands of apparently homogenous herbaceous vegetation were selected for sampling, and a plant list recorded for each one using Braun Blanquet cover/sociability scale (aufname) (Braun Blanquet, 1961). An area of approximately 4 m^2 was marked out and 6 or 7 sub-sites randomly located within this area. In general, vegetation types which were not considerably larger than the above mentioned 4 m^2 were not examined, but in some ombrotrophic areas small scale mosaics were encountered (c. 1 m^2), these were sampled by locating one sub-site in each of several similar patches of the mosaic: Sphagnetum cuspidatae and Sphagnetum magellanicae are examples of vegetation types where this procedure was used.

A sample of the above-ground vegetation was cropped from each stand, in order to obtain an integrated result for the vegetation chemistry at various locations along the ecocline. At each sub-site the vegetation in about 30 cm sq. was cropped to the level of the peat. (Some ombrotrophic vegetation was cropped to the lower limit of obvious photo-synthetic tissue). A core of peat 15 cm sq. and 20 cm deep was then extracted from each of the sub-sites with a long knife. A total of approximately 300 vegetation samples and 300 peat cores were collected from the 50 vegetation stands. All the vegetation samples from the sub-sites of each stand were bulked, as were the peat cores, so that one composite sample of peat and one of vegetation from each stand was obtained. The 100 composite samples were stored in plastic bags, those containing peat being hermatically sealed.

In order to reduce the effect of daily and seasonal variation, samples of vegetation and peat were collected between 10.00 a.m. and 2.00 p.m. during July. Field work connected with nitrogen fixation was not restricted to July, and was carried out between 1971 and 1974; all other aspects of field work were completed during the summers of 1972 and 1973. In 1972 a limited range of vegetation and peat was collected and analysed. In 1973 the survey was repeated and extended.

7(b) Preliminary laboratory procedures

The vegetation samples were sorted into species which were dried for 48 h, and then weighed. After weighing, the vegetation samples from each stand were reconstituted by re-combining the species. Each sample was then ground and stored in preparation for chemical analysis. The biomass of each species was utilised in the phytosociological analysis.

Each peat sample was divided into two parts, one of these was used to estimate exchangeable and available elements, water content, and pH. The other sample was dried at 90°C for 72 h, then ground and stored for measurements of total elements and ash. Volume to weight ratios were determined by weighing a known volume of fresh peat.

Estimation of available and exchangeable elements was started as soon as the peat samples reached the laboratory.

Ash content was determined by loss on ignition at 360°C for 24 h; pH was measured by inserting the electrode of a pH meter directly into the fresh peat, and total carbon in the 1972 peat samples was estimated by gas chromatography.

The fresh peat was sealed in plastic bags, and stored at 4°C between the various sets of analyses.

All other experimental details are described in the appropriate chapters.

7(c) Phytosociology

The main objective of this research was a detailed investigation of the relationship between inorganic nutrients, and vegetation associated with the successional change from fens to bogs; therefore a detailed discussion of the various schools of phytosociology would be out of place in the present context. However, it is necessary to consider the various techniques in order to achieve the most effective analysis of the results. Reviews of the philosophy, together with evaluations of the methods, have been presented in many recent publications (e.g. Tuxen, 1973; Gauch and Whittaker, 1972; Whittaker, 1967). The following section deals with the method used in this project, and reasons for the choice.

A study of the interrelationships between peat chemistry and the chemical constitution of the vegetation as such, can be handled by numerous statistical procedures; however an investigation of chemical interaction along an ecocline requires the vegetation samples (aufnamen) to be arranged in a sequence (ordinated), such that the ordinated aufnamen become a true reflection of the coenocline. Direct ordination of the aufnamen on the basis of some chemical factor would give information in relation to that particular factor, but this would not relate to the fen/bog coenocline, unless the factor chosen was the criterion of this gradient. There are several other reasons why I considered this type of direct ordination unsuitable, the first being that in the present context it could lead to circular arguments. Secondly, it is likely that many factors interact along the fen-bog ecocline (i.e. it is a compositional gradient). Finally, it is the available minerals in the peat, rather than total amounts, that are more important in controlling the vegetation, in which case seasonal fluctuations could lead to considerable errors. Vegetation manifests an integration of all the environmental parameters, therefore an ordination based upon vegetation relationships offered a more reliable way

of arranging the aufnamen such that they become a reasonably accurate representation of the fen-bog coenocline.

Having accepted that an ordination based upon floristics was required, then a number of methods were available. A subjective ordering of the aufnamen based upon previous experience might be considered satisfactory when only a few samples widely placed in the coenocline are involved, but this method is unlikely to be successful when more than a few vegetation types are being investigated.

An indirect ordination based upon floristic relationships to polar aufnamen was also considered. This method, developed by the Wisconsin School of phytosociology (e.g. Bray & Curtis, 1957), selects the two most dissimilar aufnamen by examining a full matrix of comparisons. The rest of the aufnamen are geometrically located on the axis by comparing each one in turn with the two polar aufnamen. However, there are several problems when the β diversity (rate of floristic change along the coenocline) is high. The first is that several pairs of aufnamen are likely to have the same degree of dissimilarity, and the pair selected may not represent end points of the axis. Another problem which arises when the polar aufnamen are very dissimilar is that many aufnamen, not associated with either pole, will cluster in the middle of the axis. The standard Wisconsin procedure would be to utilise this cluster as the basis of a second axis representing another environmental parameter, but when the polar aufnamen of the first axis are floristically very different, this clumping is merely an artifact of the method, and a second axis is not necessarily justified.

Maycock and Curtis (1960) have suggested that the aufnamen could be divided into 2, 3, or 4 sets with known relationships to each other, each then being ordinated by means of a partial matrix of comparisons (one matrix for each set). After each section has been ordinated they

are then fitted together. They also consider it more satisfactory to select the polar aufnamen by utilising previous experience whenever possible. Many, but not all of the aufnamen recorded during this research could have easily been placed into the category of rheotrophic, intermediate or ombrotrophic mire types, therefore this method was attempted. However, the resulting ordination placed many aufnamen in positions bearing no relationship to their location in the mire coenocline, this was probably due to the fact that several aufnamen could not be placed with certainty into one of the three categories. The method was eventually abandoned.

An ordination based upon the degree of rheotrophy or ombrotrophy exhibited by aufnamen was then considered; such a method was used by Whittaker (1960) in his studies of the Siskiyou Mountain vegetation. Whittaker (o.c.) gave each of the major species a coefficient of 0, 1, 2 or 3 depending upon its hydrophily. These coefficients were then utilised to derive a weighted average for each aufname (with respect to moisture), these averages were then used for locating the aufnamen on the moisture gradient. The main problem with this technique is that a knowledge of species tolerance is required before a weighting can be applied, such data were not available for the sites being investigated. It would have been possible to utilise the accumulated information of species distribution on mires in other parts of Europe, but the reliability of such methods would be questionable in view of the facts presented in Chapter 6.

In order to discover the approximate range of species, aufnamen recorded during the 1973 survey of Pfrule Moss were first arranged in an approximate order from fen to bog. This was achieved by organising a matrix of similarity values so that the highest values were near to the axis and the lowest at the corners. Two categories of similarity value are available: (1) a similarity measure based upon presence and absence,

or (2) a similarity measure based upon some quantitative measure. The indices based upon presence or absence are greatly influenced by species which are not abundant in the vegetation; conversly rare species have very little affect on an index utilising a quantitative measure. The final ranking was based upon the average of two matrices: one utilising presence and absence

$$= \frac{2C}{A+B} + 100 \text{ (Sørenson, 1948)}$$

where C is the number of species common to both aufnamen, A and B the number of species present in each aufname; the other, based upon the quantitative measure is a modification of the above equation,

$$= \sum_{i=1}^{n} \min (A_i B_i) \text{ (Bray and Curtis, 1957)}$$

where A_i and B_i are performances of the ith species expressed as a percentage of the total performance in each of the aufnamen, A and B which are under comparison.

Detailed data on species phytomass were obtained and applied to the second of the above formulae, but the similarity values obtained with these figures were much too low to be useful in organising the matrix. Several workers have concluded that a subjective estimate of abundance, such as the Braun-Blanquet cover/abundance values provide a better basis for the ordination of vegetation types than detailed measurements of every species (Banister, 1968; Moore, et al., 1970; Gauch and Whittaker, 1972). Two of the possible reasons for this are: (1) these subjective estimates give more weight to rare species than purely objective measurement; and (2) the subjective estimate is based upon observation of the

Ordination based upon presence or absence.

	1	2	3	4	6	5	7	8	11	10	9	13	12	14	15	16	18	19	17	20	21	22		Final weighting
1	100	32	25	24	24	13	0	0	8	0	0	0	0	0	0	0	0	0	0	0	0	0	1	3
2	11	100	49	37	20	14	12	13	11	0	7	15	6	6	6	11	7	6	6	6	0	0	2	15
3	18	20	100	43	34	29	20	13	6	6	15	28	53	0	6	6	8	0	0	0	0	0	3	6
4	21	18	21	100	33	49	31	13	16	19	6	25	6	6	6	11	7	12	6	6	7	6	4	16
6	5	11	26	49	100	19	19	17	28	25	10	19	16	9	8	14	21	16	0	9	0	11	6	37
5	0	7	24	47	35	100	24	27	29	35	30	32	25	18	40	29	44	17	9	0	0	22	5	29
7	4	5	14	35	23	36	100	27	24	31	19	45	17	18	21	33	25	22	8	10	5	0	7	47
8	0	1	13	0	27	16	7	100	26	23	43	41	30	0	21	27	19	15	8	0	10	0	8	35
11	12	1	9	2	34	23	7	29	100	45	15	46	44	47	30	57	38	56	45	47	15	46	11	74
10	6	0	3	23	12	15	13	32	32	100	26	47	30	40	43	40	38	44	31	22	20	38	10	61
9	0	5	0	4	10	14	11	26	26	42	100	27	26	0	24	23	35	17	9	19	12	0	9	43
13	0	7	10	22	25	30	17	15	15	13	15	100	29	48	50	29	48	69	39	61	34	34	13	68
12	0	1	1	7	9	9	4	4	4	27	42	14	100	23	34	39	45	36	22	23	9	18	12	61
14	0	1	0	16	22	18	15	0	0	11	9	36	44	100	59	69	30	77	72	67	30	60	14	82
15	0	4	0	1	1	3	0	4	4	28	6	43	31	53	100	69	52	69	57	51	35	27	15	73
16	0	5	4	4	14	11	4	6	6	48	27	18	24	27	36	100	56	77	73	69	40	48	16	80
18	0	5	1	1	11	9	1	6	6	11	34	12	30	6	10	18	100	57	38	38	25	25	18	76
19	0	6	1	2	8	11	1	6	6	14	34	24	38	24	19	19	37	100	81	85	56	55	19	87
17	0	4	3	4	9	10	4	8	8	27	13	54	43	63	70	36	18	30	100	60	47	27	17	86
20	0	5	0	1	0	3	1	5	5	9	11	29	32	15	50	19	13	27	54	100	60	48	20	89
21	0	0	0	1	0	1	0	4	4	31	4	24	2	17	32	41	11	17	26	25	100	27	21	84
22	0	0	0	4	8	6	4	0	0	11	8	11	43	60	32	12	50	13	40	33	20	100	22	92
	1	2	3	4	6	5	7	8	11	10	9	13	12	14	15	16	18	19	17	20	21	22		

Aufname number

Ordination based upon semi-quantative measure

FIG. 15 FIRST ORDINATION OF AUFNAMEN FROM PFRULE MOSS (1973), BASED UPON SIMILARITY MATRICES.

entire vegetation unit, whereas an objective measurement is usually based upon a very small sample. Therefore the quantitative measure of performance finally used was the Braun Blanquet cover abundance value transformed according to the scale suggested by Moore et al. (1970), as follows:

+	1	2	3	4	5
0.2	1	4	6	8.5	10.5

Each transformed value was then expressed as a percentage of the sum of the transformed values for its aufname. The resulting matrices are displayed in Figure 15.

The next step was to examine the performances of each species in relation to the ranking obtained from the average of the two matrices shown in Figure 15. This was done for all the important species, and the distributions of those occurring in 4 releves or more are shown in Figure 16. The standing crop of each species expressed as a percentage of the total standing crop was used for this purpose. Both range and modal position were considered before assigning any species to a weighting. Five weightings were used: 0, 25, 50, 75 and 100, corresponding approximately to species characteristic of extreme rich fen, rich fen, fen, poor fen, and bog. The weighting given to all the important species is shown in Table A23.

Finally, the aufnamen from each of the mires visited in S. Germany were ordinated on the basis of the weighted index I,

$$I = \sum_{i=1}^{n} S_i W_i$$

where S_i is the relative performance of the ith species derived from the

Braun Blanquet index as described above, and W_i is the weighting assigned to the ith species. Thus, the index reflects the position of the aufname on the nutrient ecocline without direct reference to any chemical parameters. Subjective examination of the aufnamen suggested that the distribution of the indices in relation to the mire categories of Sjörs (1950) is approximately as follows:

ERF	RF	F	PF	B
0	12	37	62	87	100

7(d) Units

Whether results are expressed in milligrams, moles, etc., is a matter of convention and does not affect comparative investigations of a single element, although when different elements are being compared milliequivalents may be considered more satisfactory; however whether soil results are expressed in terms of unit volume, dry weight, organic content, area, fresh weight, etc., can affect the conclusions reached. In most ecological work the quantity of any element available in soil is usually required, but the unit which expresses this most accurately is not known. In an examination of some 20 papers in which peat chemistry is reported in relation to availability to plants I found that 2 expressed results in terms of per unit area, 14 per unit volume, and 14 per unit dry weight. One also reported the results in terms of per unit organic content. Only one discussed the problem of units, this was Sjörs (1961) who considers per unit volume a measure of total amount available, and per unit organic content a measure of the intensity of an element. It is noteworthy that Sjörs results show little difference between values obtained when results are expressed in terms of per litre of soil, and per 100 g humus. Many of the results obtained in this investigation were examined in both per

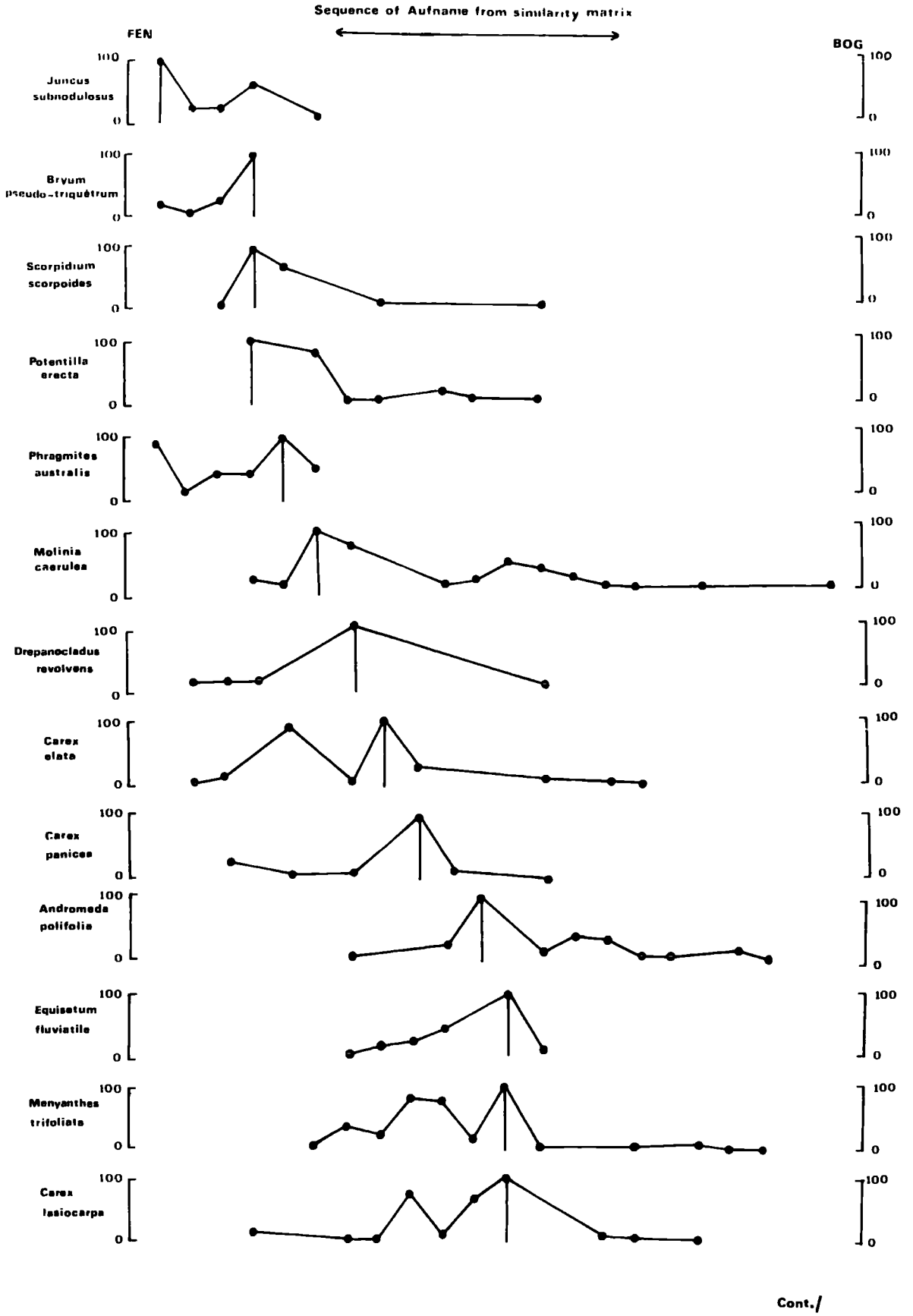

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FIG. 16 RELATIVE PERFORMANCE OF VARIOUS SPECIES IN DIFFERENT PARTS OF THE MIRE ECOCLINE. FIRST APPROXIMATION OF AUFNAME SEQUENCE BASED ON SIMILARITY MATRIX (FIG. 15). VERTICAL AXIS IS RELATIVE PERFORMANCE OF SPECIES IN EACH COMMUNITY AS PERCENTAGE OF MAXIMUM RELATIVE PERFORMANCE.

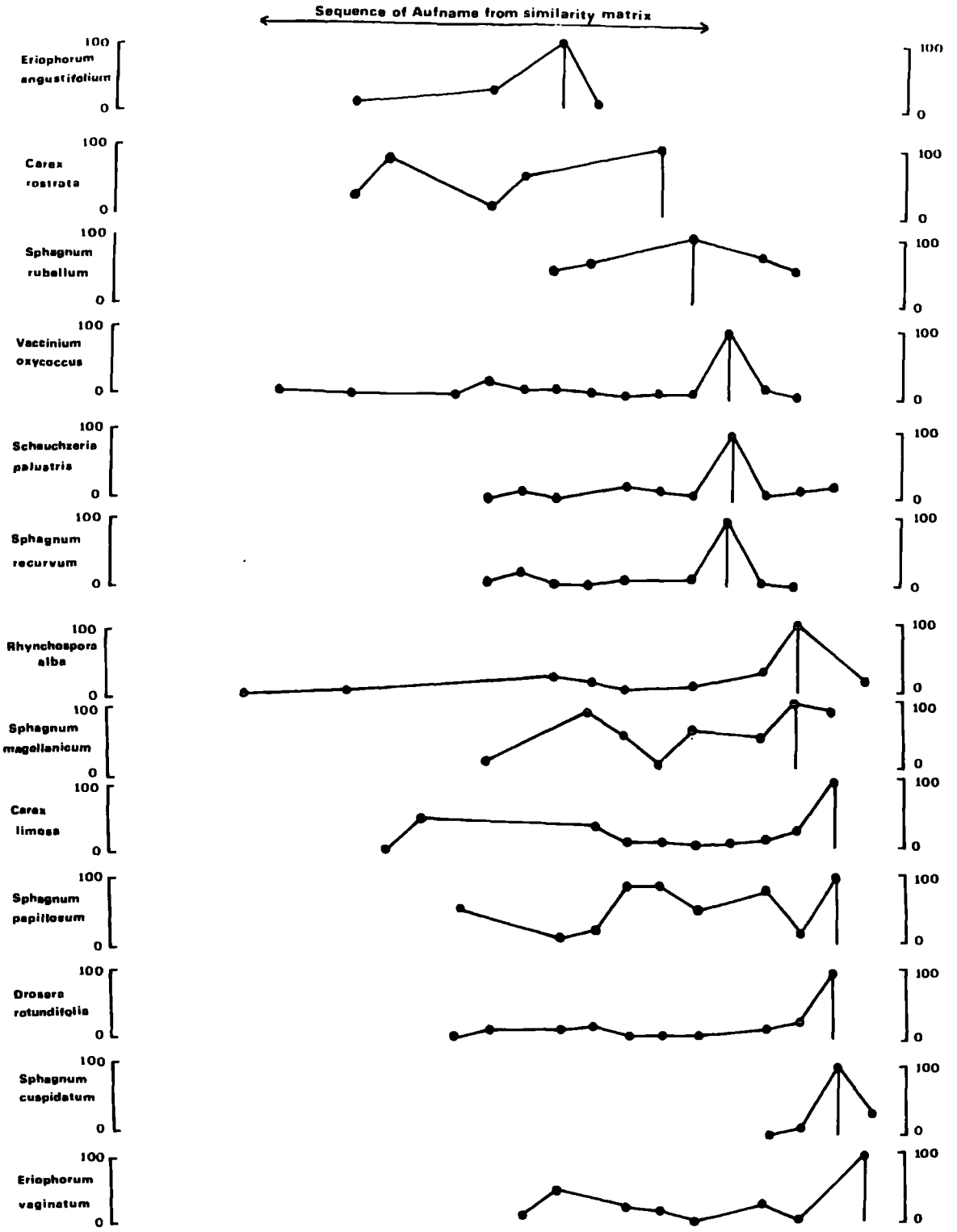


FIG. 16 CONTINUED.

unit humus and per gram dry weight of peat, and little difference was found, except for a few extreme fen samples. In these cases the clay coloids can form a large fraction of the peat or 'muck', and the slightly higher figures obtained when expressed in terms of organic content may misrepresent the intensity with which an element is retained. Furthermore, in order to express results in terms of volume or organic content, it is necessary to make several more laboratory measurements, thus compounding the experimental errors.

In view of the above considerations, all data in the diagrams relating to the ecocline have been expressed in terms of unit dry weight, however information on volume and ash contents are reported in the appendix so that the reader may convert to these units if he so wishes. The one exception to this is in Chapter 8 where potential nitrogen fixation has been expressed (a) in terms of volume, in order to provide an indication of the total contribution beneath each vegetation type; and (b) in terms of organic content for detailed analysis; this is a more accurate measure of the intensity of activity because the inorganic fraction could not possibly contribute to living nitrogen fixation.

7(e) Notes on the statistics used

In Figure 17 a selection of data have been plotted in the form of frequency distributions, it can be seen that many of these show marked skewness. Therefore certain statistical tests which require normal distribution of the error component are precluded, unless a transformation is carried out. The right hand set of figures illustrates the effect of logarithmic transformation, it can be seen that in most cases normality is reasonably approximated; a more complete list of deviations from normality and the effect of log transformation is shown in Table A20. Therefore, in all instances where normality is required, the distributions were

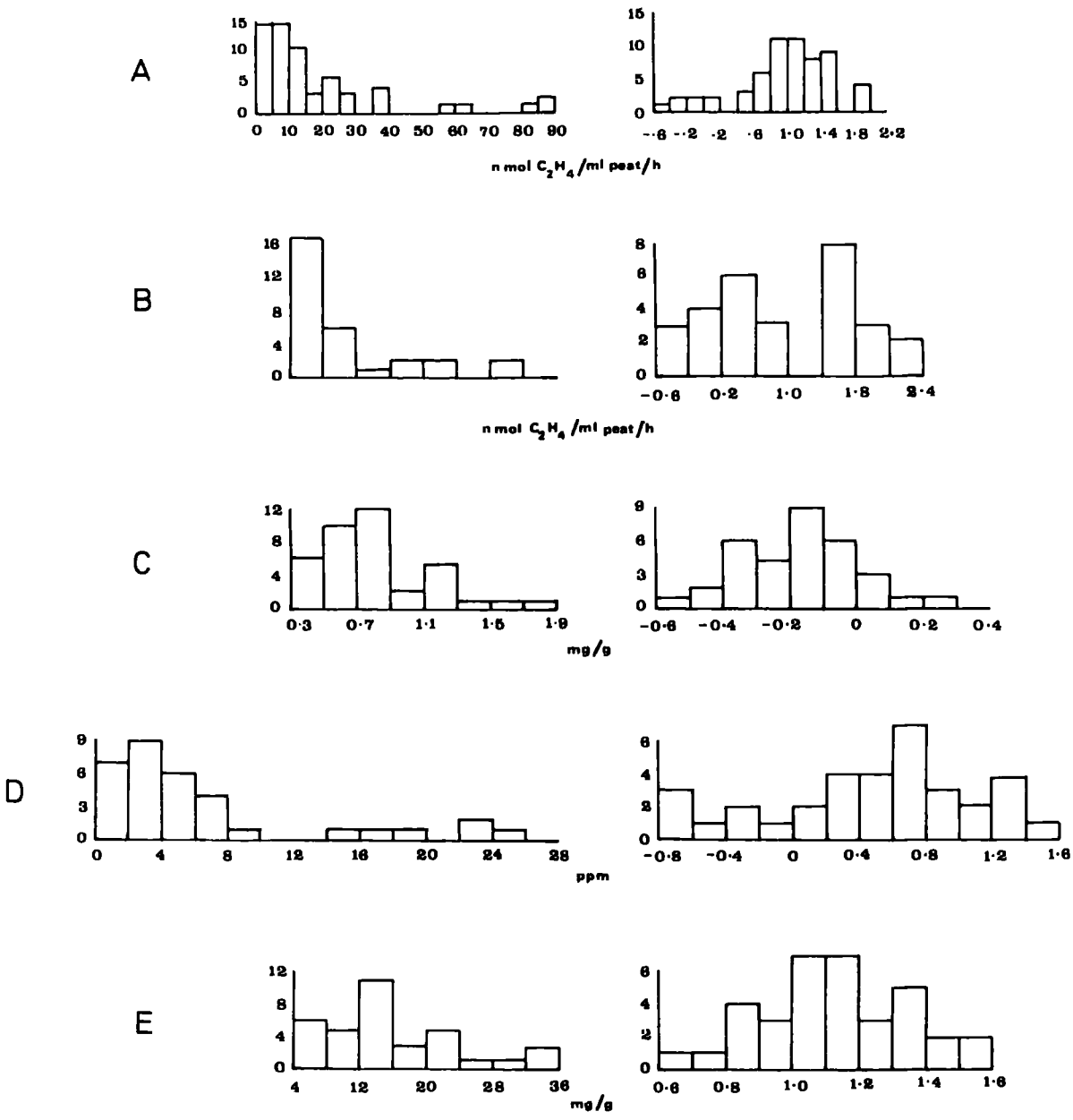


FIG. 17 EFFECT OF LOGARITHMIC TRANSFORMATION ON SOME SETS OF DATA. A, ACETYLENE REDUCTION DATA FROM EAST ANGLIA; B, ALL 1973 ACETYLENE REDUCTION DATA; C, PHOSPHORUS IN VEGETATION 1973 DATA; D, SOLUBLE PHOSPHORUS IN PEAT (AMMONIUM CHLORIDE EXTRACT) 1973 DATA; E, TOTAL NITROGEN IN PEAT 1973 DATA.

examined, and if necessary, log transformations carried out.

In view of the lack of previous data on mire nitrogen fixation, I decided that the extra labour required in order to obtain an estimate of field variability was justified, this enables statistical limits to be applied to the figures for each vegetation type. Logarithmic transformation of the data gave an approximation to normality as well as a stabilisation of the variance. Due to this transformation, data on acetylene reduction in relation to the ecocline are presented in the diagrams in the form of log. geometric means; arithmetic means can be found in the Appendix.

(Note: 1 was added to each figure before transformation in order to eliminate zeros).

The relationships of the various chemical parameters to the mire ecocline were examined by testing the significance of the trends using linear, or where necessary polynomial, regression techniques.

The manner in which various chemical factors are related to nitrogen fixation, as well as to nitrogen and phosphorous in the vegetation was investigated by means of multiple regression and partial correlation methods. The general approach used for this was as follows:

1. General screening of potential independent variables by means of simple correlation coefficients.

2. Variables with a high correlation coefficient with the dependent variable were then examined for linearity with the dependent variable, and co-linearity amongst themselves. Multiple regression cannot be carried out if there is high correlation between the independent variables, therefore where r values greater than 0.8 were found, one of the independent variables was eliminated on the basis of partial correlation coefficients with the dependent variable.

3. For the analysis of factors affecting nitrogenase activity and

phosphorus in vegetation, a log transformation was used because in addition to improving the normality, it gave rise to a more nearly linear relationship with the independent variables, and stabilised the variance, both characteristics being required by regression techniques.

4. The variables which appeared to be most important as a result of step 2 were then subjected to step-wise regression procedure each factor being included so as to maximise the explained variance.

Multiple regression becomes inaccurate when the number of variables is more than about $\frac{1}{5}$ the number of data points (Draper & Smith, 1967) and cognisance of this fact was taken during the analysis.

F values adjacent to individual factors in the tables refer to the proportion of the variance accounted for by that factor, when the variance accounted for by the other factors in the equation is controlled (i.e. they are derived from the semi-partial correlation coefficients). The significance of each step of the regression is not shown, but with n greater than about 20, and the R^2 value (coefficient of multiple determination) greater than about 0.5, then increases of 3-5% are significant in the p = 0.1-0.05 range. Therefore the general limit for increments is set at 3% above R^2 = 0.5.

Beta values shown in the tables are standardised regression coefficients: these provide a useful indication of the relative influence of the different factors in the regression equation as the effect of different units is reduced. The more important statistical data are presented in the form of tables, the rest is summarised in the text. The following symbols are used in association with the statistics: F = variance ratio; n = number of samples; d.f. = degrees of freedom; s.e. = standard error of estimate; p = the probability less than; N.S. not statistically significant; r = correlation coefficient; R^2 = coefficient of multiple determination. *$p < 0.05$; ** $p < 0.01$; ***$p < 0.005$.

All the elementary statistical techniques employed are standard ones which can be found in any statistical text, the more advanced regression procedures are from Draper & Smith, (1967). The computer programmes are those of Nie, et al., (1972).

CHAPTER EIGHT :

HETEROTROPHIC NITROGENASE ACTIVITY IN VIRGIN PEATLANDS

8(a) Methods

Peat samples, varying in number between 7 and 25, were collected from each vegetation type sampled to a depth of 20 cm. About 15 ml of fresh peat from each sample was assayed in 30 ml universal bottles with an incubation period of 4 hours in the dark at ambient field temperature. At the end of the incubation period a sample of the gas was collected and stored for analysis (Waughman, 1971).

The temperature was noted at various times throughout the assay period, and the average obtained. In order to facilitate comparisons all rates of nitrogenase activity are standardised to 20^{o}C using the technique described in Chapter 5.

In order to determine the relationship, if any, between nitrogenase activity and specific chemical factors, it was necessary to analyse the peat cores and vegetation samples. Details of these various analyses are dealth with in Chapter 7, 9, 10 and 11. The method of estimation used for nitrogenase activity has been described in Chapter 4.

8(b) The distribution of nitrogen fixation in peatlands

The results of the survey are displayed in Figures 18-20, and from these it can be concluded at once that nitrogen fixation is widespread in peatlands. Activity was easily detected in every one of the 11 systems examined. Thus it appears that nitrogen fixation in peatland soils is much more common than in mineral soils, to which an energy source must frequently be added if nitrogen fixation is to be even detected.

In peatland systems where it was possible to assay more than one

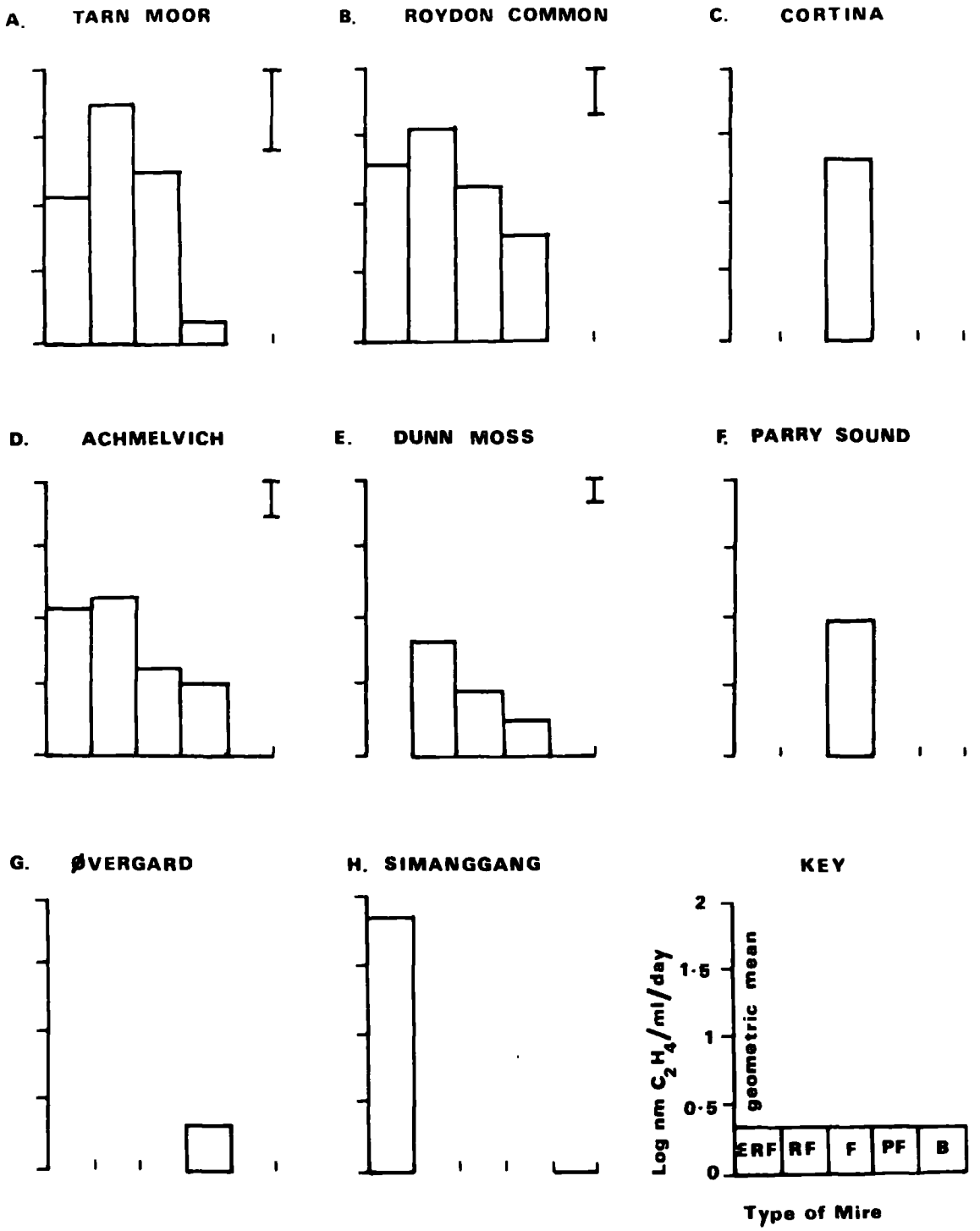


FIG. 18 ACETYLENE REDUCTION BY FRESH PEAT FROM MIRES OTHER THAN THOSE IN GERMANY, IN RELATION TO THE MIRE ECOCLINE. E.R.F., EXTREME RICH FEN; R.F., RICH FEN; F., FEN., P.F., POOR FEN; B., BOG. BARS INDICATE L.S.D., p = 0.05.

mire type, the lowest rates of activity were recorded in ombrotrophic communities, and the highest in the rheotrophic ones: this feature is apparent in the results from German mires (Figures 19 and 20), and in those from Tarn Moor, Roydon Common, Achmelvich, Dun Moss, and Simanggang (Figure 18). The rheotrophic mire at Simanggang site is in fact Riverine Mangrove, and the ombrotrophic site is the adjacent Shorea albida forest: these vegetation types are considered to have a relationship which is equivalent to the fens and bogs of Temperate lands, (Anderson, 1964).

Figures 18 A, B, D and E suggest that the rate of nitrogenase activity is in fact quite closely related to the mire ecocline, gradually increasing from bog to fen; however, these figures also suggest a marked decline in activity in extreme rich fens, as compared with rich fens (Figures 18A, B and C). The German sites permitted a more detailed examination of the relationship between nitrogenase activity and peatland type, and in these mires similar trends were observed. All the trends shown in Figures 19 and 20 are statistically significant, except those for Wurzacher Ried (Figure 19B). The general statistical significance of the quadratic term in the equations for trends in Figures 18A, B and Figure 20 supports the suggestions made above that activity in extreme rich fen is significantly lower than in fens (Table 5). The feature was not observed in the results from Taufach Moss, where no rich fens occur.

It must be emphasised that these experiments were carried out only during the peak growing seasons of 1972 and 1973, therefore it is not possible to derive a predictive equation for nitrogen fixation which is meaningful, and this was not the objective. However, hope that such an equation might be eventually obtained by a development of the methods used here is encouraged by the similarity of equation obtained for Phrule Moss on the two separate surveys:

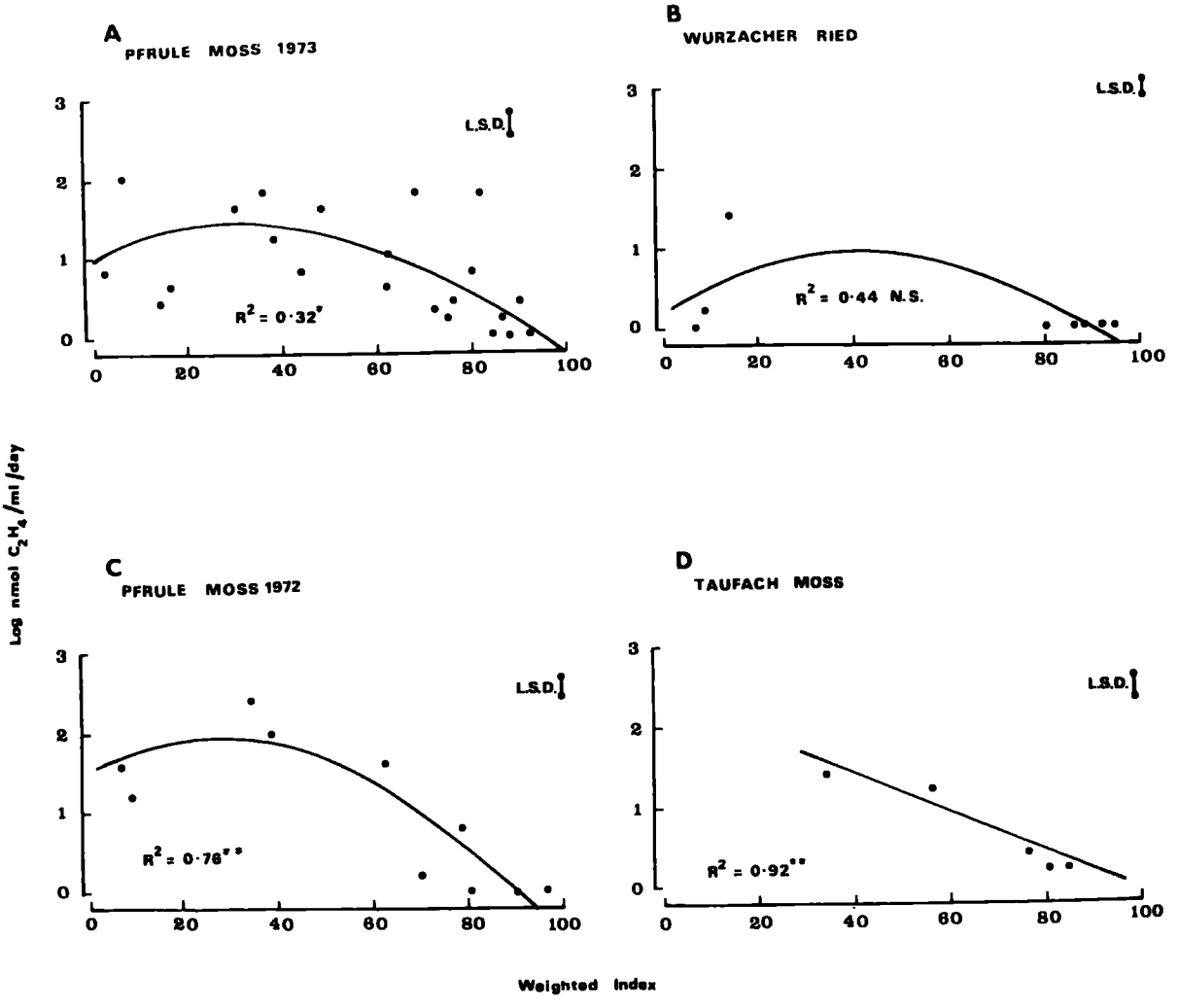


FIG. 19 ACETYLENE REDUCTION BY FRESH PEAT FROM GERMAN MIRES IN RELATION TO THE MIRE ECOCLINE. L.S.D. BETWEEN POINTS AS INDICATED, p = 0.05. R^2 VALUE OF TREND SHOWN.

Table 5

Significance of the trends of nitrogen fixation in relation to the mire ecocline. (Figures 19 and 20)

Location	Source of reduction	Reduction in sums of squares	Residual S.O.S. after reduction	Residual d.f.	F
Pfrule Moss 1973	linear	2.010	7.469	20	5.38*
	quadratic	1.068	6.400	19	3.17*
Wurzacher	linear	0.600	1.181	6	3.05 N.S.
	quadratic	0.189	0.993	5	0.95 N.S.
Taufach Moss	linear	1.182	0.0963	3	36.81**
	quadratic	0.064	0.0161	2	4.00 N.S.
Pfrule Moss 1972	linear	4.482	3.312	8	10.82**
	quadratic	1.5098	1.803	7	5.86*
All 1973 results pooled	linear	3.298	12.019	33	19.06***
	quadratic	3.543	8.469	32	13.41***

(Note: all subsequent curves in the thesis were analysed in the above manner).

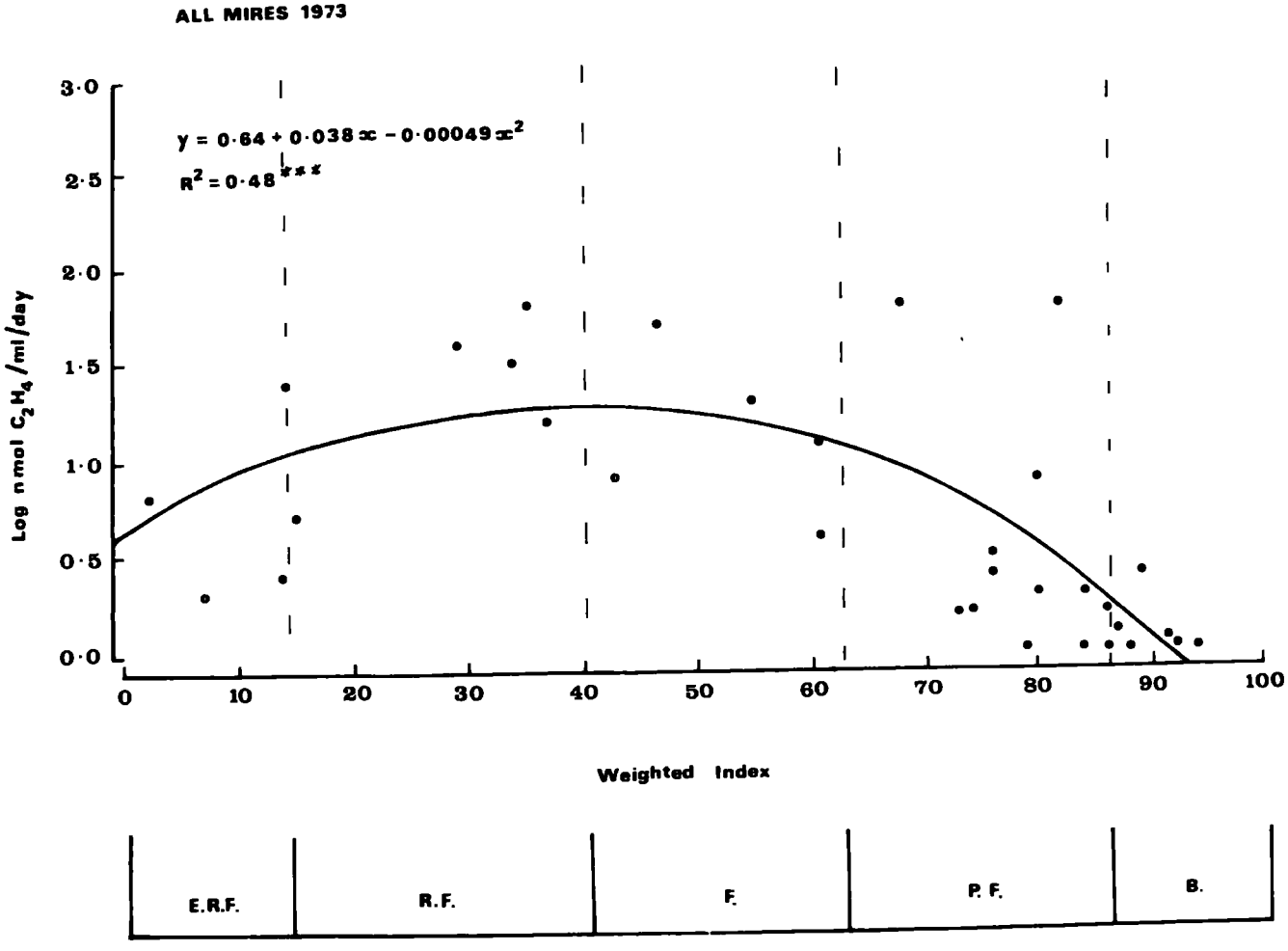


FIG.20 POOLED RESULTS FOR 1973 ACETYLENE REDUCTION TESTS ON PEAT FROM GERMAN MIRES. (DETAILS AS FIG.19).

1972 $y = 1.5 + 0.03x - 0.00053x^2$
1973 $y = 1.0 + 0.022x - 0.00034x^2$

(Symbols correspond to axes in Figure 18).

Plate counts of Azotobacter sp on elective media were performed with material collected from Tarn Moor in Westmoreland. Only a simple comparison of rheotrophic peat with ombrotrophic peat was feasible. The estimates of 71,000,000 organisms per gram for rheotrophic, and about 100 organisms per gram for the ombrotrophic peat (Table A17) are consistent with the trends described above. It is interesting to note that some Azotobacter sp. were detected in peat from the ombrotrophic site where the measured pH was 4.6. Clostridia were also detected, and although no attempt was made to count these species, the results indicate greater numbers in the rheotrophic peat (Table A16).

8(c) Chemical factors influencing heterotrophic nitrogenase activity in peat

The general relationship between the mire ecocline and nitrogenase activity has been described above, this, in part at least, is attributable to the greater metabolic activity in fens, hence faster breakdown and cycling of minerals, which in turn helps to support greater productivity providing more energy for microbial activity etc. The objective of this section of the work was to discover which, if any, specific elements might have a significant influence on the rate of nitrogen fixation. The exchangeable and extractable forms of the various elements may, or may not be available to higher plants, however they do provide some information relating to the chemical milieu in which the nitrogen fixing organisms exist. The factors which were included in the analysis were Ca, K, Mg, Fe, Mn, Zn, Al, pH and Ash content. The statistical analysis has been carried out in 3 parts: (1) utilising all the samples; (2) those from the

poorer mires only; and (3) those from the richer mires. The weighted index of 50 was used to separate the two sets.

Table 6

Relative contribution by soluble inorganic factors in peat to the explained variance of heterotrophic nitrogenase activity in peat

Factors added to equation	% variance explained	Beta	F
	Entire Mire		
pH	30	1.07	24***
Ca	16	-0.64	12***
K	12	0.42	11***
Fe	4	0.18	2.1 N.S.
p	3	-0.20	2.1 N.S.
(R^2 = 0.65***; n = 35)			
	Richer Mires		
Ca	32	-0.74	9.5**
K	23	0.67	8.7**
P	13	-0.37	3.6 N.S.
Fe	5	0.24	1.3 N.S.
(R^2 = 0.73**; n = 13)			
	Poorer Mires		
pH	32	0.83	12***
K	21	0.54	11***
Fe	5	0.29	2.3 N.S.
Mg	4	0.40	4.4*
NH_4^+	3	-0.35	0.38N.S.
Ca	3	-0.55	3.2 N.S.
(R^2 = 0.68***; n = 22)			

Soluble P = average of NH_4Cl and 0.02 NH_2SO_4 extractable

Results of the step-wise regression analysis are displayed in Table 6, and the first fact which is apparent is the appearance of potassium in all three sections of the Table, this element not only explains a considerable proportion of the variation in nitrogenase activity, but its F values indicate that potassium is probably important in its own right, as well as through interactions with other variables in the regression equation.

pH also explains a large proportion of the variation, and again the F values indicate that it may exert some direct influence. It should be emphasised that the F values for individual variables are based upon the part-correlation coefficient with allowance made for other variables in the regression equation. Therefore when an F value is significant it can be concluded that the variable in question exerts some influence independently of other variables in the regression equation (but this, of course, does not preclude the possibility of interaction with factors not in the equation, chemical or otherwise). On the ombrotrophic part of the mire pH is the main determinant, after which potassium accounts for a further 21% of the variability. The F values for these two variables are about the same, which indicates that pH must exert a considerable influence through other factors in the equation. pH does not appear to exert any significant influence on nitrogenase activity at the rheotrophic end of the mire gradient, which includes samples generally above about pH 4.8.

The best established chemical feature of the peatland ecocline is the general decrease in the alkali elements towards the ombrotrophic end, where the rooting zone is gradually removed from the influence of ground waters by growth of the peat mass. Thus the general relationship of nitrogen fixation to pH might have been predicted once the overall relationship with the ecocline was established as described above. However, a fact which is particularly interesting is that the cation which appears to exert the most positive influence overall, namely potassium, <u>increases</u> from the rheotrophic to the ombrotrophic areas which is opposite to the general direction of decline of nitrogenase activity (Figure 41B).

Calcium appears to have considerable negative influence on the rate of activity, and again the high F value suggests some direct affect at the rheotrophic end; its influence is not statistically significant at the ombrotrophic end. Exchangeable calcium declines from fen to bog, which

is the general direction of decline of nitrogenase activity, however the regression coefficients for this variable are negative, suggesting some form of inhibitory effect. Calcium appears to have little direct influence on nitrogenase activity in ombrotrophic mires, although its inclusion in the regression equation increases the explained variance by 3%, of greater interest is the fact that once again the regression coefficient is negative.

Magnesium appears to be of far less consequence with regard to the nitrogenase activity than either potassium or calcium, however it does make a small and statistically significant contribution to the results for ombrotrophic mires. Ammonium nitrogen also appears to have some small negative influence.

Iron accounts for a significant amount of the explained variability in each analytical category, however in no instance is the partial coefficient itself statistically significant.

Phosphorus appears in the results for the entire mire, and the rich mires sections only, in both cases its affect appears to be negative, and, like iron, the regression coefficients are not themselves significant.

Numerous trace elements are known to play an important part in nitrogen fixation, e.g. molybdenum, cobalt; and vanadium in the absence of molybdenum (Mortenson, et al., 1967; Burns et al., 1971; Saubert and Strijdom, 1968). These could not be detected in the acetic acid extracts, therefore total quantities of these elements were determined in the peat, and a statistical analysis performed in order to detect any indication of some effect; the additional elements included in this analysis were Co, Cd, Ni, Cu, Pb, V and Mo. Using the same partial regression technique as above, no influence of these elements could be detected.

One general conclusion which may be drawn from the statistical

analysis, is that chemical factors account for about 60-70% of the variability of heterotrophic nitrogen fixation in mires.

These results will be fully discussed in Chapter 12.

CHAPTER NINE :

NITROGEN IN PEATLANDS

9(a) Methods

Total nitrogen in peat and vegetation was determined by Kjeldahl digestion of 100 mg samples, followed by estimation of the ammonium formed using the indophenol reaction. The digesting mixture for each sample was 2 ml concentrated H_2SO_4 containing 0.1% Se, and 1 ml H_2O_2. Heating was continued for 1.5 h after the fuming had stopped. Colorometric estimation of ammonium, without distillation, was carried out following the method described by Allan and Whitfield (1965).

Soluble forms of nitrogen in peat (NO_2^- , NH_4^+ and NO_3^-) were extracted from fresh samples with 2N NaCl. NaCl was preferred to KCl or Na_2SO_4 because tests showed that the former contained the lowest NH_4^+ - N impurity: 'analar' grade chemicals were used throughout. Water content of the various peats was estimated, and peat equivalent to 2 g dry weight was added to 50 ml of 4N NaCl, the volume of each sample was then made up to 100 ml, i.e. 1 g dry weight extracted with 50 ml 2N NaCl. Soluble nitrogen in the 1972 samples was extracted using only neutral NaCl, for the 1973 samples both neutral and acidified NaCl was used. The extracting solution was acidified by adding sufficient HCl to give a pH of 2.3 prior to making up to volume, a pH meter and magnetic stirring device was used during this procedure. The samples were then shaken for 30 m and then left to stand overnight before filtering.

NH_4^+ - N in the filtered extracts was estimated by cold semi-micro distillation (Etherington & Morrey, 1967) using 1 ml of a 12% light MgO suspension for each extract aliquot, in order to create alkaline conditions. This mildly alkaline reagent is more suitable than stronger ones (e.g.

KOH, NaOH) because with it, the problem of reduction of amino acids to ammonia is minimal (Bremner & Shaw, 1955). Distillation took 24 h at 25°C. NO_3^- was reduced to NH_4^- using titanous suphate, and the NH_4 then being distilled as described above (Waughman, 1969). The distilled NH_4^+ was estimated with sodium phenate using nitroprusside as a catalyst (Lubochinsky & Zalta, 1954). NO_2^- content of the neutral 1973 extracts was estimated colorometrically using sulphanilomide and N-(1-naphthyl)-ethylenediamine as described by Hesse (1971). The methods of sample preparation, are described in Chapter 10.

9(b) Relationships between nitrogen and the mire ecocline

These results are displayed in Figures 21-27: Figures 21 and 22 illustrate the results from individual mires, and Figures 23-27 illustrate pooled data. Only a summary of the statistics is provided on each figure, the procedure used for statistical analysis of the trends was exactly the same as shown in Table 5. In Figure 22 the results for total nitrogen content in peat at Taufach Moss indicate a trend towards higher levels at the ombrotrophic end of the ecocline, this trend is statistically significant at $p < 0.05$. No other trends regarding total nitrogen content of the peat in relation to the ecocline are significant, although results from other locations indicate a slight decrease in the nitrogen content of peat towards the ombrotrophic end of the gradient (Figures 22 and 26). The range of values obtained for poor mires (5-35 mg N/g dry wt.) is greater than the range in rich mires, and may explain why some previous investigators have failed to reach any firm conclusions regarding the nitrogen content of peat and the mire gradient. Kaila et al. (1954) and Kaila (1956b) were unable to detect any differences between the different types of peat, and Sjörs (1954) concluded that the nitrogen content of peat in mire meadows was not related to pH. In a later publication Sjörs again

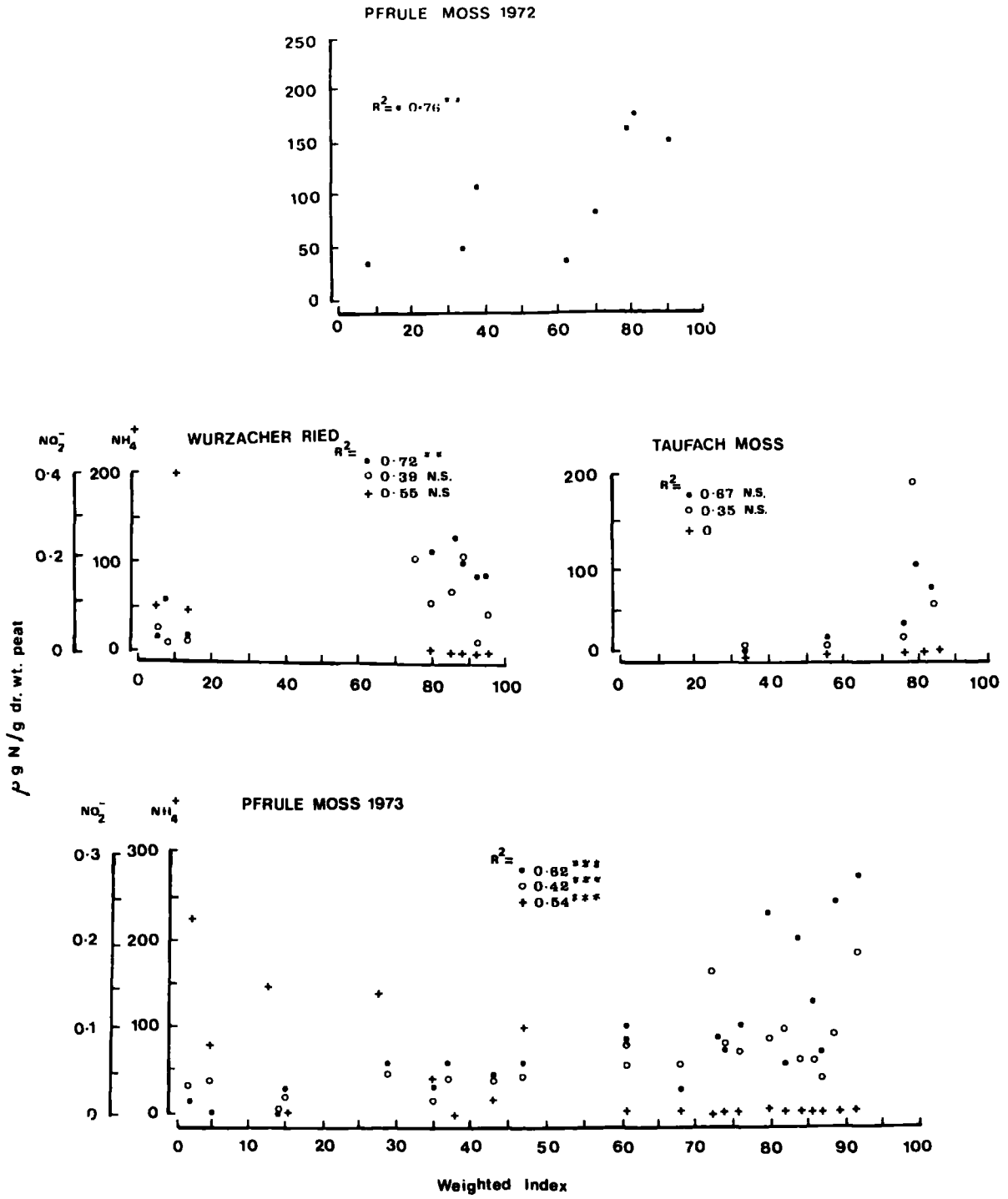


FIG 21 SOLUBLE NITROGEN IN PEAT IN RELATION TO MIRE ECOCLINE. AMMONIUM NITROGEN, ACIDIFIED EXTRACTANT (•); AMMONIUM NITROGEN, NEUTRAL EXTRACTANT (o); NITRITE NITROGEN, NEUTRAL EXTRACTANT (+). R^2 VALUES OF TRENDS AS SHOWN

reported very variable total nitrogen values, but concluded that bog peats contained less nitrogen than fen peats when the results were expressed in terms of per unit humus (Sjörs, 1961). After examining a few mires Malmer did not include total nitrogen as one of the chemical features distinguishing bogs and fens, (Malmer and Sjörs, 1955); but in a larger survey he also concluded that fen peats contained rather more nitrogen than bog peats (Malmer, 1962a). Pollett (1972) found the nitrogen content of fen peats in Eastern Canada higher than bog peats when expressed in terms of unit volume.

The consensus of results from earlier investigations indicates a slight decline in nitrogen content in peat towards the ombrotrophic end of the gradient, but this is by no means a universally obvious feature of all earlier data. Results displayed in Figure 26 illustrate the large variability in values for this element, and it is clear how the conclusions might easily be affected by choice of site in a small investigation. The decline in concentration is somewhat more obvious when the results are expressed in terms of either per unit volume or per unit humus (Table A21). Total nitrogen in peat does not appear to be a useful diagnostic parameter by which to distinguish bog and fen peats.

The concentration of soluble NO_2^- - N is greatest in rich fens, decreasing towards fens, and is non-existent in poor fens and bogs (Figures 21 and 25). Results for the concentration of NH_4^+ - N indicate that this ion is present in much greater quantities than NO_2^- - N, and has a trend in concentration which is opposite to the latter ion, i.e. it is lower in the rich mires and increases towards the ombrotrophic end of the ecocline. This trend is statistically highly significant in most instances. (Figures 21, 23 and 24). The same trend was found whether neutral or acidified NaCl was used as the extracting fluid.

NO_3^- - N was not detected in any of the samples; considering the

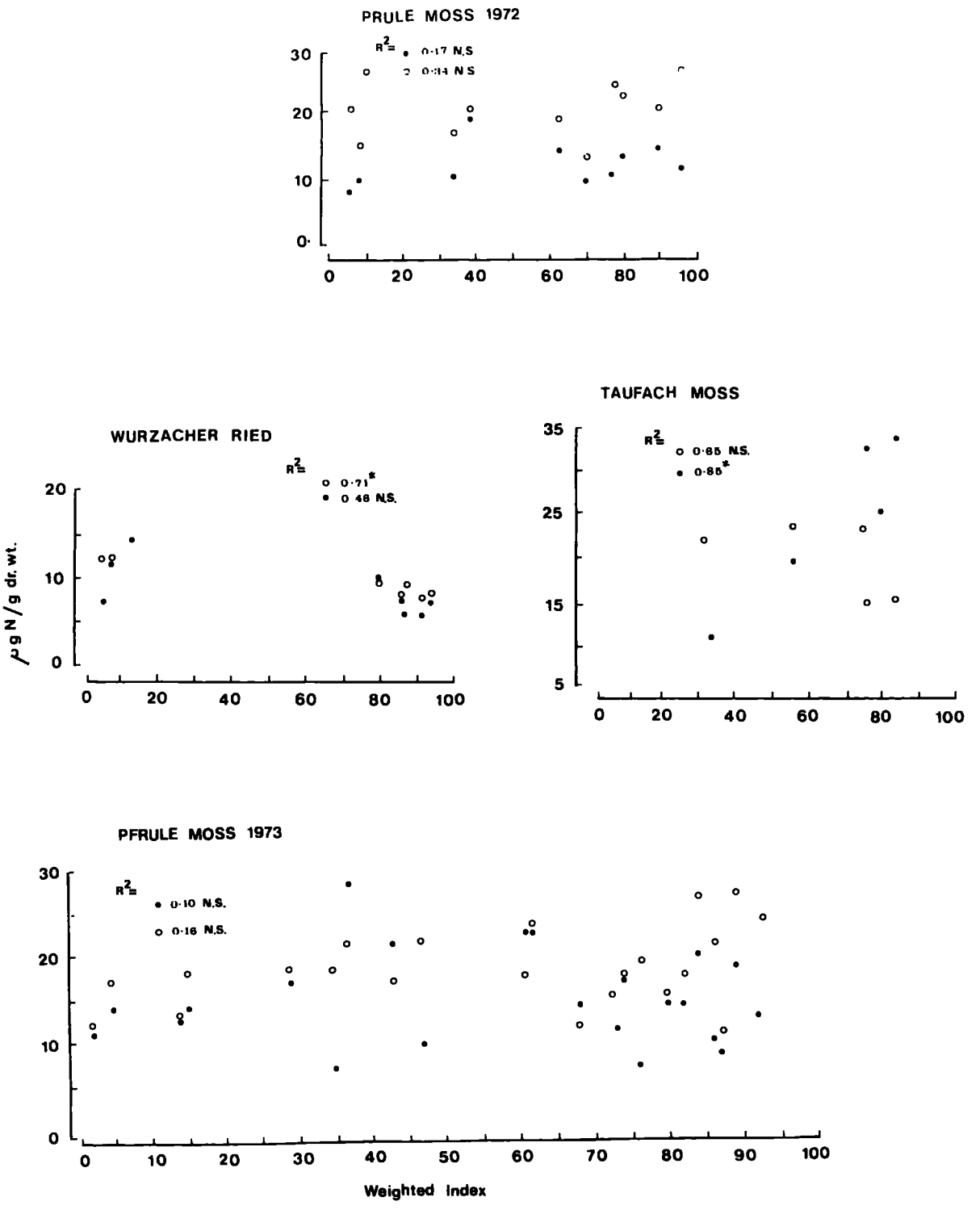


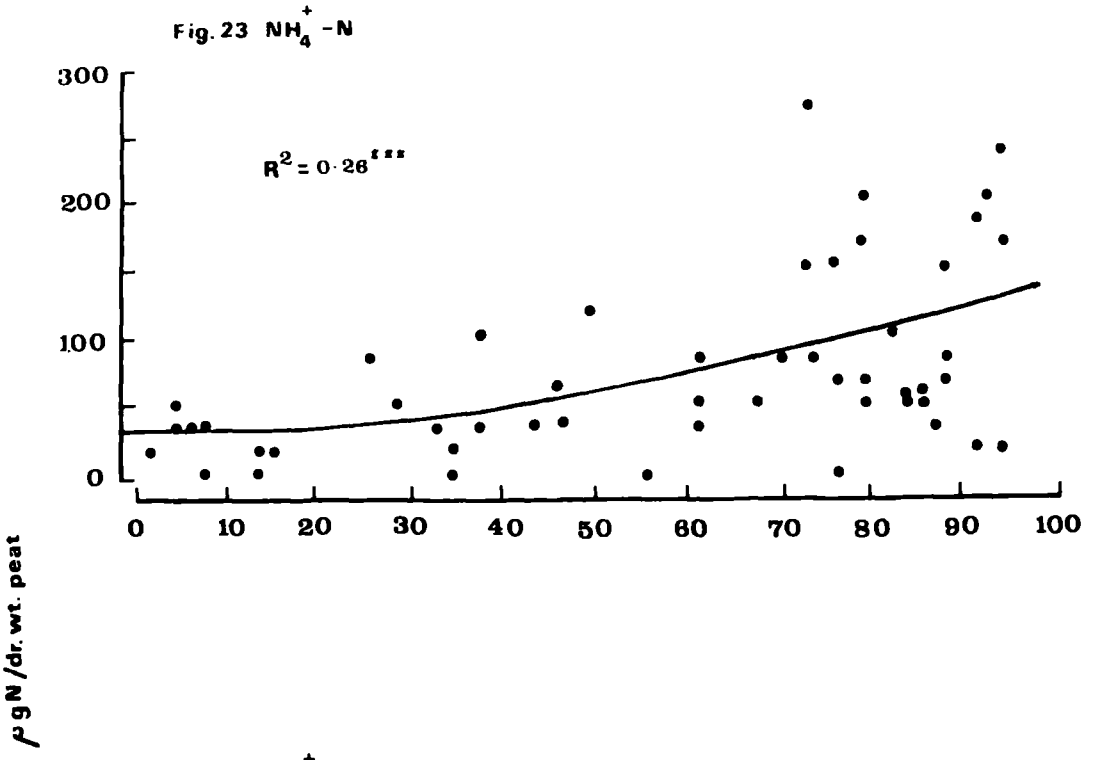
FIG 22 TOTAL NITROGEN IN PEAT IN RELATION TO MIRE ECOCLINE (•), AND VEGETATION (o). R^2 VALUES OF TRENDS SHOWN

sensitivity of the method this indicates that the concentration of NO_3^- - N in peat could not have been greater than about 3 p.p.m. (dr. wt. peat), which is much lower than the concentration of NH_4^+ - N in most of the samples. These results show that NH_4^+ - N is the most important source of inorganic nitrogen in mire systems.

Sufficient data are provided in the Appendix to permit the reader to convert results from most sites into any units desired. If the results were presented here in every possible way it would require at least a three-fold increase in the number of figures. However, some of the more important trends in relation to the mire gradient have been re-calculated in terms of alternative units, and the results listed in Table A21. It can be seen that the direction of the trends is not altered, although the levels of significance are.

9(c) The main forms of inorganic nitrogen in peat

The results outlined above show NH_4^+ - N to be by far the most important form of nitrogen in peat, this is consistent with the results of previous workers, although in some instances a small amount of NO_3^- - N has been detected. For example, Cyplenkin & Schilin (1936) found abundant NH_4^+ - N but very little NO_3^- - N in their mineralisation studies of Tundra peats. Similar results were obtained by Kaila et al. (1953), even after liming to pH 6.0; however in later experiments this group found levels of up to 150 p.p.m. fen peat. This high figure for NO_3^- - N must be questioned, because it was obtained using the phenoldisulphonic acid method of NO_3^- - N estimation: a technique which is notoriously prone to interference by many of the substances present in peat, including chloride, iron, and organic substances (Johnson & Ulrich, 1959). The phenoldisulphonic acid technique presents too many problems to be of any real value for nitrate estimation in peat. Persson (1962) examined NO_3^- - N in rich fens and



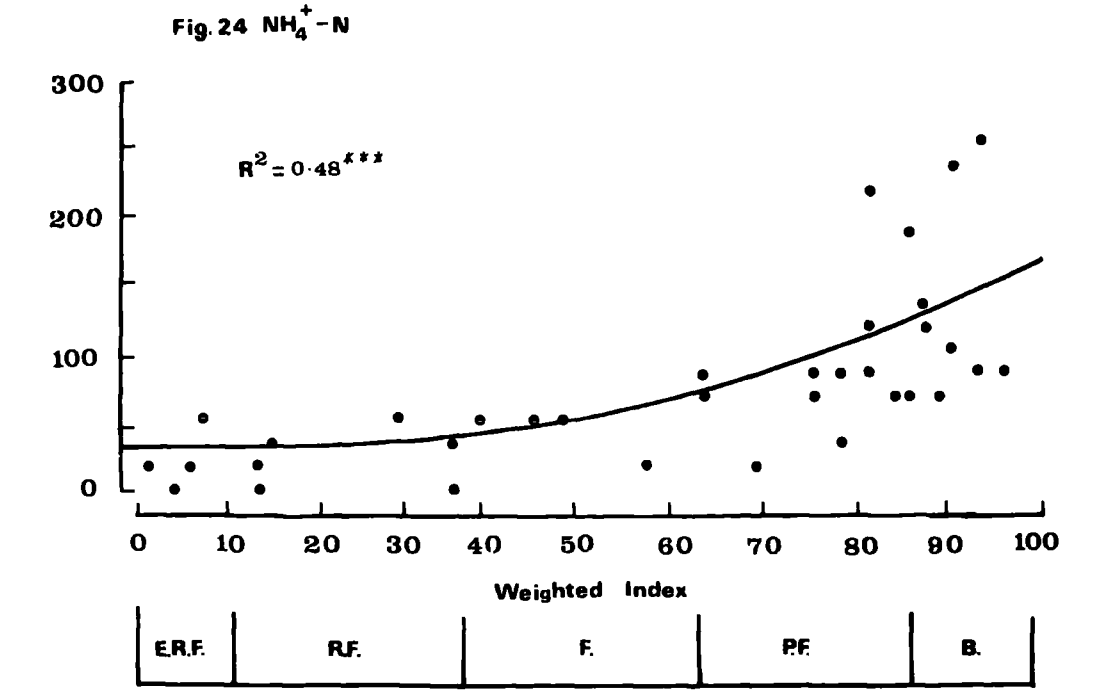


FIG. 23 POOLED DATA FOR SOLUBLE AMMONIUM NITROGEN IN PEAT IN RELATION TO MIRE ECOCLINE, NEUTRAL EXTRACTANT. R^2 VALUES AS INDICATED.

FIG. 24 POOLED DATA FOR SOLUBLE AMMONIUM NITROGEN IN PEAT IN RELATION TO MIRE ECOCLINE, ACIDIFIED EXTRACTANT. R^2 VALUES AS INDICATED.

found the concentration generally less than about 0.05 m mol NO_3^- - N per litre of fresh peat (about 3 p.p.m. dr. wt. of peat).

There are several possible reasons why NH_4^+ - N predominates in mires, especially bogs. The most obvious is that nitrifying bacteria are inhibited below about pH5, whereas ammonifying bacteria are tolerant of acid conditions (Harmsen and Kolenbrander, 1965). However, there are many complex reactions between inorganic nitrogen chemical species in peat, and not all of these chemical conversions are biological in origin (Mortland & Wolcott, 1965). The relative amounts of NO_2^- - N, NO_3^- - N and NH_4^+ - N reported in the previous section are quite consistent with their known chemistry in respect of pH and redox potential. Figure 28 shows a pH-redox diagram for the relative stability of these three ions, it illustrates which species is likely to predominate in any specific conditions of redox and pH. The normal ranges of redox and pH usually found in peat have been superimposed onto this diagram, and it can be seen that only NO_2^- - N and NH_4^+ - N are likely to exist within the circumscribed limits. This exactly predicts the results obtained in this project. It also shows how aerating calcareous peats and mucks might cause NO_3^- - N to increase, possibly even to toxic levels (e.g. Levin & Shoham, 1972).

If NO_3^- - N is ever to be found in abundance it would be in near neutral, or alkaline conditions. However, such conditions in mires are always rheotrophic, and as anions are not strongly bound by colloids it follows that any nitrate formed and not absorbed by the vegetation would be removed from the peat by the leaching action of moving groundwater. The fact that no NO_3^- - N was found does not mean that none had been formed, the pH-redox diagram indicates which chemical species will predominate in any set conditions, but this does not preclude the existence of others in such prescribed conditions. The greater amounts of NH_4^+ - N in poorer mires is consistent with Marthalers findings that bog plants are adapted to

Fig 25 NO_2^--N

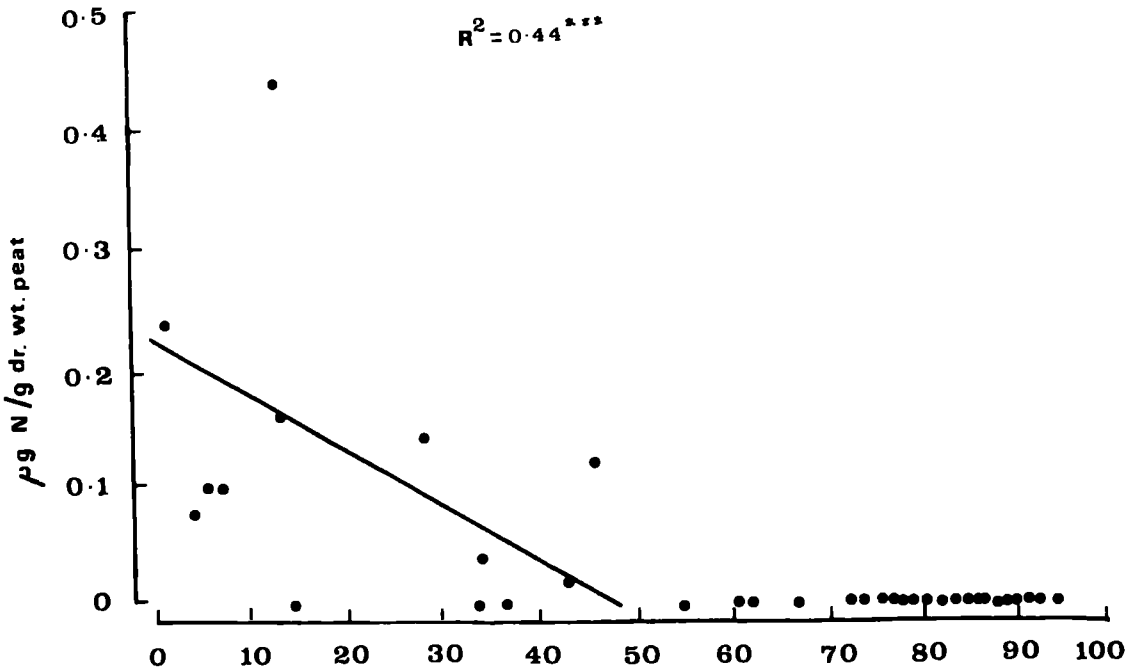


Fig. 26 Total N in peat

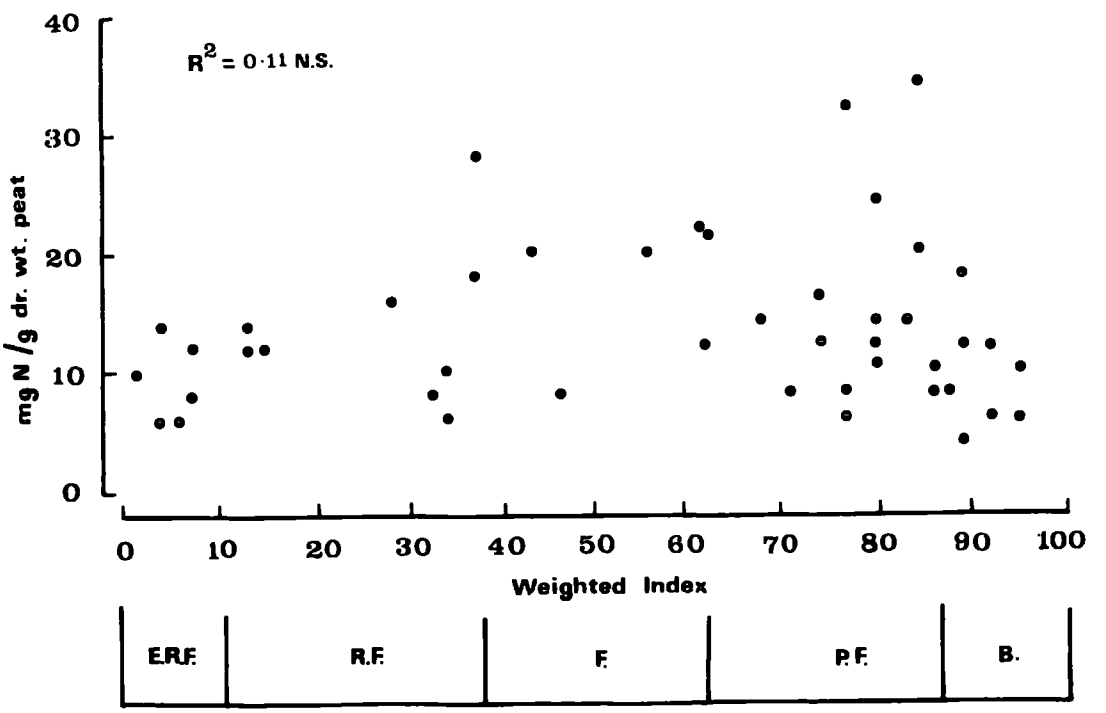


FIG.25 POOLED DATA FOR SOLUBLE NITRITE NITROGEN IN PEAT IN RELATION TO MIRE ECOCLINE. R^2 VALUES FOR TREND AS SHOWN.

FIG.26 POOLED DATA FOR TOTAL NITROGEN IN PEAT IN RELATION TO MIRE ECOCLINE. R^2 VALUES OF TREND AS SHOWN.

NH_4^+ - N uptake. He also observed that NO_3^- - N gave rise to less growth in these plants, and was even toxic in some concentrations; Marthaler noted that bog plants can tolerate high levels of nitrogen, provided that it is supplied as NH_4^+ - N (Marthaler, 1939). By contrast some plants growing in spring fens are known to accumulate nitrate in their above-ground tissue (Hesselman, 1917; Persson, 1962).

The above facts may be summarised as follows:

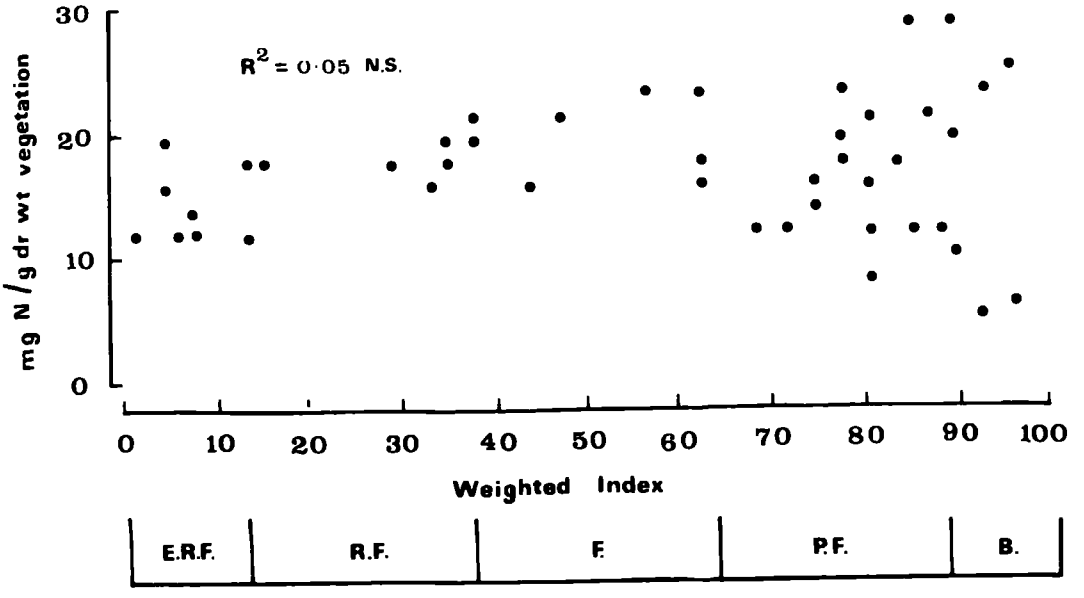
(1) It has been shown that appreciable amounts of NH_4^+ - N can be detected in mires during the peak growing season, and that amounts of this ion increase along the ecocline in the direction of poorer mires, small amounts of NO_2^- - N were detected at the rheotrophic end, but no NO_3^- - N; all these results are quite consistent with basic chemical theory, as well as known specifications for microbial nitrogen transformation.

(2) Previous work has shown that some spring fen plants accumulate NO_3^- - N; whilst bog plants require NH_4^- - N, and find NO_3^- - N toxic. Thus it is quite possible that tolerance of, and requirement for different nitrogen ion species may be an important factor in determining floristic patterns in this ecocline. Our understanding of peatland systems might well profit from any future investigations directed towards this topic.

9(d) Nitrogen in mire vegetation

The concentration of nitrogen in the vegetation varied between about 5 and 30 mg N/g dr. wt., and, as in the case of nitrogen in peat, variability was greatest at the ombrotrophic end of the gradient (Figure 27). The results from Wurzacher Ried and Taufach Moss show a slight decline in concentration in the more ombrotrophic mires, this decline is significant on the former site. No apparent trend is seen in either of the two sets of results from Pfrule Moss (Figure 22). The pooled data indicate that the concentration of nitrogen in fen vegetation is always

Fig. 27 Total N in vegetation



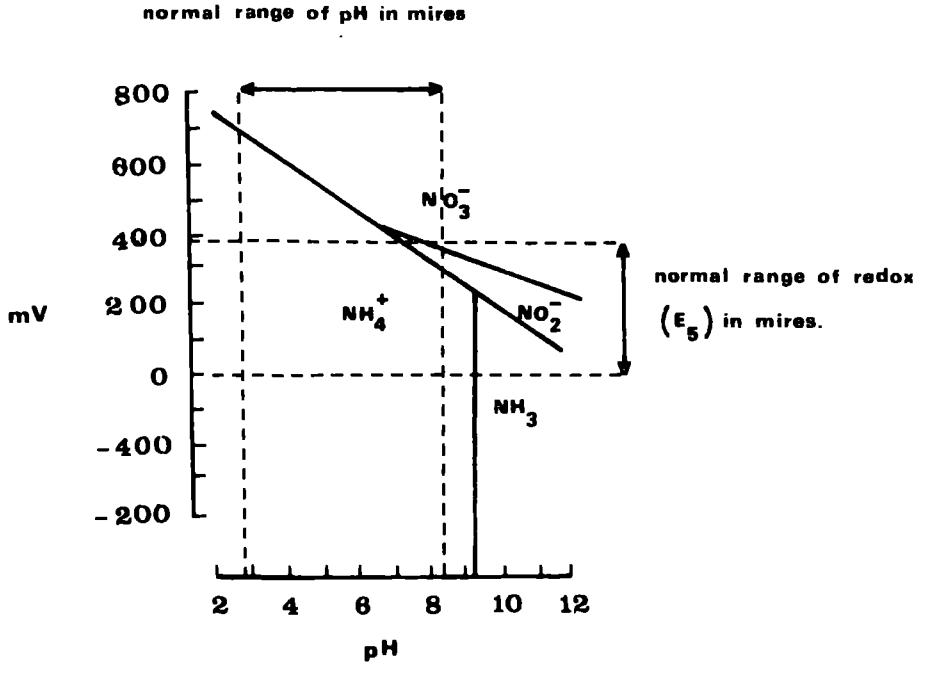


FIG. 27 POOLED DATA FOR TOTAL NITROGEN IN MIRE VEGETATION IN RELATION TO MIRE ECOCLINE. R^2 VALUE OF TREND AS SHOWN.

FIG. 28 pH/REDOX POTENTIAL DIAGRAM FOR SOME AQUEOUS NITROGEN SPECIES (FROM CAMPBELL, 1970). INFORMATION ON PEAT REDOX POTENTIAL FROM PEARSALL (1938), PERSSON (1961), and MALMER (1962a).

greater than about 12 mg/g dr. wt., i.e. very low figures are encountered only in extreme bogs. A very low nitrogen content in the tissues of sphagnum mosses has been observed by several workers. (e.g. Mattson and Karlsson, 1944; Gorham, 1953b; Malmer and Sjörs, 1955), however the relationship between nitrogen in plants, and the mire gradient has rarely been investigated. Sjörs arranged his results for the nitrogen content of Scirpus caespitosus, Carex lasiocarpa, and Menyathes trifoliata in order of phytosociology from rich to poor mires, but found no relationship between nitrogen and the gradient, in fact the nitrogen content of M. trifoliata showed a distinct decline towards rich fens (Malmer and Sjörs, 1955). Gorham (1953b) examined the nitrogen content of several species in relation to the hydrosere, and recorded a marked decline at terrestrial end; however, Gorham's results are difficult to interpret in terms of the mire gradient for several reasons: (1) only 39 of his 85 samples were growing on soil with more than 70% organic material, i.e. most of his samples were not growing in what are conventionally regarded as true peats; (2) he was primarily concerned with the hydrosere, and arranged his data on a gradient of increasing organic content; and (3) he studied many different species from many different locations.

The results displayed in Figure 27 and the data from previous investigators provide no evidence for any consistent relationship between the mire gradient and the nitrogen content of plants, with the possible exception of the fact that low values are frequently associated with sphagna.

9(e) Correlations between nitrogen in peat and the nitrogen content of vegetation

In ecological investigations of the chemical relationship between vegetation and soil, nitrogen is occasionally one of the parameters studied, therefore it is of some interest to know which of the soil (or in this case peat) nitrogen characteristics bears the closest relationship to the

nitrogen concentration in vegetation. Furthermore, it is useful to know whether a better correlation is obtained when nitrogen is calculated on the basis of peat volume, concentration, or humus. In Table 7 the simple correlation coefficients are displayed for different parts of the mire.

Table 7

Simple correlation coefficients between nitrogen in vegetation and various forms of nitrogen in peat expressed in terms of various units. (Coefficients not statistically significant unless otherwise indicated).

	NO_2^- - N	NH_4^+ - N Neutral Extract	NH_4^+ - N Acid Extract	Total -N
		ALL MIRES		
unit weight	-0.04	0.14	0.31*	0.33*
unit humus	-0.10	0.07	0.32*	0.07
unit volume	-0.12	0.07	0.32*	0.05
		RICH MIRES ONLY		
unit weight	-0.22	0.32	0.41	0.38
unit humus	-0.38	0.14	0.12	-0.23
unit volume	-0.5	0.18	0.02	-0.35
		POOR MIRES ONLY		
unit weight	-	0.17	0.41*	0.32
unit humus	-	0.17	0.42*	0.34
unit volume	-	0.16	0.47*	0.23

The first fact which can be drawn from this Table is that calculations based upon unit weight generally account for more variability in the nitrogen concentration of vegetation than when the calculations are based upon either volume or per unit humus. The only significant exception to this is in the case of poor mires where acid extractable NH_4^+ - N gives an appreciably higher correlation in terms of unit volume ($r = 0.47$). Two additional measurements are needed in order to express results in terms of unit volume: the water content, and the density. Two additional

weighings are also required if estimates are to be expressed in terms of unit humus. Likewise, if chemical content of vegetation is to be expressed in terms of standing crop, then estimates of the biomass per unit area must be made. Thus expressing results in terms of volume, humus, or standing crop, greatly increases the possibility of compounding errors. A further disadvantage of standing crop as a basis for comparison is that it does not reflect net productivity or turnover, in fact on peatlands there is evidence that a reciprocal relationship exists between productivity and standing crop (Reader & Stewart, 1972, p. 1030).

When the pooled mires are under consideration the total nitrogen in peat gives a marginally higher correlation coefficient than acidified NaCl extracted nitrogen, but when either the more rheotrophic or the more ombrotrophic situations alone are under investigation, then acidified NaCl extracted nitrogen is better, accounting as it does, for about 20% of the variation in vegetation nitrogen.

The soluble NH_4^+ - N in peat will vary according to season, whereas variation in total nitrogen is minimal. An estimation of the fluctuation due to the standing crop can be made assuming (1) an average standing crop of 400 g/m^2 (Table A41), (2) 20 mg/g N in the vegetation (Figure 27), (3) 100 p.p.m. NH_4^+ - N in the peat (Figure 24), (4) a peat density of 100 g dr. wt./L (Table AP 34) and (5) a rooting zone of 20 cm. Using these figures the nitrogen in the standing crop is about 0.6 g/m^2 compared with 2 g/m^2 of soluble NH_4^+ - N. Input from rain is difficult to assess, but data from the United Kingdom indicate the amount to be less than 1 g/m^2/year (Holden, 1966). On poor mires plant growth is slow, therefore as the supply of soluble nitrogen is high relative to the demands of the vegetation, this aspect of seasonal variation is not likely to interfere too much with the correlation. These general conclusions are based upon very simplified assumptions, and make no allowance for turnover, however they are consistent

with the results of Malmer (1962b) who found very little seasonal variation in the soluble nitrogen content of poor mires (o.c. Figure 14b). In rheotrophic mires, by contrast, the flux due to ground water may be considerable, thus increasing fluctuations in the amount of readily soluble nitrogen, and making any predictions less reliable. A quadratic term was added and the following relationships are the best obtained.

$Nv = 2.1Np - 0.049(Np)^2 - 1.3$ ..... for poor mires
$R^2 = 0.38^{**}$ s.e. = 5.6

$Nv = 0.37Np - 0.0037(Np)^2 + 11.5$ ..... for rich mires
$R^2 = 0.41$ N.S. s.e. = 2.9

where Nv = nitrogen in the vegetation (mg/g dr. wt.) and
Np = total nitrogen in peat (mg/g humus)

It can be seen that the R^2 values are too low, and the standard errors too high for these equations to be of any real predictive value.

In general it appears that none of the forms of nitrogen studied appear to provide a useful index of nitrogen in vegetation; of these total nitrogen is probably the most satisfactory where a wide range of mire types is under consideration, also in rheotrophic situations; and acid NaCl soluble NH_4^+ - N when only very poor mires are being studied. Furthermore, the results indicate little advantage in expressing the results in terms of unit volume or unit humus, unless, of course, the nature of the investigation is such that the use of these latter units is essential.

9(f) Relationships between nitrogen and other inorganic factors

The study of total peat nitrogen in relation to other chemicals in peat has not proved very rewarding in the past. For example Kaila (1956b) found only a weak correlation between nitrogen and phosphorus ($r = 0.20^{*}$),

with somewhat higher coefficients when the test was carried out on either fen or bog peat separately (r = 0.40* and 0.64*). Kaila (o.c.) commented that these values were too low to be of any interest. The simple correlation coefficient obtained in this investigation between total nitrogen and phosphorus in peat is 0.63***. In his studies of drained peatland in Sweden Holmen (1964) found only a weak correlation between total nitrogen and phosphorus in peat, and none between total nitrogen and either total calcium or total potassium; likewise my results give insignificant correlations for these last two relationships (r = -0.22 and 0.02). Koulter-Anderson (1960) examined the nitrogen in peat from profiles of 12 raised bogs, and found a statistically significant negative relationship between total nitrogen and the base content of ash. He did not find this relationship in the peat from fen profiles, and suggested that the characteristic might be useful in distinguishing bogs and fens. Sjörs (1961) however, found no such relationship in his results, he therefore re-examined Koulter-Anderson's data on an inter-stand basis, and finding only a weak negative correlation concluded that any such relationship must be a characteristic of individual bogs. Sjörs (o.c.) also found no relationship between nitrogen and phosphorus in peat. The correlation coefficients between total peat nitrogen, and bases or ash given by my results for different bogs presented here are both weakly positive (0.57 and 0.40), which rather support Sjörs conclusions.

The highest simple correlation between total peat nitrogen and other total peat factors was with phosphorus in peat from poor mires (r = 0.82***), all other coefficients were considerably lower than this, and when data from bog and fen peats were pooled, again only the relationship with phosphorus was statistically significant.

The relationship between nitrogen in vegetation and other chemicals in vegetation was examined, but the correlation with phosphorus over all

mires was the only one found to be significant ($r = 0.62^{**}$). There was no correlation between nitrogen and total bases in vegetation (c.f. Mattson & Karlsson, 1944). However the relationships between nitrogen in vegetation and soluble forms of several elements in the peat are statistically significant, therefore a more detailed stepwise regression analysis was performed, the results of which are displayed in Table 8. As in Chapter 8 this analysis was performed on three groups of data, rich mires, poor mires and all mires pooled, the weighted index of 50 was used to separate rich and poor mires.

Table 8

Relative contribution by soluble inorganic factors in peat to the explained variance of total nitrogen in vegetation

Factor added to the equation	% variance explained	beta	F
	ALL MIRES		
pH	16	0.41	4.6*
P	14	0.58	18***
N	12	0.89	26***
Nitrogenase activity	7	0.42	7.4**
Ca	6	0.41	4.3*
	5	0.30	5.3*
$R^2 = 0.60^{***}$; n = 35			
	POOR MIRES ONLY		
P	19	0.39	6.3*
pH	18	0.35	3.0 N.S.
N	18	0.74	16***
Al	5	0.29	2.2 N.S.
$R^2 = 0.60^{***}$; n = 22			

Acidity is the single factor which accounts for most of the variability in vegetation nitrogen, however the F value for its partial regression coefficient and its beta value are both relatively low suggesting that

pH has little direct effect; this conclusion is even more obvious when the results for poor mires alone are considered. The two peat chemicals which appear to have the greatest influence on the amount of nitrogen in vegetation are soluble phosphorus and soluble nitrogen, in both instances the F value attached to their partial regression coefficients is relatively high, both for all mires and for poor mires only: this indicates, as might be expected, considerable direct effect.

The only significant regression coefficient obtained for rich mires was a negative one for calcium ($r = -0.58^{*}$), therefore no further statistical analysis of the data from this group was performed. A possible explanation of this negative correlation may be found in the above-mentioned fact: that both soluble nitrogen and soluble phosphorus appear to have a strong positive influence on the nitrogen content of vegetation. It has already been observed that the concentration of soluble nitrogen in peat declines towards the direction of rich fens, also a high calcium value might cause a reduction of soluble phosphorus by the formation of insoluble phosphates.

Nitrogen fixation appears to have a small but significant influence upon the nitrogen content of the mire vegetation, and it is interesting to note that this factor does not appear in the analysis of data from poor mires only. The simple correlation coefficients between nitrogen fixation and vegetation nitrogen in poor and rich mires are, in neither case, statistically significant, but the coefficient for the relationship in rich mires is considerably higher than in poor mires (0.39 cf. 0.11). It is therefore possible that biological nitrogen fixation is of greater importance with regard to the supply of nitrogen in vegetation on rich mires, than it is on poor mires.

Soluble aluminium appears in both sections of the analysis, where in each case it accounts for 5% of the variability, and the F value for

its all mire partial regression coefficient suggests that this element may have some direct effect. There have been suggestions previously that low levels of aluminium might stimulate uptake of nutrients into plants, therefore this topic is discussed in Chapter 12 after the results for other chemicals have been presented.

CHAPTER TEN:

PHOSPHORUS IN PEATLANDS

10(a) Methods

Details of sampling and preparation of the peat have been presented in previous Chapters. Total phosphorus in peat and vegetation was extracted using a perchloric and nitric acid wet digestion procedure (Johnson & Ulrich, 1959). After dilution, phosphorus in the digest was estimated as molybdivanadophosphoric acid (Kitson and Mellon, 1944).

A great variety of chemical extractants for 'available' or easily soluble phosphorus have been utilised, and only a limited selection of these were used in this study. All of the phosphorus extracted by Truog's (1930) extractant and N NH_4Cl is considered to be available to plants; weakly acid solutions are generally favoured for acid soils, and stronger acids for extracting phosphorus which exists as insoluble phosphates. I planned to examine the relationship between the amounts of phosphorus extracted by the different chemicals and the phosphorus content of the vegetation, and, as far as possible, to compare my results with those from other investigations on peat. Taking all these factors into consideration the extractants finally selected were Truog's solution, N NH_4Cl, 0.02N H_2SO_4, 0.5N H_2SO_4. N acetic acid, and N ammonium acetate.

The extraction procedure was the same as described for nitrogen; after shaking for one hour the extracts were filtered and phosphorus estimated by the molybdenum blue method (Fogg & Wilkinson, 1958).

10(b) Peat and vegetation phosphorus in relation to the mire ecocline

The results are presented in a similar fashion to those for nitrogen: data for the individual mires in Figures 29-31, and pooled data

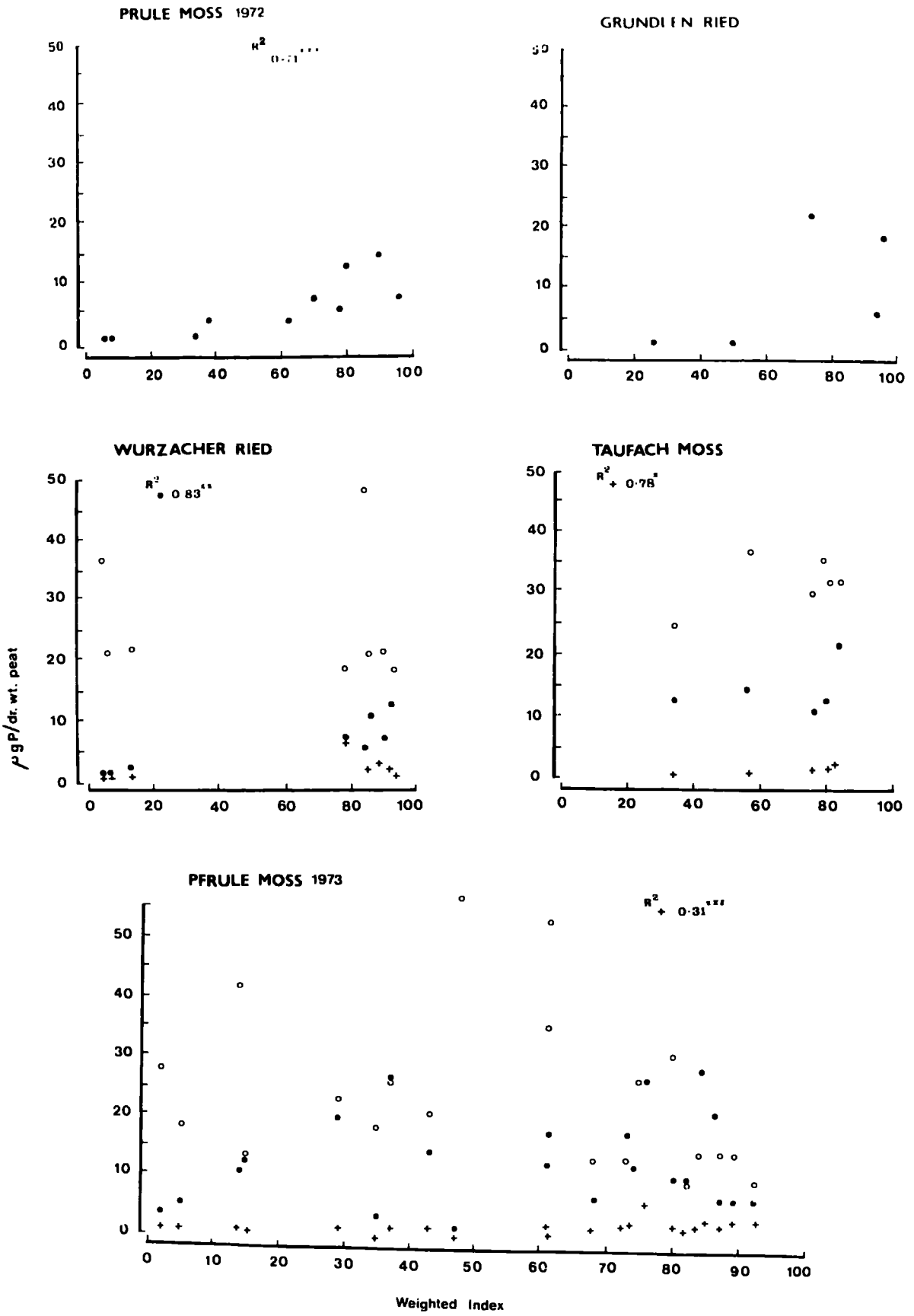


FIG. 29. SOLUBLE PHOSPHORUS IN PEAT IN RELATION TO PIRI ECOCLINE. (O) EXTRACTED WITH 0·5 N SULPHURIC ACID, (•) 0·02 N SULPHURIC ACID, (+) 0·002 N SULPHURIC ACID. R^2 VALUES OF SIGNIFICANT TRENDS SHOWN

in Figures 32-39. Trend lines in relation to the ecocline have not been drawn for separate mires, but where trends are statistically significant the R^2 values are shown; for the pooled data both significant trend lines and all R^2 values are indicated.

The general levels of total phosphorus in both peat and vegetation are approximately the same for corresponding parts of the ecocline at all locations (Figure 31). Total phosphorus concentration in the vegetation increases very slightly from extreme rich fens to fens, this is followed by a decrease in concentration at the ombrotrophic end of the ecocline (Figure 38). Malmer studied a few species from poorer mires with respect to their phosphorus content and their phytosociological position, and found a similar decrease towards the extreme ombrotrophic end (Malmer, 1958; Malmer & Sjörs, 1955). Sjörs, however, in the same publication (o.c.) stated that the phosphorus content of plants did not correlate along the phytosociological series; this difference between the two workers could be explained by the fact that Sjörs concentrated on richer mires, whereas Malmer studied the poorer mires.

The levels of total phosphorus in peat in relation to the ecocline follow a pattern which is almost identical to that for phosphorus in vegetation, except that the decline at the ombrotrophic end is even more marked (Figure 39). The low total phosphorus content of ombrotrophic peat appears to be characteristic, and has been recorded by several previous workers (e.g. Kaila, 1956a; Malmer & Sjörs, 1955; Sjörs, 1961; Pollett, 1972).

The 'easily soluble' phosphorus in peat, extracted with different extractants, appears, like total phsophorus, to be at about the same level in corresponding parts of the ecocline at all the different locations. Furthermore, the relative amounts extracted by the various extractants are similar for each location (Figures 29 and 30). These differences between

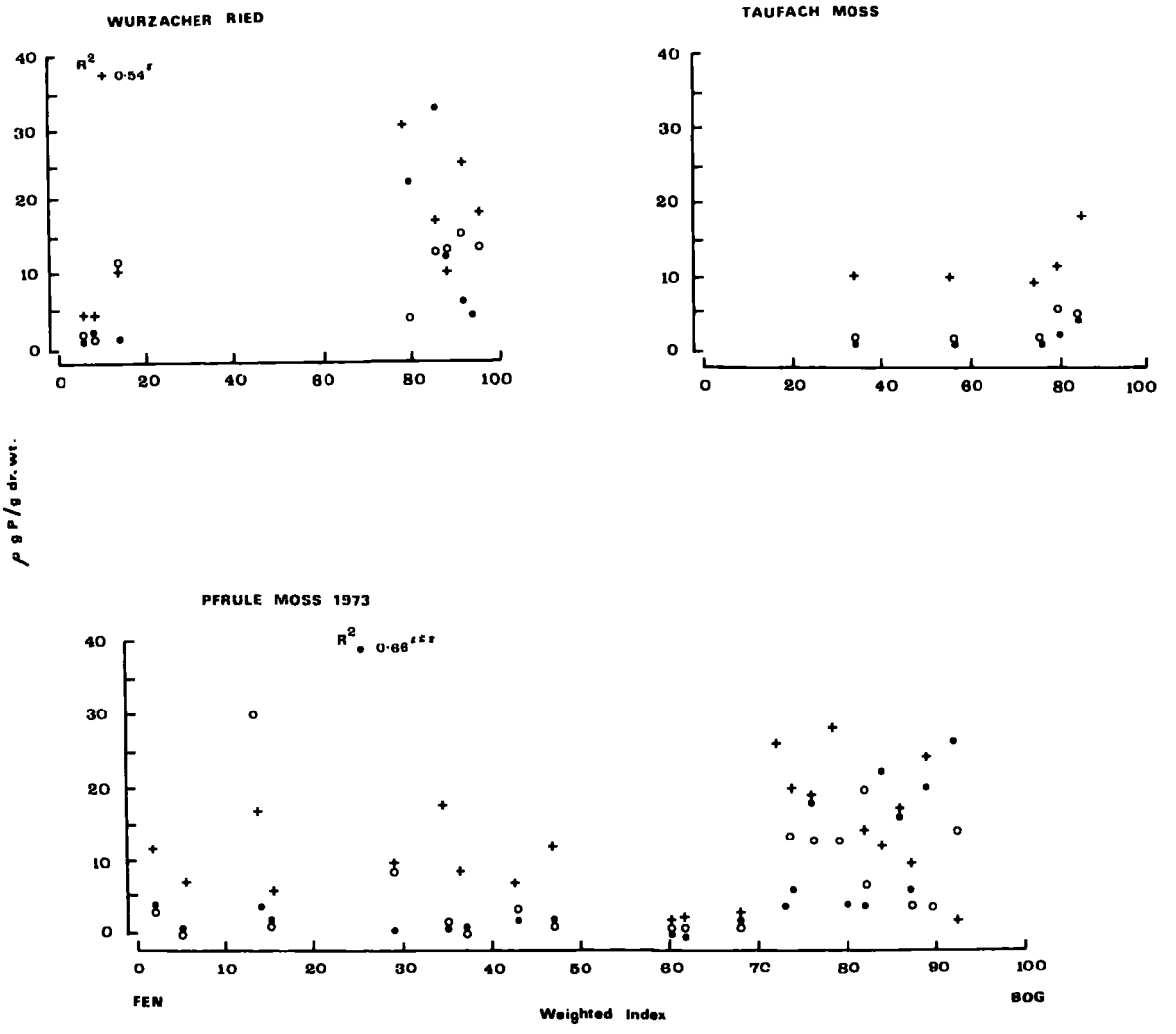


FIG.30 SOLUBLE PHOSPHORUS IN PEAT IN RELATION TO MIRE ECOCLINE. (o) EXTRACTED WITH AMMONIUM ACETATE; (+) ACETIC ACID: (•) AMMONIUM CHLORIDE. R^2 VALUES OF SIGNIFICANT TRENDS SHOWN.

extractants are best seen in the pooled data (Figures 32-37). One marked feature of these results is that the soluble phosphorus content of peat, when determined by extraction with any of the 'weak' extractants, shows an increase towards the ombrotrophic end of the ecocline (Figures 33, 34, 35, 36 and 37); this trend is statistically significant in all instances, with the exception of phosphorus extracted with ammonium acetate. There is no trend when the phosphorus is extracted with 0.5N H_2SO_4. These results for soluble phosphorus are of considerable interest with respect to the phosphorus economy of peatlands, and as such they are discussed more fully in the following section, and in Chapter 12.

10(c) Phosphate fixation in peat

At the pH values and redox encountered in peat most of the phosphorus will be in the form of ions or compounds of orthophosphoric acid, furthermore $H_2PO_4^-$ predominate over HPO_4^- (Campbell, 1970). The $H_2PO_4^-$ ion may combine with various substances in soil, and become unavailable to plants in varying degrees. In alkaline conditions it may become unavailable because of the precipitation of insoluble calcium phosphates, and in acid conditions through being bound as phosphates or hydroxyphosphates of iron and aluminium (e.g. Magistad, 1925; Gaarder, 1930; Cole and Jackson, 1950; Hsu, 1965).

Although it would be of considerable interest to have a better understanding of the nature of phosphorus compounds in peat, such an undertaking, involving detailed fractionation (e.g. Petersen & Corey, 1966), was impractical in this, essentially ecological, project. However, several extractants were utilised, and the relative amounts of phosphorus dissolved by each of these can provide some information on the matter. All of the phosphorus removed by Truog's solution is considered to be available to plants (Truog, 1930), as is the loosely bound 'saloid' phosphorus, which

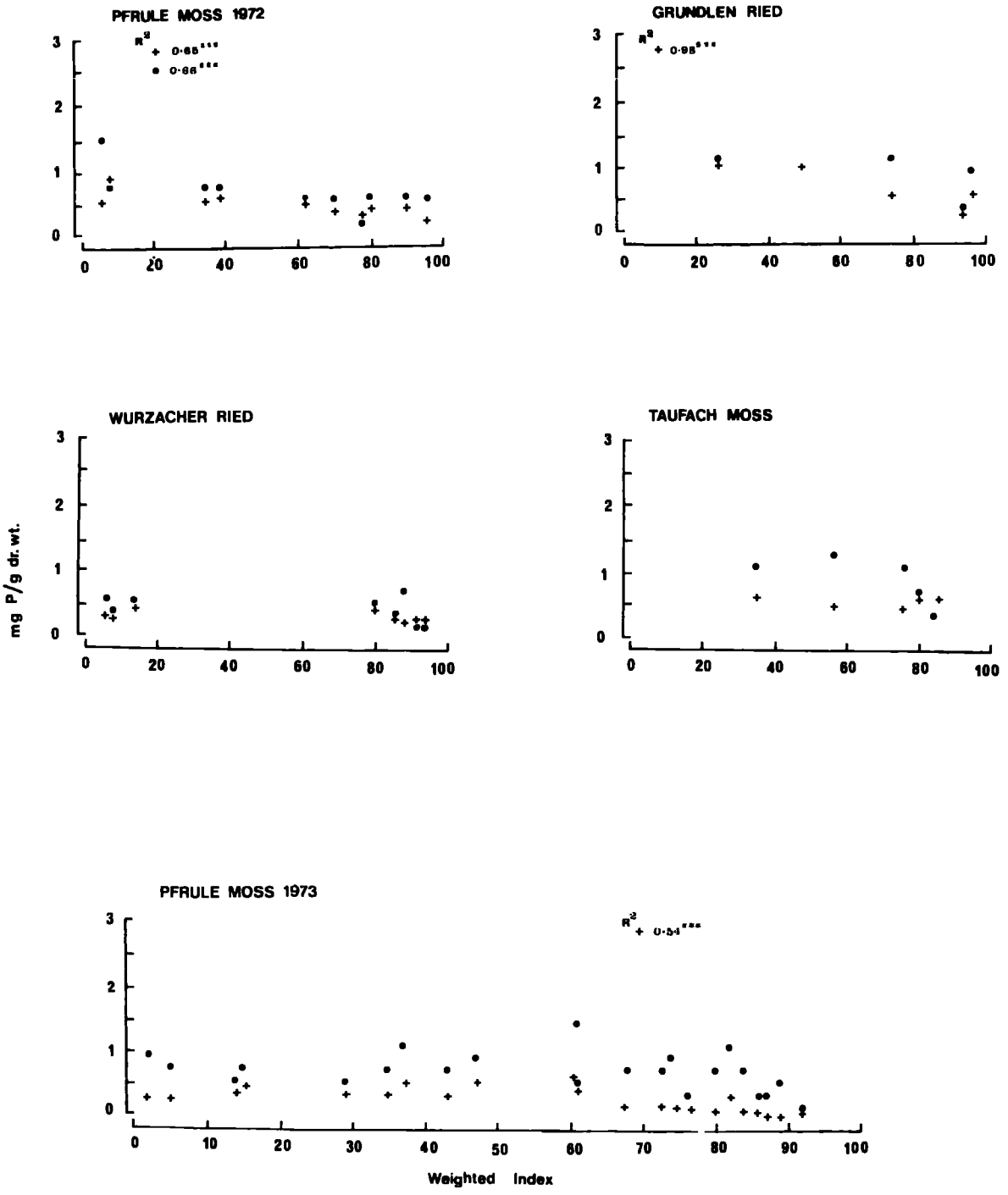


FIG. 31 TOTAL PHOSPHORUS CONTENT OF PEAT (+), AND BIRF VEGETATION (•) IN RELATION TO BIRI ECOCLINE. R^2 VALUES OF SIGNIFICANT TRENDS SHOWN.

is removed by neutral salts such as ammonium chloride or calcium sulphate (Mattson and Karlson, 1938). N acetic acid and 0.02N H_2SO_4 will include most of the aluminium and iron bound phosphorus together with a small amount bound to calcium. 0.5N H_2SO_4 will dissolve most of the insoluble calcium phosphates including apatites. The afore-going information was obtained from several sources in addition to those already mentioned, but particularly Magistad (1925), Hesse (1971), and John (1972).

A summary of results for the easily soluble phosphorus is as follows (details in Figures 29-37 and in the appendix):-

extractant	extreme rich fen	extreme bog
NH_4Cl	1	20
acetic acid	10	19
0.02N H_2SO_4	10	16
0.5 N H_2SO_4	25	21

All figures p.p.m. dr. wt. peat.

In rich fens saloid bound phosphorus is only a very small fraction of the easily soluble phsophorus, most presumably being fixed into inorganic compounds. (Figure 35). These results for rich fens are not unexpected, and are consistent with the findings of Puustjärvi (1956) who concluded that the retention of phosphorus by hydrous ferric oxide was an important factor regarding the growth of crops on reclaimed treeless fens, and with those of Doughty (1930) who showed that rheotrophic peat did not fix added phosphorus after the iron and aluminium had been removed by leaching.

The data for ombrotrophic sites is of greater interest, indicating as it does that most of the easily soluble phosphorus in the poor mires is saloid bound, this warrants closer examination of the electrostatic theories relating to phosphorus fixation in soils. In mineral soils the

Fig. 32 P in peat (0·5 N H_2SO_4 soluble)

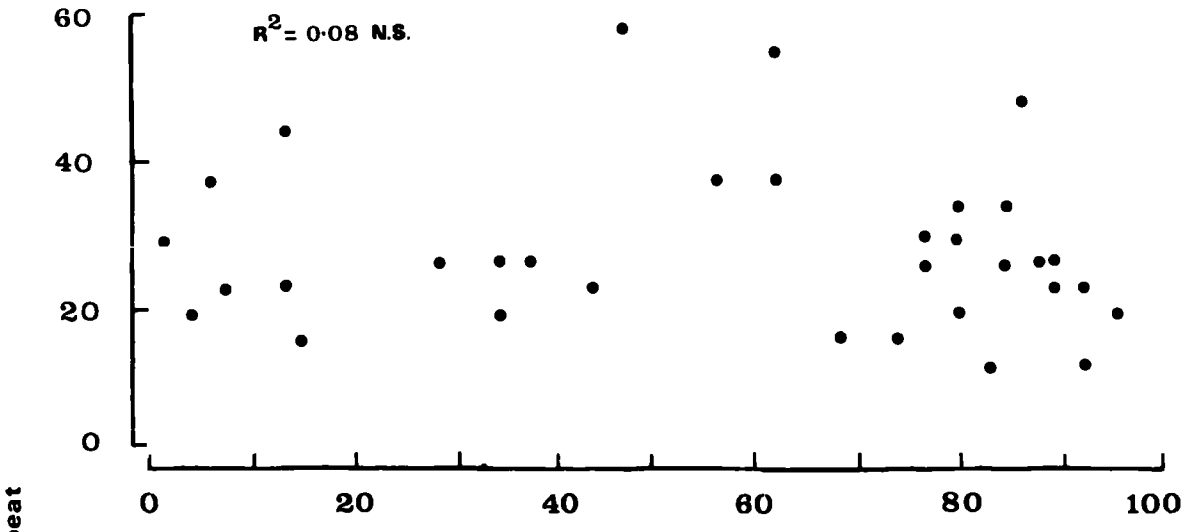
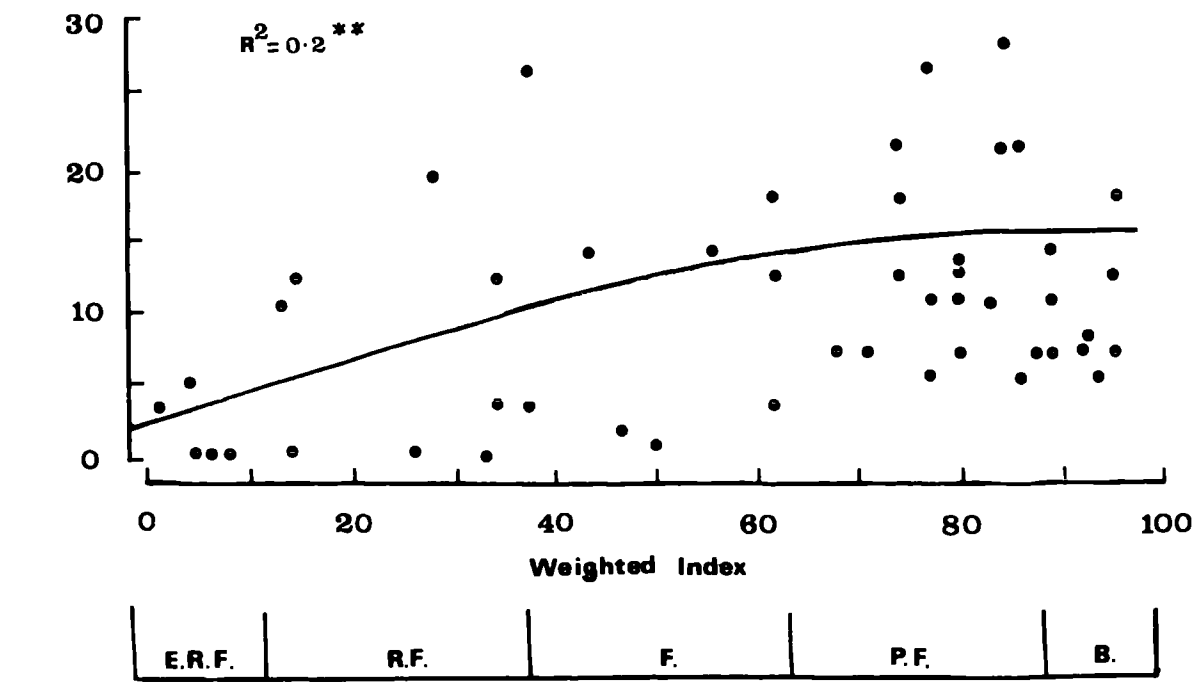


Fig. 33 P in peat (0·02 N H_2SO_4 soluble)



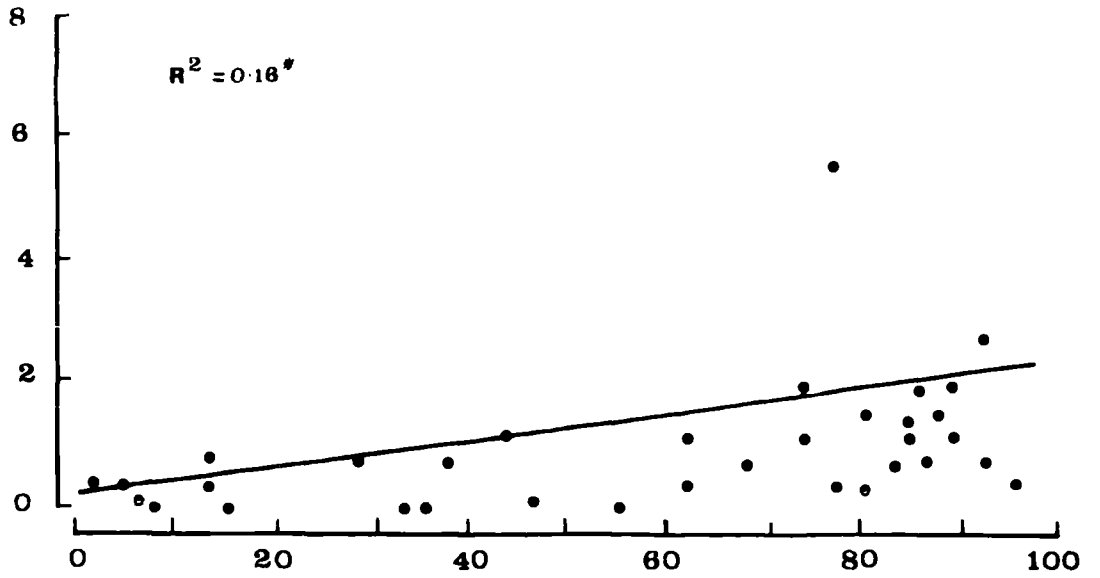
µg P/g dr. wt. peat

FIGS. 32 AND 33 POOLED DATA FOR SOLUBLE PHOSPHORUS IN PEAT IN RELATION TO MIRE ECOCLINE. R^2 VALUES OF TRENDS AS SHOWN.

binding of phosphate in acid conditions is usually attributed to aluminium and iron. The work of Gaarder (1930) showed that fixation due to these two elements increased with increasing acidity to a certain pH, below this pH the amount of soluble phosphate in equilibrium with the amount fixed increases rapidly. This point of maximum fixation is slightly on the acid side of the iso-electric point (Mattson & Karlson, 1938). The iso-electric point varies according to the ratio of phosphate to metallic oxide, those for aluminium being slightly higher than for iron (Fe^{+++}). The more phosphate that is present the more the iso-electric point is depressed, and greater are the amounts of free phosphate in equilibrium with the metal-phosphate complexes. It is difficult to assess just how much of the estimated iron, aluminium, and phosphorus in peat is free to take part in these reactions, and an additional complication is that humic acid and other organic compounds reduce the amount of PO_4 fixed (Matson & Karlson o.c.; Bradley & Sieling, 1953). However, if we simplify the situation and assume first that total amounts are involved, then the iso-electric point for iron in the richer peats is about pH 6.4, and pH 6.0 in the ombrotrophic peats; if, on the other hand the calculations are based upon only acid soluble amounts then figures are pH 5.9 and pH 5.4, (from Mattson & Karlson o.c. Figure 15A). The equivalent figures for aluminium in peats, which is believed to bind phosphorus less strongly than the ferric ion, are all about 0.5 pH units higher than those given above.

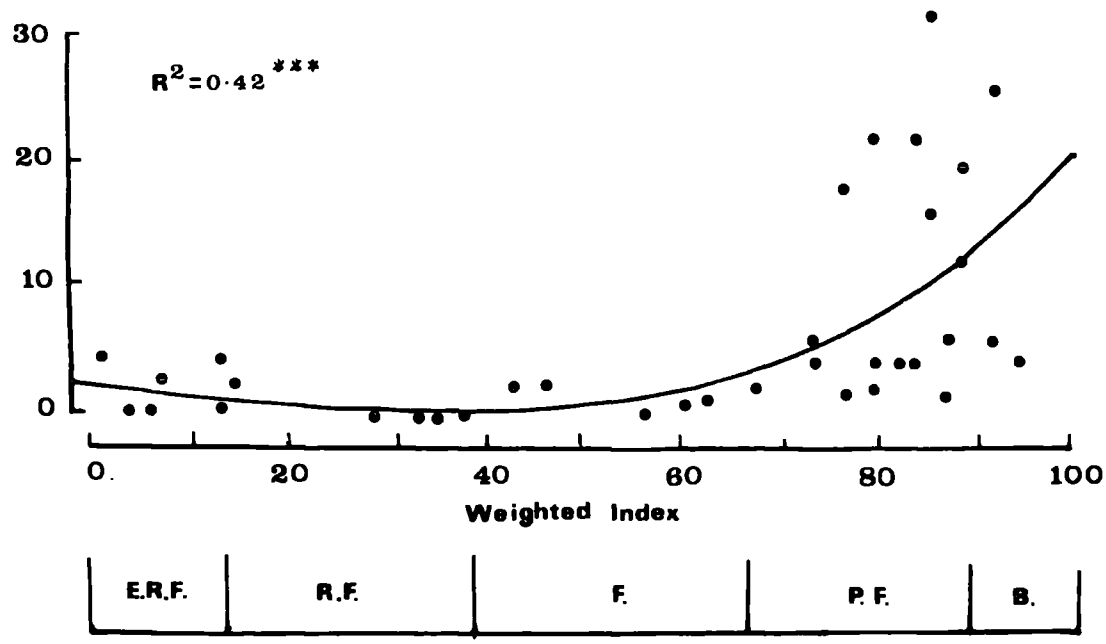
Mattson & Karlson point out that the practical experiments of Gaarder (o.c.) show the pH of maximum phosphate fixation to be on the acid side of the theoretical pH of maximum fixation: the iso-electric point. They account for this by suggesting that saloid bound phosphorus increases with increasing acidity until the point at which the cation complex breaks down. (Saloid bound is used to describe an 'atmosphere' of phosphate surrounding the amphoteric colloid complex by electrostatic attraction).

Fig. 34 P in peat (0·002N H_2SO_4 soluble)



μg P/g dr wt peat

Fig. 35 P in peat (NH_4Cl soluble)



FIGS. 34 AND 35 POOLED DATA FOR SOLUBLE PHOSPHORUS IN PEAT IN RELATION TO MIRE ECOCLINE (CONT'D). R^2 VALUES OF TRENDS AS SHOWN.

The amounts of phosphorus dissolved by the various extractants in relation to the ecocline could thus be explained by application of the above concepts. In the ombrotrophic mires the pH is below the iso-electric point of any metallo-phosphorus complexes, and much of the phosphorus is in the saloid form which is easily extractable with a neutral salt, in this case NH_4Cl. With increasing rheotrophy the pH increases to the iso-electric point (as estimated above), to the point of maximum fixation by iron and aluminium. It is theoretically possible for the amount of phosphorus in equilibrium with the complexes to rise at pH values above the iso-electric point, but at these values precipitation by calcium is likely to occur, this can start at pH values as low as pH 5.4 (Gaarder, 1930). This would explain why no saloid (or 0.002N H_2SO_4) soluble phosphorus is extractable from the more rheotrophic peat, but in these peats phosphorus can be dissolved by strong acids.

The difference in the shape of the curves for NH_4Cl extractable (Figure 35) and 0.02 NH_2SO_4 (Figure 33) is particularly interesting. One simple interpretation of these is that in the more rheotrophic direction the saloid phosphorus becomes rapidly less important, such that even in fens (i.e. before rich and extreme rich fens) forms of bonding other than saloid predominate. Assuming that 0.02N H_2SO_4 can, at least in part, extract phosphorus from these other forms then it would be expected that the curve for extractable phosphorus utilising this solvent would decline less rapidly than the curve obtained using NH_4Cl. Neither solvent can extract the strongly bound calcium phosphate of the richer mire.

These results disagree with those of Saebo (1968) who concluded, on the basis of the effect of $(NH_4)_2SO_4$ on the phosphorus content of suction water, that the phosphorus in an extremely ombrotrophic peat was not in the exchangeable form, Saebo (o.c.) found that more phosphate was extracted from the peat by acetic acid, rather than by ammonium acetate, a fact

Fig.36 P in peat (ammonium acetate soluble)

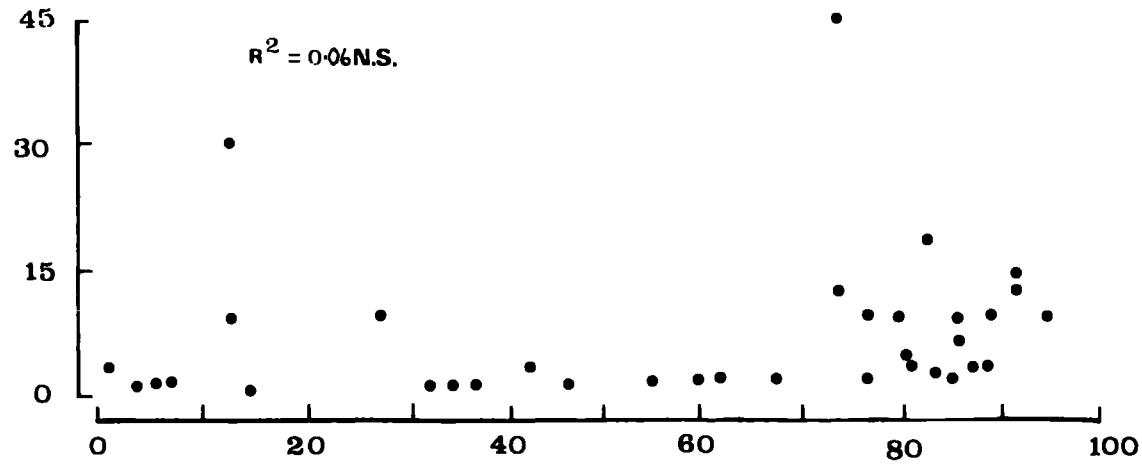
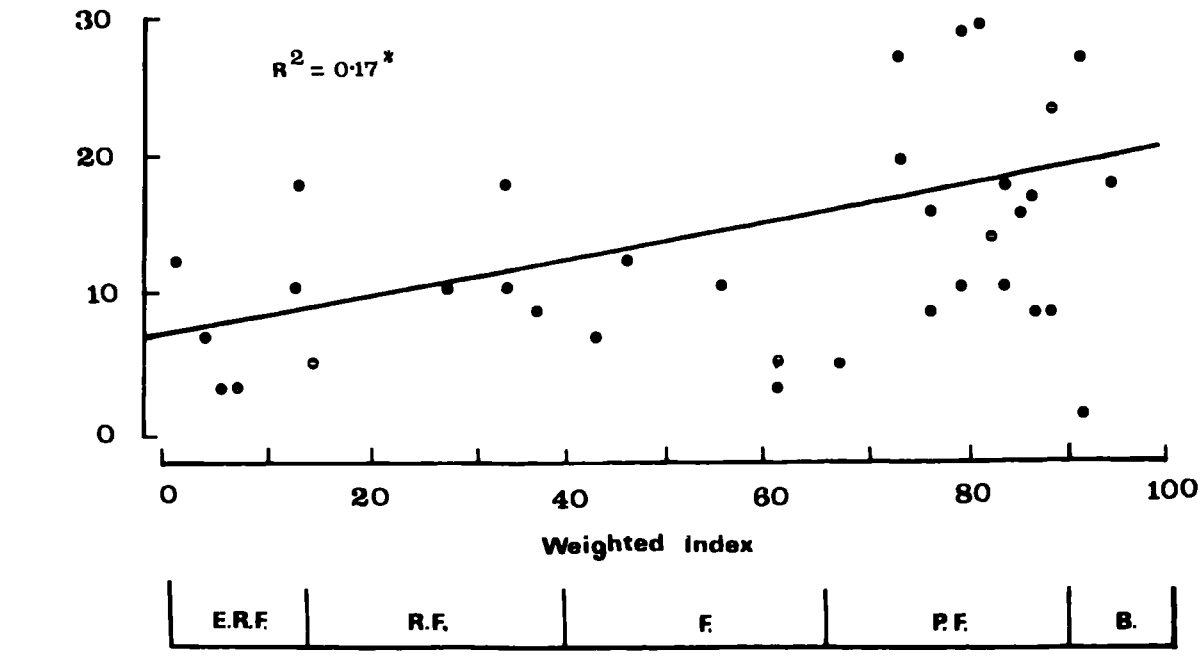


Fig. 37 P in peat (acetic acid soluble)



FIGS. 36 AND 37 POOLED DATA FOR SOLUBLE PHOSPHORUS IN PEAT IN RELATION TO MIRE ECOCLINE (CONT'D). R^2 VALUES OF TRENDS AS SHOWN.

which is in general agreement with my results. Saebo concluded that phosphate ions are attached to the peat surfaces through hydrogen bridge bonds.

Saloid bound phosphorus is easily removed by chloride, and the difference between saloid bound phosphorus recorded in the two projects could arise from higher cloride contents of the Norwegian peat studied by Saebo. This ion was not estimated in either Saebo or myself, however Bellamy (1968) has shown that there is a dramatic decline in the chloride content of ombrotrophic peats in an easterly direction from the Atlantic seaboard to the central continent of Europe (o.c. Figure 4). Therefore it is possible that the Norwegian peats contain higher levels of chloride than the South German ones, which could account for the differences in saloid bound phosphorus. In fact it is possible that saloid phosphorus in Ombrotrophic peats may be inversely related to chloride along the east-west European axis.

10(d) Correlations between phosphorus in the vegetation, and peat phosphorus

In Table 9 it can be seen that of the easily extractable phosphorus only that extracted with NH_4Cl ($r = 0.47$ all mires; and 0.51 poor mires only) gives statistically significant correlations with vegetation phosphorus. In the next Chapter it will be shown that phosphorus in the peatland system is concentrated in the vegetation to a much greater extent than nitrogen, therefore in the height of the growing season it is easy to appreciate why the easily soluble phosphorus does not correlate well with vegetation phosphorus.

Total phosphorus in peat is the only form which provides consistently useful correlations with phosphorus in vegetation, which, considering the above comment regarding concentration, is only to be expected. Thus, in any phytosociological study involving phosphorus chemistry, the only index

Fig. 38 Total P in Vegetation

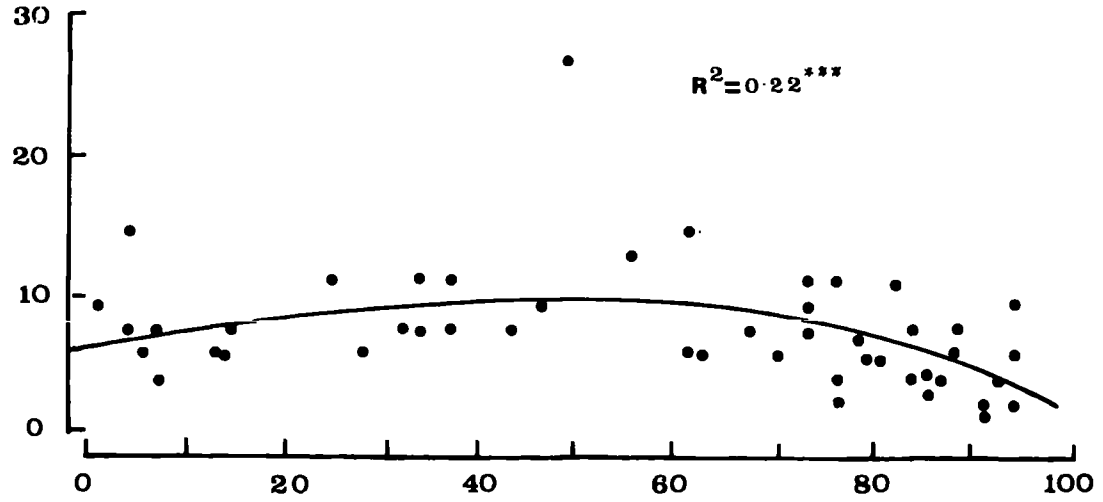
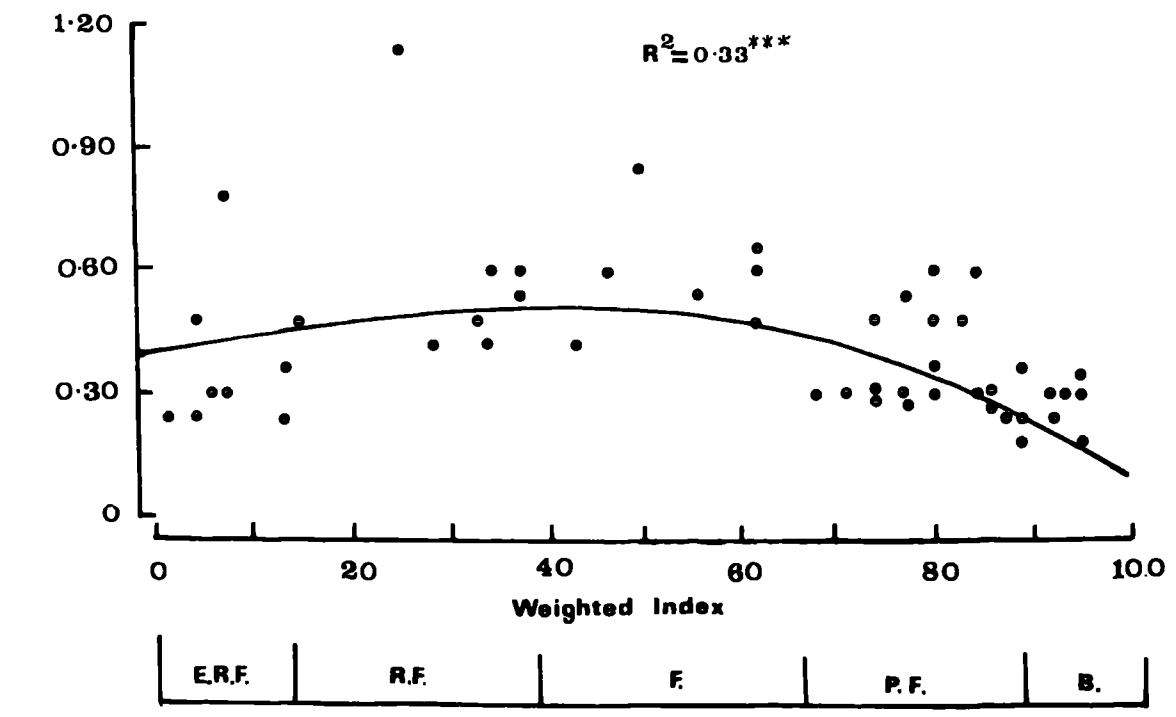


Fig. 39 Total P in Peat



FIGS. 38 AND 39 POOLED DATA FOR TOTAL PHOSPHORUS IN MIRE VEGETATION AND PEAT IN RELATION TO MIRE ECOCLINE. R^2 VALUES OF TREND AS SHOWN.

Table 9

Correlation between phosphorus in vegetation, with soluble peat phosphorus extracted with different solvents.

In terms of	EXTRACTANT 0.5N H_2SO_4	0.02N H_2SO_4	0.002N H_2SO_4	NH_4Cl	Acetic Acid	Ammonium Acetate	Total Peat
			ALL MIRES POOLED				
Unit dry Weight	0.28	0.09	0.32	0.47	0.21	0.21	0.60
Unit humus	0.24	0.01	0.23	0.32	0.07	0.11	0.41
Unit volume	0.29	0.09	0.32	0.46	0.21	0.22	0.59
			RICH MIRES ONLY				
Unit dry Weight	0.14	0.13	0.02	0.27	0.28	0.29	0.53
Unit humus	0.32	0.14	0.23	0.24	0.33	0.08	0.26
Unit volume	0.14	0.13	0.02	0.07	0.28	0.29	0.53
			POOR MIRES ONLY				
Unit dry Weight	0.35	0.10	0.32	0.51	0.26	0.17	0.73
Unit humus	0.41	0.08	0.32	0.51	0.24	0.17	0.69
Unit volume	0.34	0.11	0.33	0.48	0.26	0.17	0.73

Minimum coefficient required for statistical significance: all mires 0.33; rich mires 0.50; poor mires 0.38.

which can be recommended is that of total peat phosphorus; this has the disadvantage over the other methods of being a rather more time consuming and complex analytical technique. However, there are some considerable advantages: firstly, the analysis can be performed on peat samples collected at any time of the year with confidence in the knowledge that seasonal

fluctuations will be minimal; secondly, the levels of the correlations are fairly high, with the result that any conclusions based upon this index will be that much more valid.

As in the case of nitrogen considerable improvement in the estimate can be made by introducing a second order term, thus the best estimates are as follows:

$Pv = 2.3T - 0.82T^2 - 0.04$ ..... all mires
$R^2 = 0.37***$. s.e. = 0.34

$Pv = 1.1T - 0.49T^2 - 0.47$ ..... for rich mires only
$R^2 = 0.41*$. s.e. = 0.47

$Pv = 1 - 2.6H + 4.1H^2$ ..... for poor mires only
$R^2 = 0.71***$. s.e. 0.27

where Pv = phosphorus in the vegetation; T = total phosphorus in the peat, both in unit dry weight; and H is phosphorus in peat per unit humus.

Once again there appears to be no advantage in basing the simple correlations on anything other than per unit dry weight, although in the third of the above equations unit humus offers a 4% improvement over the equation utilising per unit weight (not given). The R^2 value for the poor mire only equation above is 0.71, with a standard error of 0.27 mg P/g humus (about 8% of the total). These figures suggest that this equation may be very useful in studies of phosphorus and mire phytosociology.

10(e) Relationships between phosphorus in the vegetation, and other inorganic factors

A step-wise regression analysis was carried out as previously described: the dependent variable being the logarithm of the phosphorus content of the aerial vegetation, and the independent variables being either the easily soluble peat inorganic chemical factors (results displayed in

Table 10), or other chemicals in the vegetation (results displayed in Table 11).

Peat chemical factors do not account for a large amount of the variability in the phosphorus content of the vegetation, however, in each case the coefficient of multiple determination (R^2) is statistically significant (Table 10). Most of the partial regression coefficients in Table 10 are not statistically significant, it must be concluded that the factors have an indirect effect. Calcium has a positive influenco in the poor mires, and a negative one in the rich mires. Ammonium nitrogen has a positive relationship in rich mires, this being the only statistically significant partial regression coefficient. Aluminium appears to exert a positive influence in both poor and rich mires.

Table 10

Relative contribution by soluble inorganic chemical factors in peat to the explanation of variability in the phosphorus content of aerial mire vegetation.

Factor	% reduction in variance	beta	F
	ALL MIRES POOLED		
Al	15	0.31	3.5 (N.S.)
Fe	8	0.31	3.5 (N.S.)
R^2 = 0.23*; F = 5.3; n = 33,			
	RICH MIRES ONLY		
NH_4^+	38	0.55	6.6*
Al	20	0.34	2.5 (N.S.)
Ca	7	-0.30	1.8 (N.S.)
R^2 = 0.65*; F = 5.6; n = 13,			
	POOR MIRES ONLY		
Ca	18	0.34	3.0 (N.S.)
Al	9	0.31	2.0 (N.S.)
R^2 = 0.27*; F = 3.5; n = 22,			

Table 11

Relative contribution by inorganic chemicals in aerial mire vegetation to the explanation of variability in phosphorus content of the vegetation.

Factor	% reduction in variance	Beta	F
	ALL MIRES POOLED		
K	32	0.46	8.9*
Zn	10	0.43	6.5*
Mn	7	0.30	4.8*
Pb	3	-0.45	7.1*
R^2 = 0.52***; F = 8.1; n = 35.			
	RICH MIRES ONLY		
Mn	64	1.2	37***
K	8	0.48	10*
N	8	-0.46	5*
R^2 = 0.80***; F = 17.2; n = 17.			
	POOR MIRES ONLY		
K	35	0.36	3.6 (N.S.)
Na	10	0.36	4.5*
Pb	9	-0.55	7.7*
Mn	7	0.27	3.1 (N.S.)
R^2 = 0.61***; F = 7.2; n = 22.			

Analysis of the relationships between phosphorus and other chemicals in vegetation has produced much higher levels of statistical significance (Table 11) than the analysis of vegetation phosphorus and soil factors, described above. In all cases the coefficients of multiple determination are highly statistically significant ($p < 0.005$). Overall, potassium appears to be the factor which accounts for most variability in vegetation phosphorus, although much of this effect is indirect: in poor mires this can be concluded from the fact that the partial correlation coefficient is not statistically significant even though this factor accounts for 35% of the variability; in the analysis of data from all mires potassium

accounts for 32% of the variability, but again the F value for its partial regression coefficient suggests that much of this influence is indirect. The 8% of variability in vegetation phosphorus accounted for by potassium in rich mires possibly reflects a more realistic figure for the direct influence of this element. Nitrogen also accounts for 8% of the variability in rich mires, but it is interesting to note (and difficult to explain) the fact that the beta coefficient is negative: opposite to that for soil nitrogen. Lead has a negative affect, and this element appears to exert its influence mostly in the poor mires where its statistically significant partial regression coefficient is the highest appearing in this section of the analysis. Sodium apparently has some small influence, but only in poor mires. Two other heavy metals occur in the final results: Manganese and Zinc, the former of these two is probably the more important, appearing as it does in all sections.

Certain features of above results can be dealt with fairly briefly. Potassium is an essential element for plant metabolism and its appearance emphasises (i) that the movement of phosphorus from soil to aerial parts of herbaceous vegetation is a metabolic dependent process, and (ii) that this element is possibly an important limiting factor in mire systems; it is notable that potassium also appears as an important influence in determining the rate of heterotrophic nitrogen fixing activity in these peats (see Chapters 8 and 12).

The negative beta value associated with rich mire peat calcium may reflect several interractions. In section 10(d) the possible importance of the iso-electric point for the P_2O_5-Fe_2O_3 complex was discussed in relation to the fact that minimum phosphate solubility occurs near to this point. Increasing levels of calcium in rheotrophic peats are likely to be reflected in higher pH, i.e. values close to the iso-electric point. Calcium can also cause a reduction of phosphorus availability by precipit-

ation of calcium phosphates, and possibly also by limiting the mobility of manganese (this latter point is discussed below). Thus for several reasons, increasing amounts of calcium in rich mires may have a deleterious effect on the supply of phosphorus to vegetation (Lundblad, 1941; Puustjärvi, 1952; Puustjärvi, 1956; Pizer, 1965).

The negative beta value for nitrogen in the fen vegetation is more difficult to explain, however a possible answer may be found by invoking nitrogen fixation. The nitrogen content of fen vegetation may be, in part at least, a function of nitrogen fixation, and intense nitrogen fixation can cause biological fixation of phosphorus in the soil (Taylor, 1946). High levels of NH_4^+ - N in fen peat might reduce nitrogen fixing activity (see Chapter 12), thus allowing more phosphorus to be absorbed by the vegetation. There is no statistical support for this idea which must, therefore, remain nothing more than a speculation. One of the most interesting aspects of this analysis is the appearance in the results of manganese and aluminium, particularly the fact that the former element appears only in the analysis relating to the chemicals in vegetation, and the latter only in the analysis relating to soluble chemicals in the peat. Discussion of the effect of aluminium on plant growth has continued for over three quarters of a century (see Magistad, 1925 for review of early literature), and it is significant that many of these debates have also involved phosphorus. At high concentrations aluminium is toxic to many plant species, and there is evidence that toxicity results from the precipitation of insoluble aluminium phosphates in plant roots (Wright & Donahue, 1952; Foy et al., 1967; etc.) However, the levels of aluminium, as well as iron, are very low in these peats, therefore of greater interest in the present context is the fact that there is a considerable body of evidence in support of the contention that low levels of aluminium can actually stimulate the uptake of phosphorus into plants. Hackett

(1964) showed that 2-25 p.p.m. aluminium in the growth medium stimulated root growth of barley from seed; Randall & Vose (1963) found that phosphorus uptake in rye grass was increased by aluminium concentrations of up to 50 p.p.m.; and Hesse (1963) concluded that the uptake of phosphorus into rice plants was stimulated by aluminium, but that the phosphorus tended to be precipitated in the roots. It is worth noting that both the above-mentioned levels of aluminium are comparable with the levels of aluminium extracted by acetic acid from peat.

If excessive precipitation does occur in the root systems of mire plants one might expect to find comparatively high levels of phosphorus in peat, but this is not so (Chapters 11 and 12); however, this is the case with aluminium. Thus there is no indication of phosphorus immobilisation within the roots; considering that the amounts of soluble phosphorus are comparatively high compared with amounts of the elements that are most likely to cause its precipitation (aluminium and iron) this is not surprising. Thus from the analyses, and the discussion, it is possible to conclude that the soluble aluminium in peat may be stimulating higher levels of phosphorus in the aerial parts of the vegetation; such an effect has been demonstrated by Randall & Vose (o.c.) for rye and by Humphries and Truman (1964) for Pinus radiata.

Although the possibility that phosphorus uptake may be stimulated by aluminium has much supporting evidence, the nature of this stimulation is by no means understood. Clarkson (1965), and Rorison (1965) both produced results which suggested that the stimulation was not metabolic, whereas the enzyme inhibitor investigations of Randall & Vose (o.c.) lead to the opposite conclusion. The work of Randall & Vose may also help to provide an explanation for the appearance of manganese in results of the analyses of the relationship between phosphorus and other chemicals in vegetation. They found that manganese stimulated the translocation of

phosphorus from root to shoots, thus off-setting any tendancy for aluminium to precipitate phosphorus in the roots. Manganese is an important element in many metabolic processes, especially those involving biochemical phosphate transformations (Hewitt, 1958; Nason & McElroy, 1963), and a direct link between phosphate uptake and phosphorylation has been demonstrated in barley (Jackson et al., 1962).

It is interesting to note that manganese, in contrast to aluminium, is not concentrated in the roots, it appears to be generally more mobile than aluminium (see Chapter 11). Buckman and Brady (1962) present data which suggest that a total manganese content of 2 mg/g dry weight is normal in mineral soils, and in their examination of 36 Wisconsin mineral soils Dolar & Keeney (1971) found an average of 630 p.p.m. total, and 90 p.p.m. (dr. wt.) extractable manganese; by comparison the average for the peats studied in this investigation are much lower at 64 and 24 p.p.m. respectively. All of these facts suggest that manganese may be at a premium in peatland ecosystems. (See also Mitchell, 1954; and Wiklander, 1965). High levels of peat calcium appear to be related to lower levels of vegetation phosphorus. If manganese is deficient in peatland systems, it is possible that this deficiency is exacacerbated by high calcium levels reducing mobility of manganese (which has a positive influence on vegetation phosphorus) between roots and stems (Vlamis & Williams, 1962; Swanback, 1932; Lohnis, 1960). Similarly, at least part of the influence of potassium on the phosphorus content of vegetation may be due to potassium increasing the mobility of manganese (Swanback, 1932; Belle-Jones, 1955).

Comparative data for different peats show that levels of lead obtained in this study (Table 5, column 1 and 6) are considerably higher than those in peats from Finland and Russia (Table 5 columns 2-5). The fact that lead affects animal metabolism is well known (e.g. review by Dulka & Risby, 1976); data relating to the effect of lead on higher plant

metabolism are sparse by contrast; however there is some evidence that it does affect plant phosphorus metabolism (Woolhouse, 1968; Jeffrey, 1969).

In summary, the above evidence lends support for the following general suggestions concerning variability in the phosphorus content of vegetation and other chemicals. Aluminium is generally present at levels which may actually stimulate phosphorus uptake; and translocation from root to shoot may be considerably dependent upon manganese, which appears to be potentially deficient in peatland ecosystems. Potassium may be important not only in stimulating metabolism in general, but more specifically by increasing the mobility of manganese. In rich fens calcium may restrict phosphorus movement into and through the vegetation, this would explain the slight decline of the phosphorus content of aerial vegetation at the extreme rich fen end of the mire ecocline; the decline at the extreme ombrotrophic end may be due to a general lowering of metabolic activity, and perhaps also to a reduction in the potassium content of the aerial vegetation.

In view of the fact that the statistical analysis suggests that much of the influences of potassium and calcium is via other chemicals, a special statistical examination of potential interactions between either potassium or calcium, with manganese was carried out. This can be done in step-wise multiple regression analysis by first eliminating the variability due to each of the individual factors, then finally including a special variable computed as the product of the two factors suspected of interaction: a further significant reduction of explained variance is indicative of potential interaction. The results of this analysis are presented in Table 12 where it can be seen that calcium and manganese do not appear to interact, which suggests that most of the effect of calcium on vegetation phosphorus in rich fens is through the immobilisation of phosphorus in the peat. (More correctly the null hypothesis is that

Table 12

Statistical test for possible interaction between either calcium or potassium with manganese.

		POOR MIRES ONLY		RICH MIRES ONLY	
Step No.	Variable	Beta	% reduction in explained variance	Beta	% reduction in explained variance
1	Mn	-0.087	2	0.675	64***
2	Ca	0.162	7	0.058	2
3	Mn x Ca	0.223	0	0.177	0
1	Mn	-0.93**	2	-0.872	64***
2	K	0.04	41***	0.060	8*
3	Mn x K	1.30***	17**	1.711	4

Statistical parameters not significant unless otherwise indicated.

manganese and calcium do not interact, and this hypothesis must be accpeted). The analysis involving potassium, however, does provide evidence of interaction. In poor mires the interaction between potassium and manganese is statistically highly significant, accounting for a further 17% of the variance when the computed variable is added as the last step; furthermore although potassium accounts for 41% of the variability when added as the second step, addition of the computed variable completely eliminates the significance of potassium's standardised partial regression coefficient, whereas the standardised partial regression coefficient for the computed variable itself is highly significant. Using data from rich mires, none of the standardised partial regression coefficients in the potassium section of the analysis are statistically significant, but it is noteworthy that the coefficient attached to the computed variable is much higher than the other two. This statistical analysis strongly supports the hypothesis relating to a possible interrelationship between potassium and manganese. (Or, again more correctly, the null hypothesis is that there is no interaction between potassium and manganese, but the statistics

indicate that the null hypothesis must be rejected).

The exact way in which potassium influences the phosphorus content of mire vegetation must remain somewhat speculative, but the results presented in Table 11 provide evidence, not only that this influence eixsts, but also that it is considerable. Two reports from peatland afforestation studies provide further information on this point: Dickson (1972) concluded that correction of potassium deficiency permitted better utilisation of phosphorus by sitka spruce grown on oligotrophic peat; and Ferda (1972) found that the application of phosphorus fertiliser had little effect on the growth of various conifers on poor fen soil after three years, unless potassium deficiency was corrected.

CHAPTER ELEVEN :

METALLIC CATIONS

There is a larger body of information concerning the major metallic cations in peatlands than exists for the subjects discussed in previous chapters, therefore the results presented in this chapter are dealt with more briefly, emphasising particularly distributions within the ecosystem.

11(a) Methods

Total quantities of the various elements in peat and vegetation were extracted by wet digestion with perchloric acid (Johnson and Ulrich, 1959); cations were also extracted from peat using N ammonium acetate, and N acetic acid (1 g dr. wt. to 100 ml of extractant, shaken for 2 h). Amounts of the various cations in all of these extracts were estimated by atomic absorption spectroscopy, after filtration.

11(b) Distribution of cations in relation to the ecocline

Results from the various analyses are displayed in Figures 40 to 47, the total amounts of each element in peat and vegetation, together with the exchangeable quantity in peat are all shown in the same figure, so that the distribution is easily seen. The ranges of many elements are very great, therefore in order to facilitate comparisons, a logarithmic scale for the amounts of each element has been used in every case. Whilst examining these results the following points should be borne in mind: (1) a straight line indicates a curved relationship on the untransformed scale; (2) a slight curve was probably sharper on the original scale (more rarely a slight curve may represent a linear relationship on the original

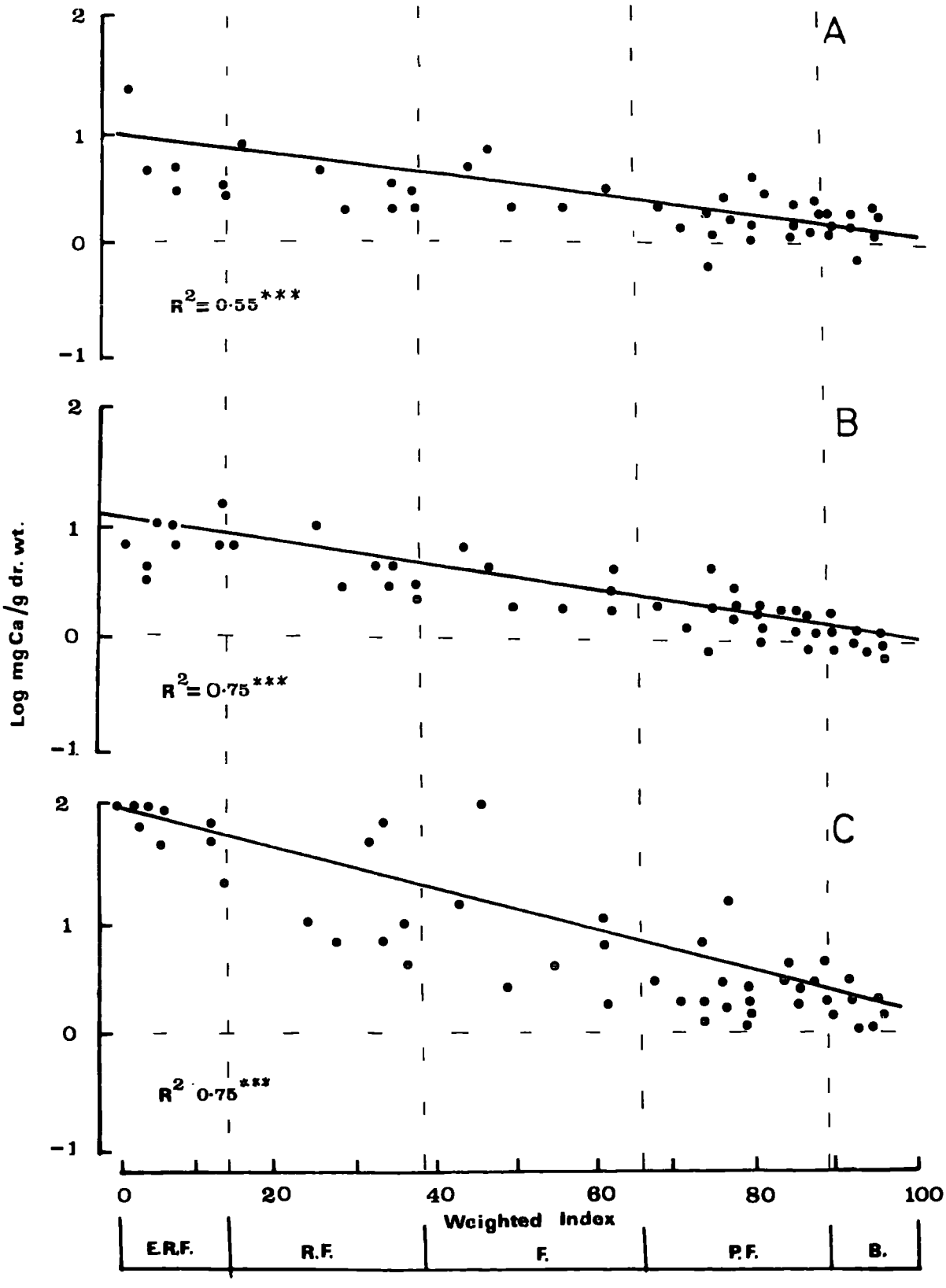


FIG. 40 POOLED DATA FOR CALCIUM IN PEAT IN RELATION TO MIRE ECOCLINE. A, IN VEGETATION; B, AMMONIUM ACETATE SOLUBLE IN PEAT; C, TOTAL IN PEAT.

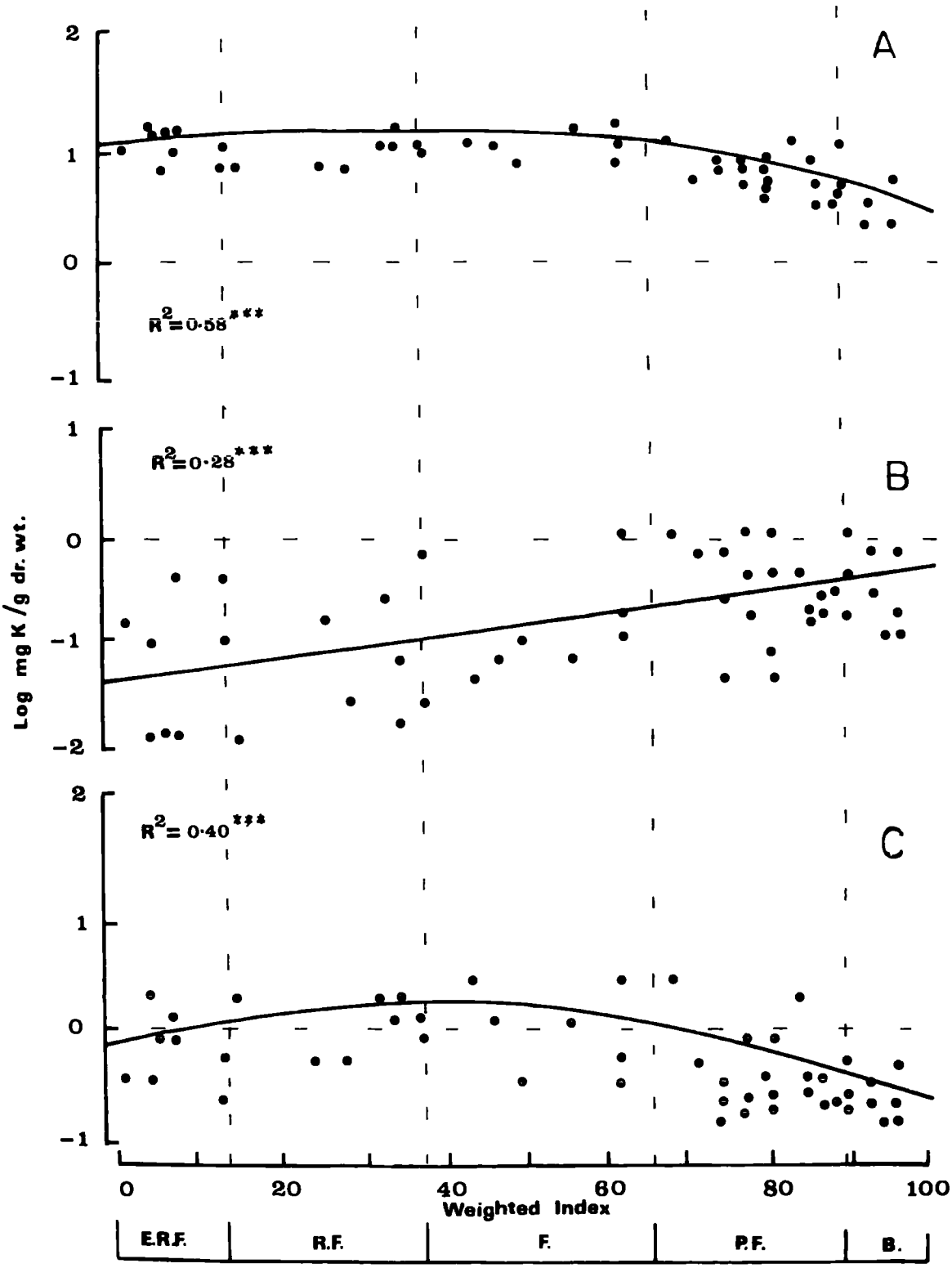


FIG. 41 POOLED DATA FOR POTASSIUM IN PEAT IN RELATION TO MIRE ECOCLINE. A, IN VEGETATION; B, AMMONIUM ACETATE SOLUBLE IN PEAT; C, TOTAL IN PEAT.

scale); (3) a single unit of change on the log scale represents a ten-fold change on the untransformed scale.

Total amounts of calcium, Magnesium, and potassium in both peat and vegetation are greatest in the more rheotrophic parts of the ecocline, and decline towards the ombrotrophic end (Figures 40A, C; 41A, C; 42A, C) decline extends over the entire length of the ecocline in the cases of calcium and magnesium, but the curves in Figure 41 indicate that potassium declines only between fens and bogs. Concentrations of total sodium in peat and vegetation do not appear to be related to the ecocline (Figures 43A, C).

Exchangeable amounts of calcium and magnesium in peat have a distribution which reflects the total quantities (Figures 40B and 42B), but with potassium the opposite is the case: exchangeable amounts of this latter element increase in the direction of ombrotrophic mires (Figure 41B). The explanation of this feature probably depends, in part at least, upon the fact that potassium is far more highly concentrated into the vegetation than calcium: in rheotrophic mires over a hundredd times more so (discussed below). Microbial activity in peat gives rise to an increase in hydgrogen ions which can replace calcium absorbed to humic and clay colloids, therefore as a rheotrophic peat mass grows calcium is free to be lost from the system by leaching and moving ground water. This leaching effect is much less marked with potassium because most of it is trapped in the living biomass. In poor mires, with much calcium having been lost by leaching during the earlier part of peat growth, and a decline in potassium uptake (at the ombrotrophic end of the ecocline potassium is only ten times more concentrated into the vegetation than calcium), potassium becomes an important ion in the exchange complex surrounding the peat colloids (c.f. rheotrophic peat where it is an insignificant member of the exchange complex).

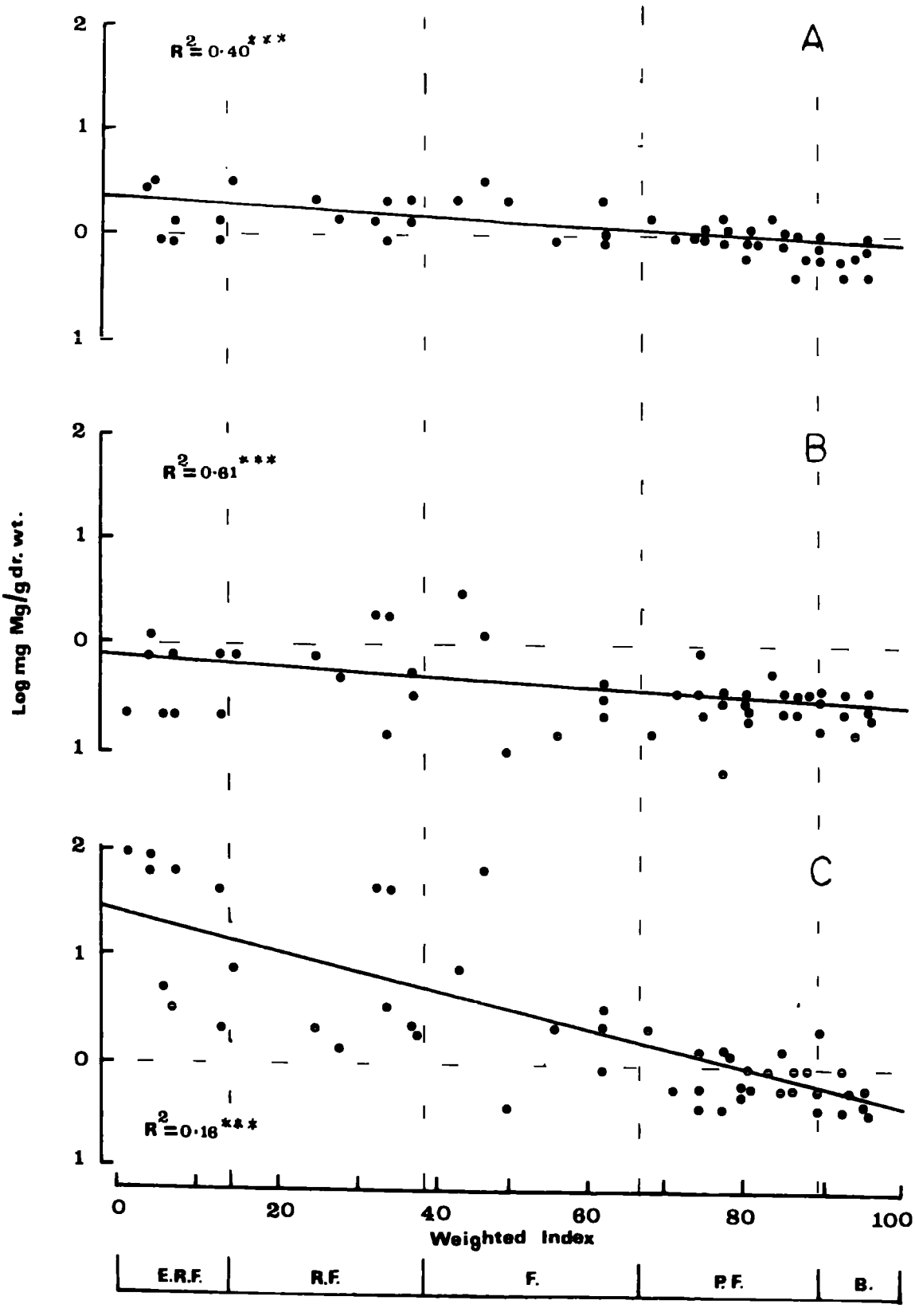


FIG. 42 POOLED DATA FOR MAGNESIUM IN PEAT IN RELATION TO MIRE ECOCLINE. A, IN VEGETATION; B, AMMONIUM ACETATE SOLUBLE IN PEAT; C, TOTAL IN PEAT.

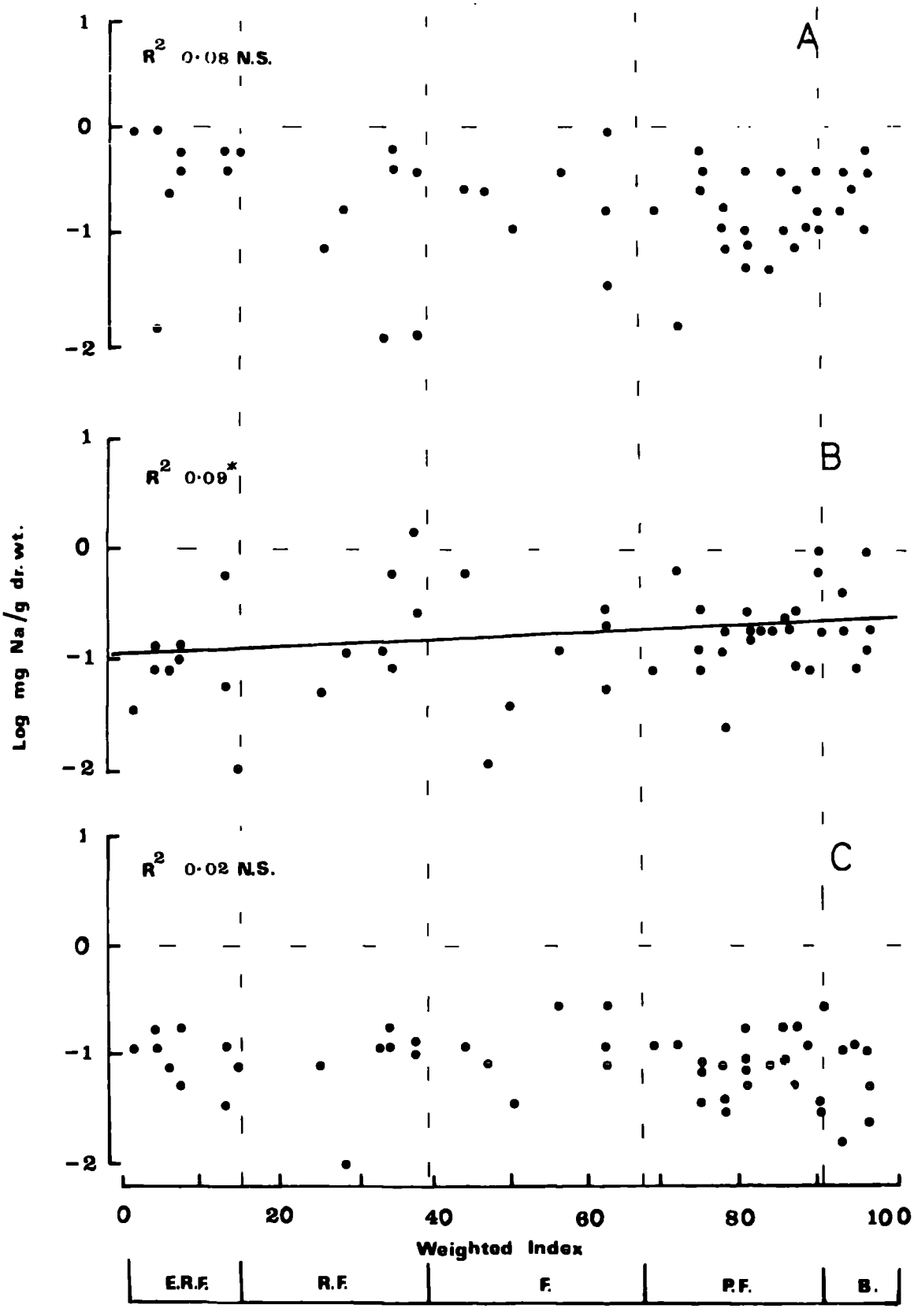


FIG. 43 POOLED DATA FOR SODIUM IN PEAT IN RELATION TO MIRE ECOCLINE. A, IN VEGETATION; B, AMMONIUM ACETATE SOLUBLE IN PEAT; C, TOTAL IN PEAT.

The distributions of iron and aluminium in relation to the mire ecocline are almost the same (Figures 44 and 46) and probably reflect the similar solubility characteristics of their compounds. In the very acid conditions of bogs, and some poor fens, compounds of these two elements become mobile, it is also in these peats that losses into the ground water are much reduced. Thus the greater mobility and retention by the system means that larger amounts of iron and aluminium are available to be absorbed by the vegetation.

The distribution of manganese is different to those of iron and aluminium. The amounts of manganese in mire vegetation increase steadily between extreme rich fens and fens (Figure 45A). This is possibly a result of the decrease in aeration making manganese more soluble in its reduced state. Iron is also more soluble in its reduced form, but the redox potentials for these couples would permit manganese to change valency at greater levels of oxygenation that iron, all other things being equal.

e.g. $\frac{1}{2}MnO_2 + 2 + 1^+ + e^- = \frac{1}{2}Mn^{++} + H_2O$

redox potential for the above couple is about 1.2 v

$Fe^{+++} + e^- = Fe^{++}$

redox potential for this couple is about 0.7 v

There is no apparent trend of manganese concentrations in the vegetation between fens and ombrotrophic mires (Figures 45A). Malmer (1962a) concluded that the concentration of manganese in Narthecium ossifragum was inversly proportional to the amount of this element in the peat (o.c. p 216). However this conclusion appears to be based upon a scattergram which displays little or no correlation (o.c. Figure 62): in fact in the afore-mentioned figure a single point creates the illusion of a relationship. Malmer also states that the manganese content of the

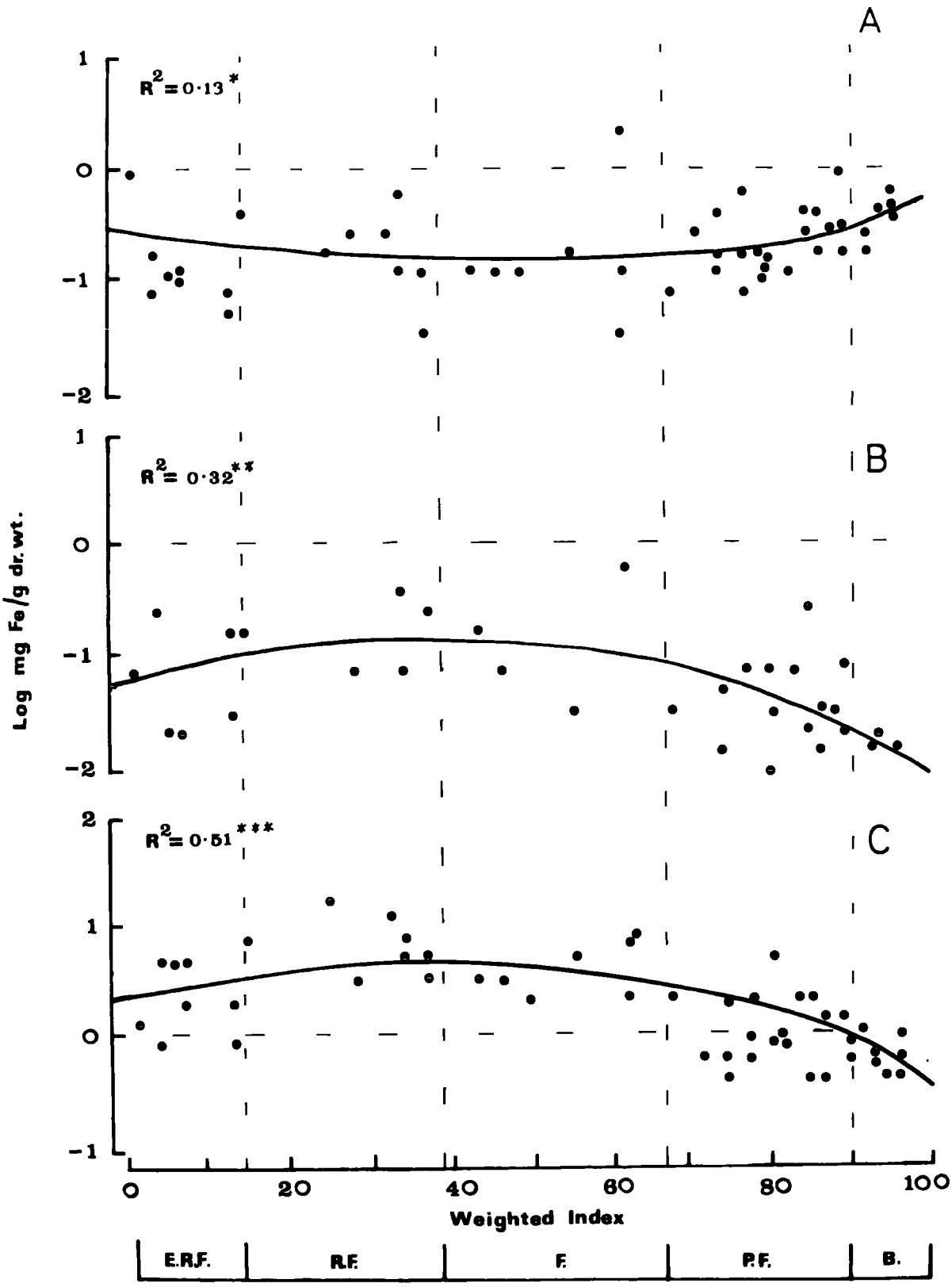


FIG. 44 POOLED DATA FOR IRON IN PEAT IN RELATION TO MIRE ECOCLINE. A, IN VEGETATION; B, ACETIC ACID SOLUBLE IN PEAT; C, TOTAL IN PEAT.

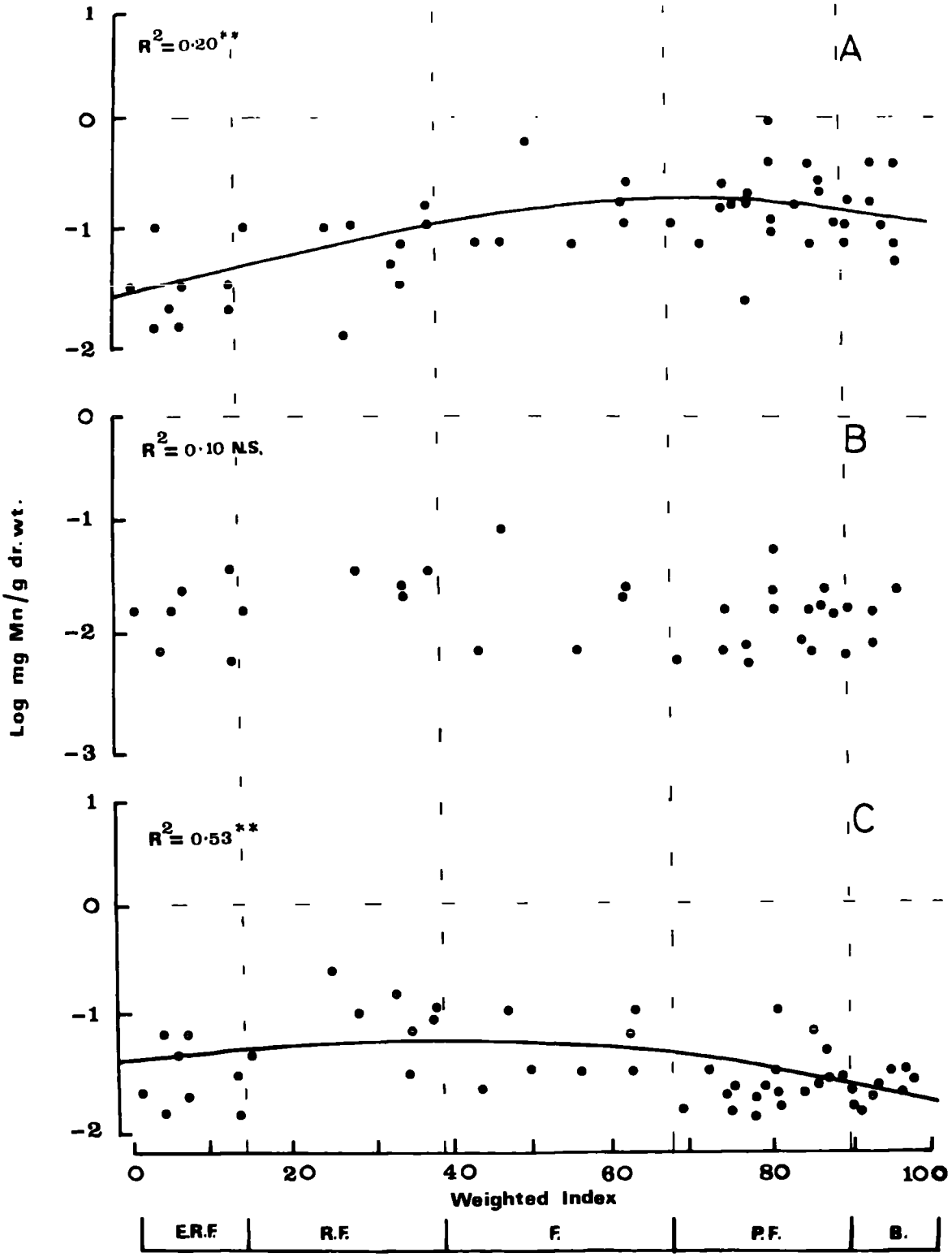


FIG. 45 POOLED DATA FOR MANGANESE IN PEAT IN RELATION TO MIRE ECOCLINE. A, IN VEGETATION; B, ACETIC ACID SOLUBLE IN PEAT; C, TOTAL IN PEAT.

plants is correlated with pH, being highest at low pH values (o.c. p 215 and Figure 62). The correlation apparent in this figure is strong, but is largely due to the spread of points between pH 4.2 and pH 5.2; the relationship at pH values lower than about 4.2 is much weaker. This trend is almost exactly the same as shown in Figure 45A, between weighted indices 38 and 70.

Sonnesson (1970b) has also studied both exchangeable and total amounts of metals in different peats, and ordinated his results according to an approximate floristic gradient (o.c. Table 3). Sonnesson's results for iron, aluminium and manganese are all in general agreement with those presented here.

Heavy metals have been less frequently studied than other cations in peatlands, therefore a summary of data obtained in this project together with results obtained by other workers have been collated in Table 13. It can be seen that concentration of metals in ombrotrophic peat are the same, or lower than concentrations in rheotrophic peat; by contrast the quantities in vegetation of ombrotrophic mires are similar or higher than in the rheotrophic vegetation. This general pattern follows the same outline as that discussed above for iron and aluminium; however lead is a notable exception, being significantly higher in ombrotrophic vegetation and in ombrotrophic peat. My figures for lead in peat are much higher than those of other workers (Table 13), this could be due to the fact that my sites were fairly close to industrial areas, whereas those of other workers were in very rural parts of Finland and Russia. Mosses accumulate exceptionally large quantities of airborne lead (Rühling and Tyler, 1968), therefore the abundance of this plant group, together with the poor drainage, may account for the higher lead concentrations in the ombrotrophic peat from South Germany.

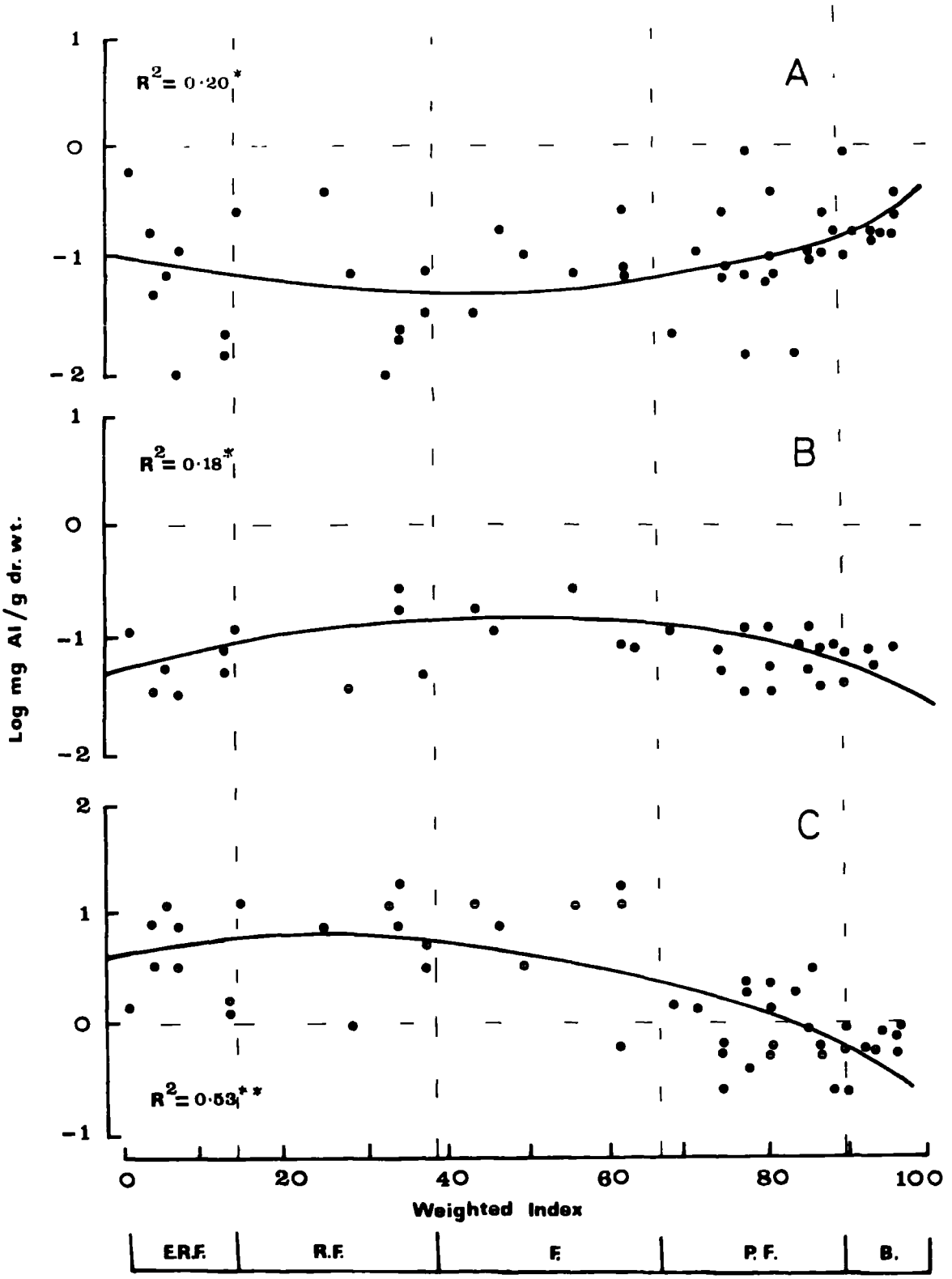


FIG. 46 POOLED DATA FOR ALUMINIUM IN PEAT IN RELATION TO MIRE ECOCLINE. A, IN VEGETATION; B, ACETIC ACID SOLUBLE IN PEAT; C, TOTAL IN PEAT.

TABLE 13. Comparative summary of the total contents of some trace elements in peat and mire vegetation.

	PEAT							VEGETATION			
Peat type	R	R	R	R	O	O	O	R	O	O	O
Source	1	2	3	4	3	1	5	1	1	6	7
n	20	c.63	12		2	30	c.60	20	30	4	10
Fe	5758 (1064)		11886			2216 (426)	2447	279 (68)	417 (95)	50	580
Al	8079 (1100)		3520		700	2694 (797)	1150	155 (46)	254 (52)		
Mn	101 (22)	99	229	284	50	43 (5)	18	133 (43)	278 (41)	190	150
Zn	140 (47)	108	8	14	4	165 (39)	20	77 (20)	78 (12)		
Pb	51 (5.7)	8	10	5.8	1.4	73 (4.7)		26 (8.7)	33 (4.5)		
Ni	10 (1.6)	9	14	56	0.6	4.8 (0.5)					
Cu	14 (3.0)	30	80	11	8	26 (5.3)	6.4	39 (17)	25 (15)		
Cd	2.7 (0.2)	0.4				3.1 (0.8)		0.10 (0.005)	0.21 (0.03)		
Co	9.4 (1.4)	6	14	3.8	0.4	2.3 (0.3)					

1, This work; 2, Salmi (1950); 3, Salmi (1955); 4, Largin, et al. (1971); 5, Sonesson (1970b); 6, Tamm (1954); 7, Malmer & Sjörs (1955), n, number of sites or samples; R, peat from rich mires; O, peat from poor mires. All figures p.p.m. standard error in brackets.

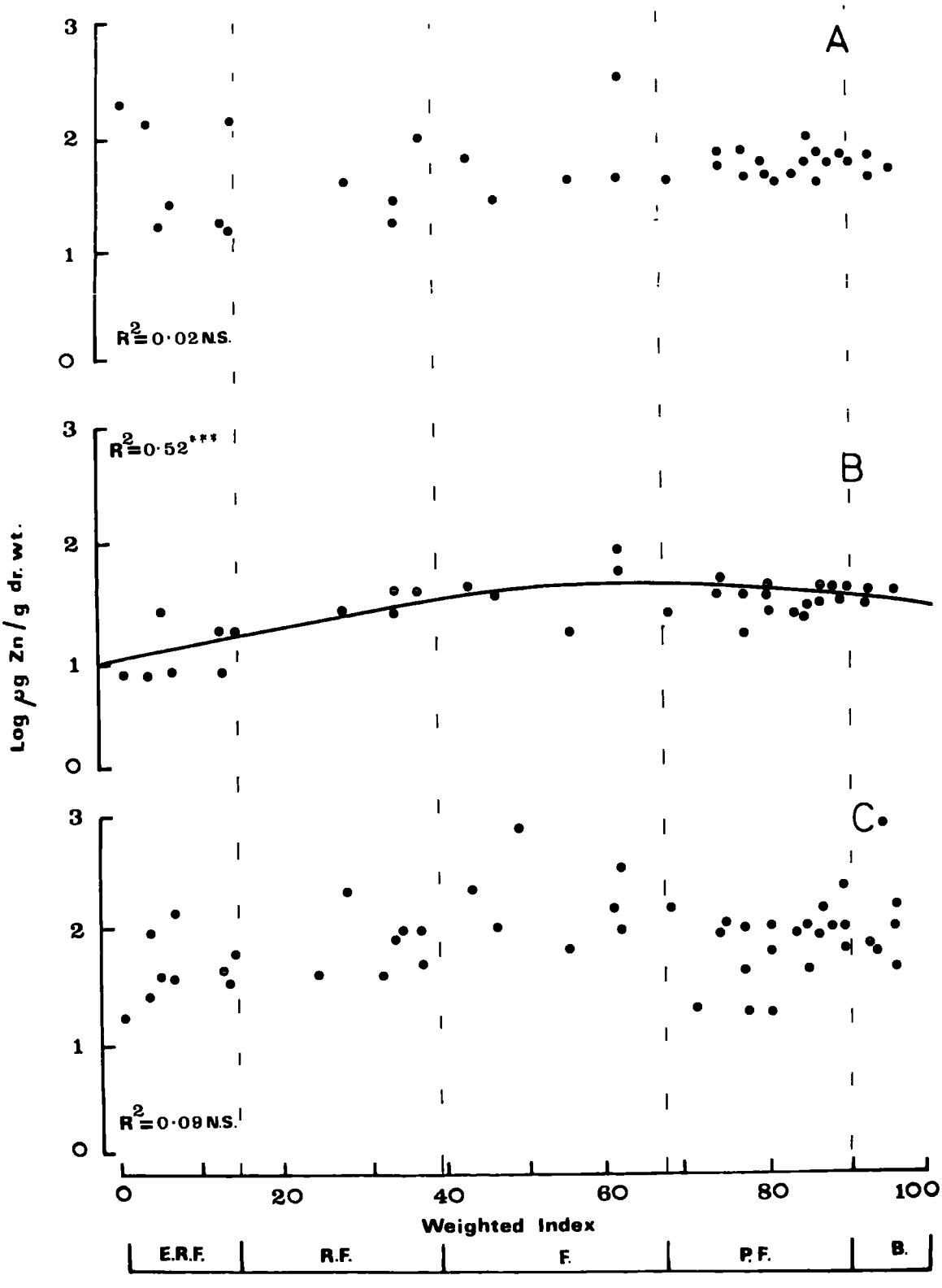


FIG. 47 POOLED DATA FOR ZINC IN PEAT IN RELATION TO MIRE ECOCLINE. A, IN VEGETATION; B, ACETIC ACID SOLUBLE IN PEAT; C, TOTAL IN PEAT.

11(c) Ionic ratios

Ratios of certain alkali cations have occupied the interest of peatland ecologists for several decades now, and one in particular, the Ca/Mg ratio has received more attention than the rest. Mattson, Sandberg, and Terning (1944) suggested that the Ca/Mg ratio would characteristically be less than one in ombrotrophic peats, reflecting the ratio of these two elements in precipitation. Mattson et al. obtained an average figure of 0.48 for ombrotrophic peat from the Ramna bog, which is situated about 20 K from the west coast of Southern Sweden, their average figure for rheotrophic peat from the same bog was 1.38 (o.c. Table 26a). Results from Coom Rigg Moss in Northumberland (Chapman, 1964), Western Ireland (Boatman, 1957), some Scandinavian data (Malmer, 1962a; Malmer and Sjörs, 1955) and Sarawak (Waughman, unpublished) are consistent with the concept: however there have also been several reports to the contrary. Sjörs (1948) found ratios greater than one in bog water; and later, utilising exchangeable amounts of these two elements, Sjörs obtained ratios lower than one in coastal bogs, but greater than one in bogs situated inland (Sjörs, 1961 p 23 and Table 4). In their detailed examination of a single Sphagnum fuscum hummock Bellamy and Rieley obtained values of 1.5-2 for the Ca/Mg ratio based upon total amounts in the ombrotrophic parts of the hummock, and generally higher than three for 'ombrotrophic water' (Bellamy and Rieley, 1967, Tables 1 and 2). The Ca/Mg ratio of water in some mires of the north eastern U.S.A. is actually higher in ombrotrophic situations than it is in rheotrophic ones (Heinselman, 1970, Table 3).

Sonnesson (1970b) found Ca/Mg ratios of less than one in peat from bogs located near to the western coast of Northern Scandinavia, but generally greater than one in peat from bogs located inland (o.c. p 91). Sonneson also showed that the amount of magnesium in precipitation declined

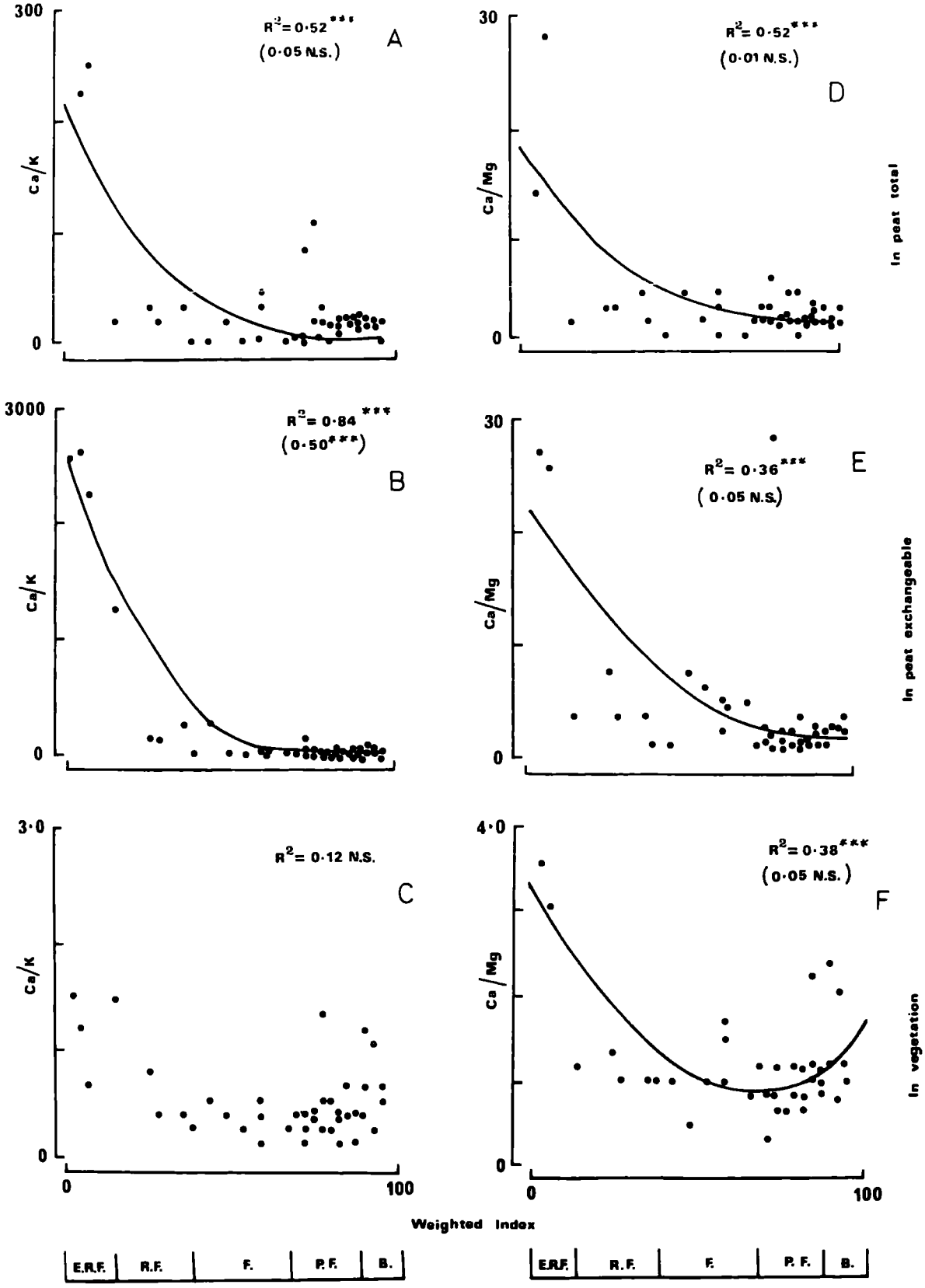


FIG. 48 SOME IONIC RATIOS (M EQUIV.) IN PEAT AND VEGETATION IN RELATION TO THE MIRE ECOCLINE.
R^2 VALUES SHOWN, FIGURES IN BRACKETS EXCLUDE E.R.F. AND R.F.

more rapidly than either calcium or potassium as distance from the coast increased (o.c. Figure 5). The influence of sea water on the composition of precipitation is the most widely accepted explanation for these results, but Saebø (1973) considers that differential leaching might be just as important (o.c. pp 2-9). In this context Mörnsjö's (1968) results for two peat profiles are interesting in that ombrotrophic peat in the profile from Fjölmossen has Ca/Mg ratios of less than one, whereas ombrotrophic peat in the Skoggardänger profile has ratios higher than one: and the former site has about 40% more rainfall than the latter. It is perhaps also worth mentioning that the Ca/Mg ratio in tropical peats, is 0.23, lower than any value that I have found in the literature.

The Ca/Mg ratios in peats from Southern Germany are displayed in Figure 48, where it can be seen that very few of the values for this ratio are below one (Figure 48D and E). Thus in Central Europe a Ca/Mg ratio of less than one is no criterion of ombrotrophy. There is a general trend of increasing values along the ecocline in the direction of richer mires, but this trend is entirely due to the values for peats from rich, and extreme rich fens; when the trend is calculated on values in peats from the fen to bog part of the ecocline it is not significant (note the R^2 value in brackets in Figure 48D and E). The Ca/Mg ratio of mire vegetation is only weakly related to the ecocline, and does not reflect the ratio of exchangeable amounts in peat (Figure 48F). This illustrates the fact that vegetation has considerable power of selectivity with regard to ion uptake.

The Ca/K ratio is also displayed in Figure 48 (A, B and C). The values for this ratio based upon both total and exchangeable amounts in peat are much higher than for the Ca/Mg ratio. The trend of the Ca/K ratio in vegetation in relation to the ecocline is not significant, and the trend for the ratio based upon total quantities in peat is much the

same as for the Ca/Mg ratio. However, the trend for the Ca/K ratio based upon exchangeable amounts in peat is particularly interesting. First, the statistical significance of this trend is much higher than any of the others shown in Figure 48, and second, when the extreme rich fens and rich fens are excluded from the calculations the trend is still highly significant (Figure 48B). This trend reflects the fact that potassium is strongly bound into the ecosystem so that the exchangeable amounts actually increase in the ombrotrophic direction of the ecocline (Figure 41B), whereas exchangeable calcium decreases in this same direction (Figure 40B). One point which is not apparent in Figure 48B is that the Ca/K ratio in all ombrotrophic sites is less than 10, in fact most of the values are less than 5. A Ca/K ratio of less than 10 cannot be described as defining bogs, because such values also occur in poor fens and fens; however all parts of the ecocline other than bogs also contain peats which are mostly higher than 10, often much higher. The Ca/K ratio in peat appears worthy of further investigation by those seeking a chemical index of ombrotrophy.

The Ca/K ratio in mire vegetation is much lower than the same ratio of exchangeable quantity in peat. This, like the Ca/Mg ratio again illustrates the powers of ion selectivity possessed by mire vegetation.

11(d) Comparative mobility of some elements in the mire ecosystem

Any attempt to acquire precise information on the movement of chemicals in an ecosystem requires detailed measurements on a small part of that system, preferably utilising radio-active, or otherwise easily identifiable isotopes of the elements being studied. This project was not designed to obtain such facts, however careful examination of the data to hand can provide some information of comparative mobility.

It must be emphasised from the outset that in the following dis-

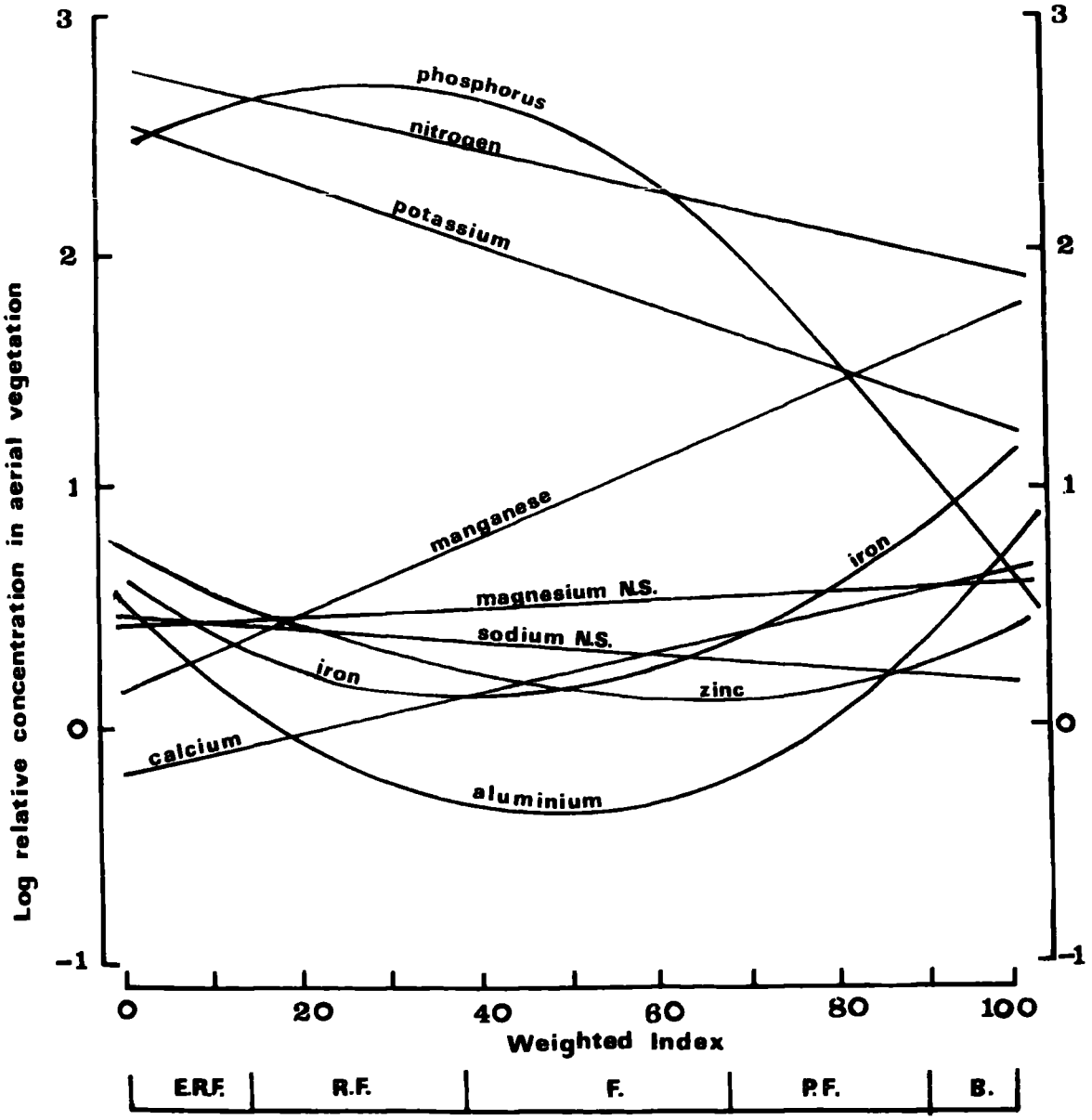


FIG. 49 RELATIVE CONCENTRATION OF VARIOUS ELEMENTS INTO AERIAL PARTS OF THE VEGETATION IN RELATION TO MIRE ECOCLINE. TRENDS SIGNIFICANT UNLESS OTHERWISE INDICATED; EQUATIONS FOR TRENDS IN TABLES A23. SEE TEXT FOR EXPLANATION.

cussion one important measurement which is missing in the quantity of each element stored in the living roots; the measurements which are available are (1) the concentration of each element in the aerial vegetation, (2) the amount of each element in peat which is easily soluble, and (3) the total quantity of each element in the rooting zone of the peat. Data are available to convert these measurements into absolute quantities, but as these data are the same for each element at each site, there is little to be gained by such conversion in a comparative study.

Two basic aspects of mobility were examined, firstly the extent to which the various elements are concentrated into the vegetation, and secondly the rate of mineralisation.

An index of concentration I_c, was calculated as follows:

$$I_c = \log\left(\frac{\text{concentration of element in aerial vegetation}}{\text{concentration of element in easily soluble extract}}\right)$$

Both the numerator and the denominator are in p.p.m. Calculated values have been plotted as trend lines in Figure 49. The logarithmic transformation is, as in other parts of this chapter, merely for convenience of presentation. Individual values have not been plotted, but regression parameters, including standard errors of predicted values, are tabulated in Table A22.

If the index I_c for an element is high, it means that this element is concentrated into the vegetation to a considerable extent, and is therefore, potentially at least, a limiting factor in that part of the ecocline. Where the index is comparatively low the interpretation is more ambiguous because it might indicate either that supply of the element is adequate, or that there are problems of uptake.

The high concentration of phosphorus and potassium into peatland vegetation has been observed by several previous workers including Kaila

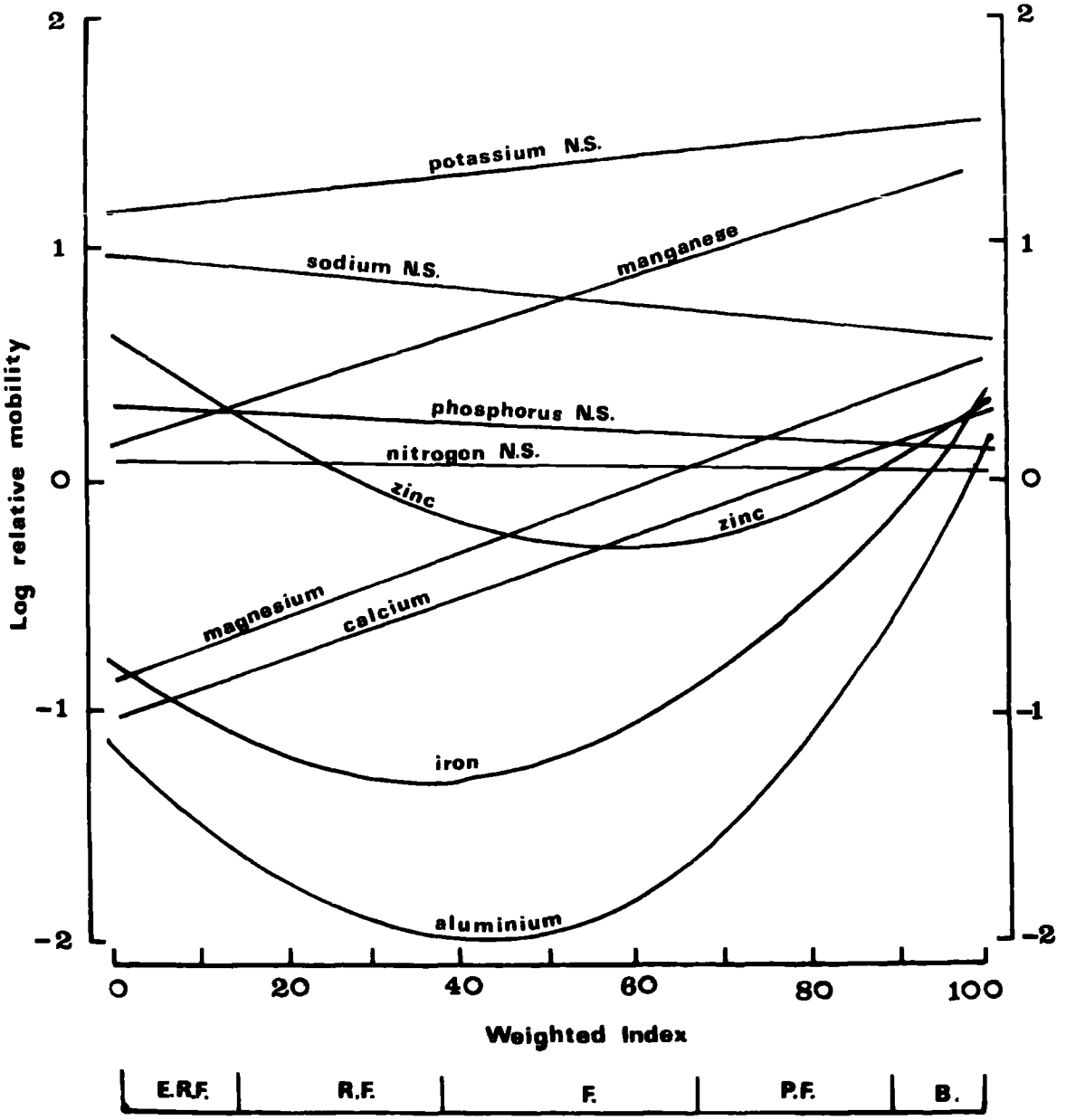


FIG. 50 RELATIVE MOBILITY OF VARIOUS ELEMENTS IN RELATION TO MIRE ECOCLINE. TRENDS SIGNIFICANT UNLESS OTHERWISE INDICATED; EQUATIONS FOR TRENDS IN TABLE A23. SEE TEXT FOR EXPLANATION.

and Kivekäs (1956), Malmer (1958), and Holmen (1964). Malmer arranged the concentration data from five mire communities into a phytosociological gradient, and records a decrease in concentration of both potassium and phosphorus towards the ombrotrophic end of the ecocline (Malmer o.c., Table 2). Nitrogen is also very highly concentrated into the vegetation, and, like potassium and phosphorus, shows a decline in concentration towards the ombrotrophic end; this decline is most dramatic in the case of phosphorus, which changes from being the most highly concentrated element in fens and rich fens, to being one of the least concentrated in bogs. The pattern for manganese is almost the reverse of that for phosphorus. Manganese is one of the least concentrated elements in richer parts of the ecocline, and the most concentrated (together with nitrogen) in ombrotrophic mires.

In short it appears that the available supply of phosphorus, potassium and nitrogen in rich mires may be limiting as compared with the supply of other elements; and in poor mires the same may be said of manganese, nitrogen, and possibly potassium. The word 'supply' has been underlined in the preceding sentence, in order to re-emphasise the point that problems of uptake into the vegetation have not been accounted for.

Another aspect of mineral cycling which is related to mobility is mineralisation. Once again it must be stated that absolute measurement of mineralisation requires detailed and specialised investigation. However, the difference between the concentration of an element bound into the organic part of the peat, and the concentration in the vegetation, does provide some measure of the comparative speed with which each element is cycled. Lack of information regarding the amount of each element bound into the living roots is, again, a source of error, as is the quantity of each element in peat in the inorganic form, whether introduced by ground-water, precipitation, or mineralisation. This second complication was

corrected by utilising information on the readily soluble quantity of each element in the peat. (i.e. soluble without acid digestion).

The index of mineralisation, I_m, was calculated as follows:

$$I_m = \log \left(\frac{\text{concentration of element in aerial vegetation}}{\text{total concentration of element in peat minus concentration in soluble extract}} \right)$$

All figures in p.p.m.

The log transformation is again merely for ease of presentation of results (in Figure 50). High values for the index indicate rapid mineralisation and/or high mobility; low values indicate slow mineralisation and/or low mobility.

The general level of the index I_m for both nitrogen and phosphorus is particularly interesting, because it shows that in spite of the fact that these two elements are highly concentrated into the aerial vegetation, the speed of mineralisation appears to be comparatively slow. Potassium sodium and zinc have the highest values for the index of mineralisation in rich mires, whereas in the poorest mires the values of potassium and manganese are about ten times greater than for any of the other elements. Iron appears to be more generally mobile than aluminium, which may reflect greater precipitation of aluminium in living root systems.

Both Figures 49 and 50 emphasise that ecological generalisations regarding classes of elements should be treated with caution: not only does the mobility of each element differ from most other elements at any one location, but trends of change along the mire ecocline are also different.

I hope that Figures 49 and 50 contribute to the general understanding of mineral mobility and cycling in open peatlands. However, the manner by which these figures were produced places severe contraints upon any

more detailed deduction. Although these figures provide only a starting point, I feel that development and improvement of the approach used in their production, may lead to a real understanding of mineral dynamics in relation to the ecology of mire ecosystems.

11(e) Summary of relationships between inorganic factors and the mire ecocline

Many inorganic aspects have been discussed in some detail throughout the second half of this thesis, and it will be appropriate to conclude with a general summary of the relationships between various inorganic factors and the ecocline. This summary is presented as a tabulation of the simple correlation coefficients, utilising the weighted index as a measure of ecological distance. From numerous factors dealt with in the preceding sections it is apparent that simple linear functions do not always adequately describe these relationships, with the phytosociological gradient so defined, however the coefficients are a useful quick guide to mire chemistry. The overall importance of the alkali cations is very clear. (Table 14).

The relative importance of various chemicals in relation to the ecocline is of greater interest than the simple correlations discussed above. To obtain information on this point a step-wise multiple regression was performed, using the weighted index as the dependent variable. The data in Table A20 suggest that the distribution of this variable approximates a normal distribution; and the independent variables were log transformed in order to improve linearity and homogeneity of variance. The objective of this exercise was to gain understanding of the chemistry of the coenocline, rather than the mire system as a whole, therefore this particular analysis was performed on the vegetation chemistry only. No significant partial regression coefficients were obtained for the 'rich

Table 14

Simple correlation coefficients between inorganic factors and the mire ecocline

Soluble in peat		Total in peat		Total in vegetation	
pH	-0.94	Ash	-0.79	Ash	-0.68
Ca	-0.80	Ca	-0.77	K	-0.64
NH_4^+	0.67	Co	-0.72	Ca	-0.55
NO_2^-	-0.66	Mg	-0.61	Mg	-0.49
P	0.47	Al	-0.52	Cd	0.37
Zn	0.41	Pb	0.49	Mn	0.37
Mg	-0.38	Ni	-0.47	Na	-0.37
K	0.37	Fe	-0.46	P	-0.28
Fe	-0.25	K	-0.32	Pb	0.23
Mn	-0.20	Mn	-0.32	Cu	-0.19
Na	0.16	P	-0.30	Al	0.19
Al	-0.13	Cu	0.19	Zn	-0.12
		Zn	0.14	Fe	0.10
		Cd	0.11	N	0.06
		Na	-0.09		
		N	0.07		

Notes: (1) negative correlation coefficient indicate that factor decreases towards the ombrotrophic end of the ecocline.

(2) Soluble NH_4^+ and soluble P are averages for the various extractants used.

Table 15

Relationships between elements in mire vegetation, and the mire phyto-sociological gradient

Factor	% reduction in variance	beta	F
	Entire mire		
Ca	55	-0.70	60***
Zn	15	0.37	20***
K	11	-0.37	18***
R^2 = 0.81***			
	poor mire only		
K	50	-0.58	10**
Mn	8	-0.27	3.1 NS
Ca	4	-0.22	2.2 NS
Pb	4	0.23	1.7 NS
R^2 = 0.65***			

Note a negative beta value indicates that the factor increases towards the ombrotrophic end of the ecocline.

mire only section of the analysis, therefore only the results for 'all mire' and 'poor mire only' are shown in Table 15. The most significant fact to emerge from this analysis is that although calcium is the major determinant when the mires are considered as a whole, potassium exerts the major influence when poor mires are considered alone. (Poor mires, as defined here, include almost all of what is usually considered fen to bog; the analysis was repeated including all of the sites in the fen-bog section of the ecocline, with no appreciable difference in the results). This change of influence is consistent with the ionic ratios, in so far as a change of predominating ion occurs at the weighted index approximately equivalent to poor fen: calcium dominating in the richer mire, and potassium in the poorer ones. This is expressed both in ratios for vegetation chemistry (Figure 48C) and exchangeable amounts in the peat (Figure 48B). No other factors are significant in the 'poor mire' section of the analysis, although in view of the discussion in Chapter 10 it is interesting to note that manganese accounts for 8% of the variance.

CHAPTER TWELVE :

GENERAL DISCUSSION

12(a) Nitrogen and phosphorus

One of the most interesting features which has emerged from the results presented in Chapter 9 is the fact that soluble nitrogen in peat increases along the ecocline in the direction of poor mires. I have discovered only three previous reports which consider soluble nitrogen in peat in terms of mire type: Pollett (1972) Malmer (1962a) and Kaila, et al. (1954).

Pollett condensed his data into only seven categories, and in those categories which contained what might be regarded true peats, there is no apparent trend in soluble nitrogen (Pollet, o.c. Table 1).

Malmer's work on the Åkhult mire included peatland which ranged from poor fen to bog, and, like Pollett, Malmer expressed his results in terms of volume. It is difficult to consider Malmer's results in relation to the mire gradient, because so few vegetation types were studied, but the average figure for the poorest zone in the Sphagnum cuspidatum - inundatum series (Eriophorum vaginatum) is 1.6 mol NH_4^+/l fresh peat, whereas the average figure for the richest zone (Menyanthes trifoliata) was 1.3 mol NH_4^+/l fresh peat, i.e. a slight decline in the rheotrophic direction. (By comparison the equivalent figures for approximately the same zones in the South German mires studied in this project are 1.0 and 0.5 mol/l fresh peat respectively).

Kaila et al. (1954) studied the mineralisation of nitrogen in some virgin peats from Northern Finland, and summarised the data simply in terms of bog and fen peats. Their results for soluble NH_4^+ - N are as follows: fens 0.11-0.25 mg/g dr. wt. and bogs 0.14-0.35 mg/g dr. wt. Their figures for

mineral nitrogen as a percentage of total nitrogen are fens, 0.6-1.2% bogs 0.7-1.7%.

The evidence from this project and the literature provides no reason to believe that bogs are substantially more deficient than fens in available nitrogen, indeed the results suggest that the reverse could well be the case. It follows that the term 'poor mires' when used with reference to nutrient supply in bogs c.f. fens does not apply to nitrogen. Kaila's group apparently came to a very similar conclusion regarding nitrogen, because they wrote: "The results reported in the present paper are not in accordance with the general opinion that fen peats are markedly superior to Sphagnum peats." (o.c. p. 93).

Further evidence of a possible surplus of soluble nitrogen in ombrogenic peat is shown by the extent to which nitrogen is concentrated into the vegetation, which is much less on ombrotrophic mires than on rheotrophic ones (Figure 52). This trend is simply a manifestation of the fact that soluble nitrogen is highest in ombrotrophic peat, whereas the nitrogen concentration of mire vegetation exhibits no significant trends (Figures 23, 24 and 26).

It is frequently stated in the literature that peats, especially bog peats, contain only small amounts of soluble nitrogen, but concentrations of more than 250 p.p.m. (dr. wt.) were obtained in this project, and by other workers quoted in Chapter 9. These values are much higher than those found in mineral soils: for example grassland soils rarely excede 5 p.p.m., and even the most fertile mineral soils rarely contain more than about 40 p.p.m. (Harmsen and Kolenbrander, 1965). Assuming that the total nitrogen content of the mineral soil is about 0.3%, this means that about 0.1% of the total nitrogen in mineral soils is in the soluble form, with about 1% in the most fertile soils. By comparison the percentage of the total nitrogen in peat which is in the soluble form is much higher,

some quoted figures are as follows: Kaila et al. (1953) up to 5%; Kaila et al. (1954) 0.6-1.7%; Malmer (1962) up to 3%; Saebo (1970) 2.5%; this project, 1-3%. All the evidence points to the possibility that, if anything, virgin peat soils are superior to mineral soils with regard to available nitrogen.

If nitrogen is ever a limiting factor to the growth of vegetation on virgin peats, Figure 52 suggests that this is more likely to occur in rheotrophic situations. There are certain obvious differences in the nitrogen budgets of bogs and fens: thus although ombrotrophic mires receive only a limited influx of nitrogen in precipitation, the loss through drainage into the groundwater is also very slow. By comparison rheotrophic mires have additional input from groundwater, however these mires also lose more soluble nitrogen to the groundwater, furthermore, the greater productivity of rich mires must also exacerbate any potential shortage by immobilising larger quantities of nitrogen within the living biomass. The greater microbial activity will accelerate the mineralisation of organic nitrogen, but a high level of microbial activity can itself give rise to problems of nitrogen shortage because bacterial protoplasm contains large amounts of nitrogen, possibly as much as 12% (Harmsen and Kolenbrander, 1965).

In view of the potential for nitrogen shortage which exists in rheotrophic parts of mire ecosystems, the abundance of heterotrophic nitrogen fixation in rheotrophic peat, as compared with the peat from poor mires, makes perfectly good ecological sense. There appears to be no evidence of excessive nitrogen shortage in ombrotrophic mires, therefore if widespread nitrogen fixation occurred in these situations it would be a redundant activity. Furthermore such activity in ombrotrophic peats could lead to a toxic accumulation of soluble nitrogen. In addition to direct toxicity, high concentrations of the NH_4^+ ion could add to the problems of

Fig. 51

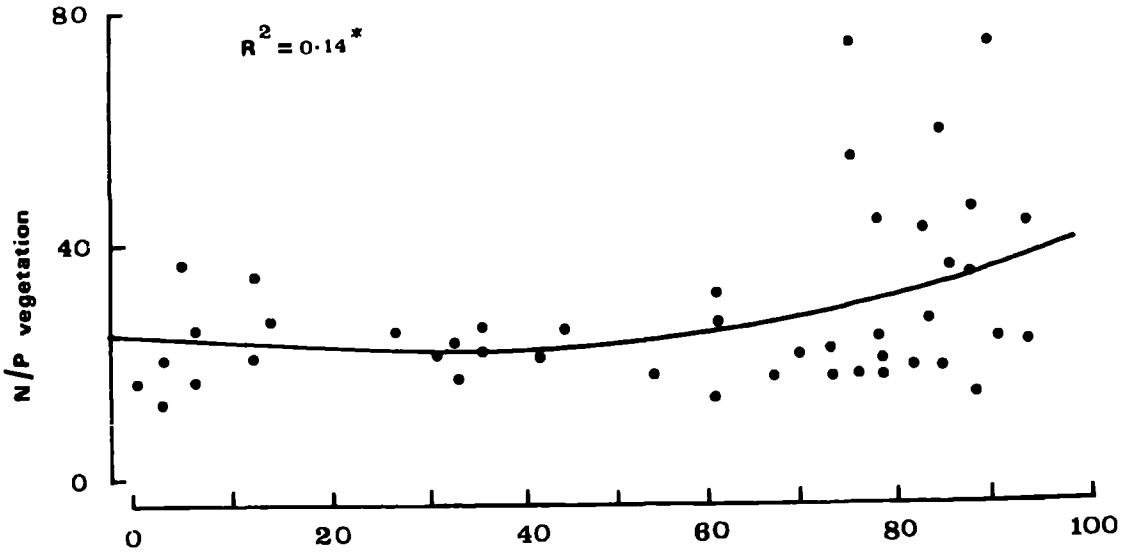


Fig. 52

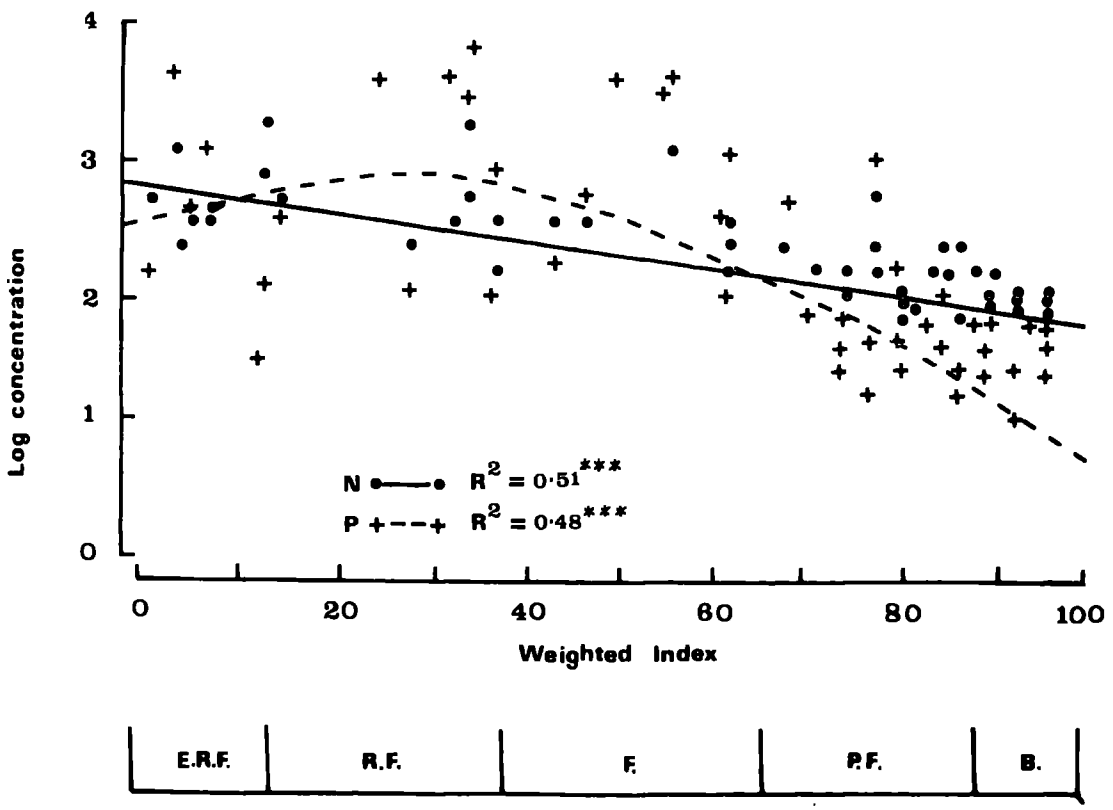


FIG. 51 N/P RATIO IN MIRE VEGETATION IN RELATION TO MIRE ECOCLINE. R^2 VALUE OF TREND SHOWN.

FIG. 52 RELATIVE CONCENTRATION OF NITROGEN AND PHOSPHORUS IN AERIAL VEGETATION IN RELATION TO MIRE ECOCLINE. R^2 VALUES OF TRENDS SHOWN. SEE CHAPTER 11 FOR DETAILS.

uptake of other cations, through competition for absorption sites on the roots. It is interesting to note that the correlation between nitrogenase activity in peat and the nitrogen content of vegetation in rich mires is much higher than the equivalent correlation in poor mires (r = 0.39 c.f. 0.11), although neither correlation is statistically significant.

As in the case of soluble nitrogen, the relative amounts of soluble phosphorus in peats from rich and poor mires, are inconsistent with the widely held view regarding this aspect of mire chemistry. If anything, this trend for soluble phosphorus is even more marked than the trend for soluble nitrogen. Once again the question of whether such a trend is a widespread feature of mire ecosystems must be examined. Although the literature contains more data on peat phosphorus than on peat nitrogen, the problem is that few workers have examined soluble phosphorus with respect to the phytosociological gradient of mires; comparison is further complicated by the fact that the phytosociological spectrum shifts with respect to pH along the NW-SW European axis. Another problem is created by different investigators using different units in which to express their results. I have attempted to overcome these difficulties by (1) expressing the results of an individual as a percentage of the maximum level of soluble phosphorus found by that worker, and (2) by ranking the data from peat beneath different plant communities according to the pH of the peat: pH being the best chemical indication of the phytosociological gradient. The collated data are presented in Figure 53, where the richer mires are represented on the left of the diagram, and poor mires on the right. Two of the investigators obtained relatively high figures in extreme rich fens, but overall the trend towards higher levels in poorer mires is quite clearly seen.

Comparison of the actual amounts of soluble phosphorus found in peat by different workers is even more difficult than comparison of soluble

Fig. 53

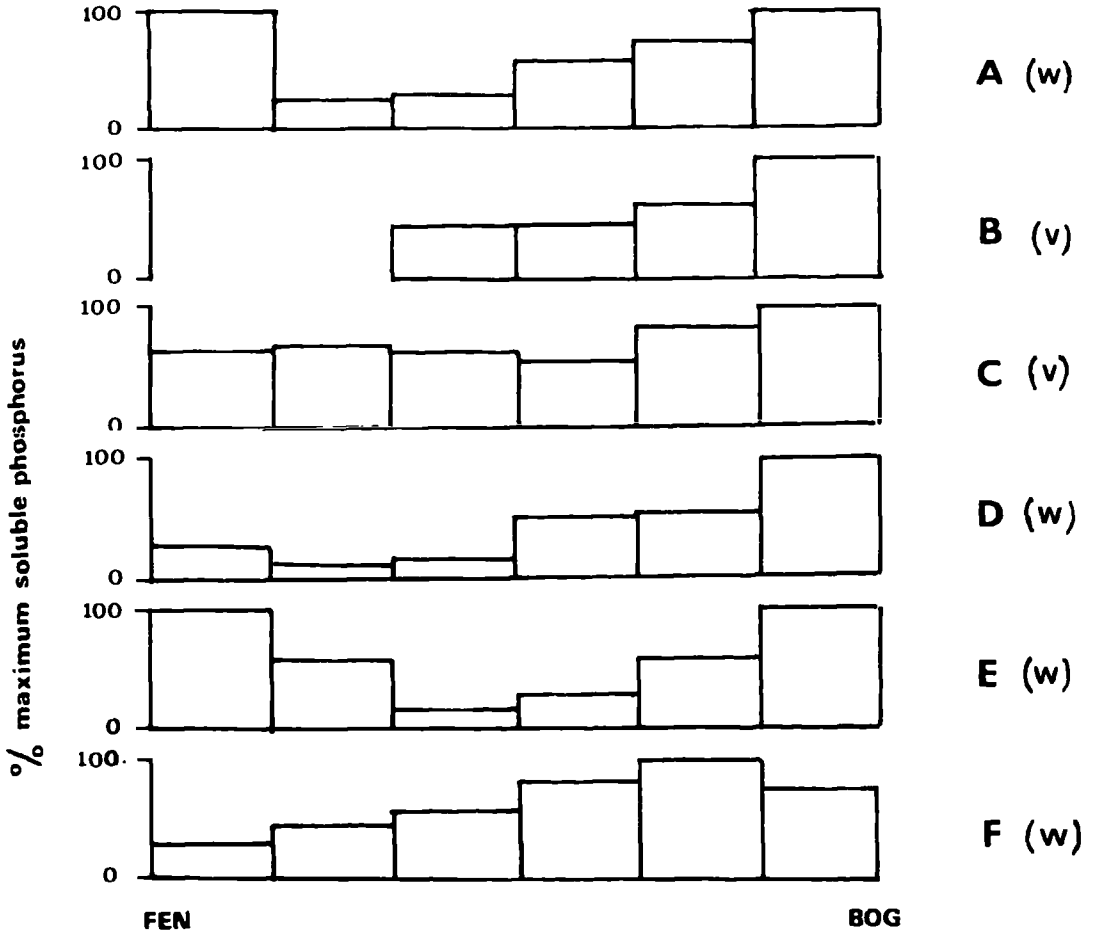


Fig. 54

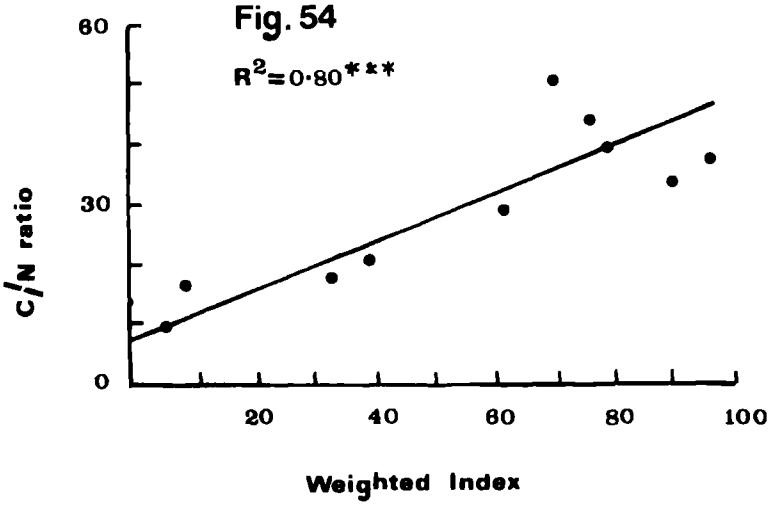


FIG. 53 COLLATION OF DATA FOR EASY SOLUBLE PHOSPHORUS IN PEAT IN RELATION TO MIRE ECOCLINE. RANKING ACCORDING TO pH, SEE TEXT FOR DETAILS. A, POLLETT (1972); B, MALMER and SJÖRS (1952); C, KURKI (1972); D, KAILA (1956a); E, KAILA (1956b); F, HOLMEN (1964). (w) per unit weight; (v) per unit volume.

FIG. 54 C/N RATIO IN PEAT IN RELATION TO MIRE ECOCLINE FROM PFRULE MOSS 1972.

nitrogen, because in addition to the problem of units, there is a multitude of extracting fluids. However, with this point in mind, the following figures provide some information regarding the level of soluble phosphorus in widely separated peatlands: Scotland, 20 p.p.m. (Reith and Robertson, 1971); Canada 30 p.p.m. (Pollett, 1972); Sweden 64 p.p.m. (Holmen, 1964), 50 p.p.m. (Sjörs, 1961); Finland, 19 p.p.m. (Kaila, 1956b); Borneo, 20 p.p.m. (Waughman, unpublished). (Note, some of the above concentrations are averages of several figures in the quoted publication, and in certain instances I have converted the results from the original units into p.p.m.). These figures are remarkably similar to each other, and to the results presented in Chapter 10. John (1972) examined soluble phosphorus in 360 different mineral soils, and found that the average value for NH_4Cl soluble phosphorus was 0.41 p.p.m., which is much lower than any of the above figures. Normal agricultural soils have been classed according to soluble phosphorus contents (Pizer, 1965), and the levels of soluble phosphorus found in peat soils would place them in the 'high' to 'very high' categories.

The aforegoing discussion was based upon chemical methods, and it can be argued that the figures obtained using chemical extractants do not truely reflect amounts available to plants. The problems of availability and absorption into the vegetation are discussed below, but for the moment suffice it to say that these widely used chemical methods do not provide any evidence to support the contention that the supply of phosphorus in ombrotrophic mires is lower than the supply in either rheotrophic mires, or mineral soils: they do in fact suggest that it is better.

The phosphorus content of mire vegetation is highest in rheotrophic sites, although it appears to decline very slightly towards extreme rich fens (Figure 38), this may be due to the very high productivity of some of these communities, together with a reduction of available phosphorus through

fixation at the high pH values. The decline of phosphorus in vegetation along the ecocline from fens towards bogs is much more marked, and this trend is the reverse of that for soluble phosphorus in peat, which increases from fens towards bogs.

At this point a reasonably clear picture of the distribution of nitrogen and phosphorus in relation to the main chemical gradient of mire ecosystems can be produced. In order to discover the relative extent to which either of these two elements might limit the growth of vegetation on peatlands, it is necessary to examine this picture in the light of information which has been obtained from various types of experimental investigation. There is a vast literature on the cultivation and fertilisation of peatlands for forestry and agriculture, however as this is not the place for a comprehensive survey, I will quote just a few pertinent examples.

Rieth and Robertson (1971) found that both nitrogen and phosphorus improved the yield of grass on deep oligotrophic peat in Scotland, but they also noted that nitrogen was not essential for establishment of the sward. O'Toole (1965) applied nitrogen fertiliser to Irish blanket peat, and observed that although rye grass responded to this treatment, the native bog species did not. Ferda (1962) cultivated three different conifer species on poor fen soil (pH 5.3), and found that application of both potassium and phosphorus was beneficial, but that nitrogen produced no improvement. The requirement for phosphorus and potassium in cultivated peats is widespread in Finland, although the need for nitrogen is not so great (Pessi, 1973). Nitrogen applications had no effect on either sown grass or native species on moorland peat in Southern Ireland (Murphey, 1960), but sown grasses did respond to phosphorus. Murphey (o.c.) noted that the application of phosphorus fertiliser actually depressed the frequency of some native species, however this was probably an indirect

effect of competition from sown grasses rather than a direct chemical response.

Chemical fertilisation is generally acknowledged to be necessary for successful crop production on blanket peat, however there are certain aspects of the work of Grennan and Mulqueen (1964) which, in the present context, deserve close attention. Their experiments showed that phosphorus application was essential if crops were to be established on blanket peat, and that if fertilisation was stopped, native species re-established themselves. These native species included Schoenus nigricans and Monilia caerulea, plants found on bogs in Ireland, although more characteristic of fens and poor fens in other parts of Europe. The main point of interest, however, is what happened in the control plots. In the plots with added lime but no phosphorus, only Schoenus appeared, but where a light dressing of phosphorus was applied in addition to the lime, Monilia appeared. Thus it seems that of these two native species, the one with the greater phosphorus requirement was the one which in most parts of Europe grows in the more oligotrophic situations.

Most of the above studies have been performed for purposes of agriculture or forestry, thus requiring drainage and other forms of ground preparation, which complicates extrapolation to the natural ecosystem. Unfortunately, there have been very few experiments directed towards understanding peatland as a natural ecosystem. One such experiment was carried out on poor mire vegetation dominated by Eriophorum vaginatum (Tamm, 1954), from which it was concluded that a shortage of phosphorus was limiting growth of the vegetation. However the design of this widely quoted experiment gives rise to certain problems of interpretation. For example Tamm applied all, minus one, or minus a few (details unspecified) elements to each of 15 plots, the elements included P, N, Ca, Mg, K, Mn, Fe, S, Cu, B, Zn and Mo. With only one level of treatment this would require

a 2^{12} factorial, or 4096 treatment combinations; even with confounding and factorial replication it is difficult to see how this was reduced to 15 plots! But perhaps a more serious criticism arises from the fact that nitrogen and phosphorus were applied in approximately equal amounts, whereas the N/P ratio of the vegetation was about 23. In fact only $\frac{1}{15}$ of the phosphorus fertiliser was utilised compared with $\frac{1}{3}$ of the nitrogen, and $\frac{1}{4}$ of the potassium: on this basis it might be concluded that the vegetation had a greater requirement for the two latter elements. In such experiments it is wiser to apply the elements in a ratio which bears some resemblance to that in the peat, or in the vegetation.

Gore found that neither treatment with calcium, nitrogen nor phosphorus stimulated the growth of Monilia caerulea when grown on ombrotrophic peat, although in one series of experiments higher levels of both nitrogen and phosphorus were present in the tissues of the treated plants (Gore, 1961a, b).

The insectivorous plants which are commonly found growing in ombrotrophic mires, have been taken by many observers to indicate a shortage of nitrogen in such locations; the fact that many plant species on bogs exhibit xeromorphic features has also been attributed to nitrogen deficiency (topic reviewed by Saebø, 1970). But insects also contain phosphorus, and as a result of both survey and experimental studies, Beadle (1966) concluded that xeromorphic characteristics of certain Australian species were due to phosphorus shortage.

The N/P ratio in rich peats is about 29/1, and in peats from ombrotrophic mires about 37/1 (data in Appendix), for mineral soils the ratio is nearer 10/1 (Sjörs, 1961, Tables 1-3). These ratios provide no indication of nitrogen shortage in mire ecosystems, especially in ombrotrophic situations.

All of the data and evidence discussed so far in this chapter can

be summarised as follows. (1) The supply of nitrogen to open virgin peatlands is less likely to be a factor limiting vegetation growth than the supply of phosphorus; in fact the surplus of soluble nitrogen in ombrotrophic peats, and the abundance of biological nitrogen fixation in rheotrophic ones, suggest that in most parts of the open mire ecosystem the supply of nitrogen is adequate for the demands of the vegetation. (2) Phosphorus may be deficient in richer mires, but the comparatively high levels of soluble phosphorus in poor mires indicates that the supply per se of phosphorus in poor mires is adequate. However, many trials have demonstrated that the supply of phosphorus in most peats is not adequate for cultivation purposes, furthermore the lower concentrations of phosphorus in bog vegetation, as compared with the phosphorus content of vegetation in richer mires, suggests that vegetation in ombrotrophic situations may encounter problems of uptake with this element (see next section).

12(b) Phosphorus uptake by mire vegetation

In Figure 52 it can be seen that phosphorus is more highly concentrated into the vegetation of rich mires than is nitrogen, but along the gradient in the direction of poor mires this changes, until in extreme ombrotrophic mires phosphorus is considerably less concentrated than nitrogen. This trend is based upon the amounts of phosphorus in vegetation and in soluble extracts from peat at each site. Thus the decline of phosphorus concentration at the ombrotrophic end of the gradient is seen to be due to both a reduction in amount of vegetation phosphorus, coupled with an increase in the amount of soluble phosphorus in peat (Figures 33, 35, 38 and 53). Under these circumstances it must be concluded that the lower concentration of phosphorus in ombrotrophic vegetation of virgin mires is a result of limitations on uptake, rather

than limitation of supply.

Phosphorus is known to accumulate in water-logged mineral soils (Glentworth, 1947; Paul and De Long, 1949), but such accumulation has been attributed to microbial fermentation (Gasser and Bloomfield, 1955). It is unlikely that fermentation is responsible for the accumulation of phosphorus in ombrotrophic peat because the level of microbial metabolism in such peats is very low: the small number of nitrogen fixing bacteria (Tables A16 and A17), the increase in C:N ratio (Figure 54) and the small amounts of nitrogenase activity, all indicate a low level of microbial activity in ombrotrophic peat. Indeed, this low intensity of ecosystem metabolism may be another manifestation of the factors which restrict phosphorus uptake into poor mire vegetation.

The possibility of the phosphorus concentration in vegetation being determined by certain other elements has been discussed in Chapter 10, and the decline in vegetation phosphorus along the mire ecocline in the ombrotrophic direction may, in part at least, be due to concomitant changes in these other elements. However, there is one very important feature of the peatland ecosystem which has not been included in this study, but which might exert some indirect influence on the uptake of phosphorus: this is waterlogging, or more precisely, the decrease in water movement which is a chracteristic trend of the gradient from rheotrophic to ombrotrophic situations. In his studies of two perennial grasses Humphries (1962) found that waterlogging restricted the uptake of phosphorus to a greater extent than the uptake of nitrogen. Greenhouse experiments have demonstrated that the bog plant Eriophorum vaginatum grows better in water-logged conditions than the more rheotrophic species Monilia caerulea (Gore and Urquhart, 1966), although the differences in performance were not matched by differences in phosphorus content of the plants. By contrast Armstrong and Boatman (1967) observed that stagnant conditions caused Molinia

plants to be stunted by comparison with plants growing where water movement occurred, moreover this stunting was accompanied by a great reduction in the phosphorus content of the leaves. Armstrong and Boatman suggested that uptake of phosphorus might be physically curtailed by the accumulation of ferrous sulphide which surrounded the roots. McVean (1959) found that alder seedlings, and numerous native species, made a better response to the application of phosphate fertiliser in sites on blanket bog where there was some influence of moving ground water. Finally it might be mentioned that anion uptake by rye grass in liquid cultures can be increased as much as twenty times merely by stirring the medium (Olsen, 1953).

Moving water in peatlands, has a variety of influences, amongst the most important of which are: on pH (Bellamy, 1968); on the removal of toxic substances (Rutter, 1955); on the nutrient budget; and on redox. It is perhaps significant that nitrogen in peat is in the cationic form whereas phosphorus is anionic, and in conditions of reduced oxygenation which accompanies the diminished ground water movement in ombrotrophic peats, a more restricted uptake by vegetation of anionic phosphorus compared with cationic nitrogen, is just as would be predicted by the theory of anion respiration propounded by Lundegårdh and others (e.g. Robertson and Wilkins, 1948; Lundegårdh, 1952; Burström, 1957). This theory states that cation accumulation by plant roots proceeds largely without the expenditure of energy, whereas anion accumulation requires both energy, and the oxidation of a carrier near to the cell surface. The theory also requires an oxidation gradient, with the highest partial pressures of oxygen on the outside of the root. Accordingly, mire plants would experience greater problems of anion uptake from peat in the more stagnant ombrotrophic situations, as opposed to uptake from rheotrophic peats where ground water movement provides some degree of aeration.

Armstrong (1964) demonstrated that the roots of bog plants release oxygen into the surrounding growth medium. This would help to create the oxygen gradient required by the theory described above; furthermore roots of the more rheophilous Monilia released about 15 times less oxygen than either Menyanthes trifoliata, or Eriophorum angustifolium which have modal positions in a considerably more ombrotrophic part of the ecocline than Monilia (Figure 16). It would be very interesting to know whether or not high rates of oxygen diffusion from roots is a widespread feature of species comprising ombrotrophic vegetation.

The details of Lundegardh's theory were, and to some extent still are, matters of contention. In its original form it is no longer considered acceptable, however in his 1972 review Epstein observed that certain essential features of the original hypothesis have been retained by all subsequent changes: notably that there is charge separation, associated with which is a process of oxidation, and that only one ion is moved by active transport (Epstein, 1972, p. 325). It is generally accepted the anion uptake is demanding of energy, involving oxidation either in the form described above, or in oxidative phosphorylation (Jackson et al., 1962).

It is likely that the lower levels of phosphorus in vegetation of poor mires are due to a general reduction of metabolic activity though the lack of oxygen and/or the lack of an adequate supply of various metal cations (e.g. Calcium Magnesium etc.) which are necessary if metabolic activity is to be maintained at a high level. The data indicate that the supply of phosphorus to ombrotrophic mires is adequate, therefore such terms as 'poor mire' and 'oligotrophic' with reference to peatland nutrition should be used carefully as they appear inapplicable to both nitrogen and phosphorus.

12(c) a posteriori note on statistics

A brief note on the statistical problems encountered in this project was given in Chapter 7. With the various multiple regression analyses completed, the computer provides a very painless way to validate the results, by examination of residuals. (A residual is the difference between the measured value of a predicted variable, and the value predicted by the regression equation). If probabilities based upon the normal distribution are to be applied to statistics produced by regression analysis, e.g. beta, R^2, then certain assumptions concerning the error component must be satisfied. The errors must be independent, have a normal distribution, a mean of zero, and be of constant variance throughout the whole range of values of the independent variable. If any of these assumptions are seriously violated then the application of probability levels placed upon the normal distribution is innapropriate.

The residual analysis is an indirect way of testing the above requirements. Residuals from each of the analyses were examined, and in every case they proved to be satisfactory. Two examples of the distribution of residuals are shown in Figure 55: for the phosphorus content vegetation as predicted by other elements in vegetation, and nitrogenase activity as predicted by soluble chemicals in peat. Moving averages and histograms have been superimposed onto these diagrams in order to aid interpretation. In both cases logarithmic transformation of the dependent variable was necessary. In the case of phosphorus the transformation has improved the normality of the distribution of residuals, and stabilised the variance. For nitrogenase activity the normality is marginally improved by the transformation, but two more serious violations of the assumptions have been completely corrected: (1) the variance has been stabilised and (2) the linear relationship between the dependent variable and size of residuals has been eliminated.

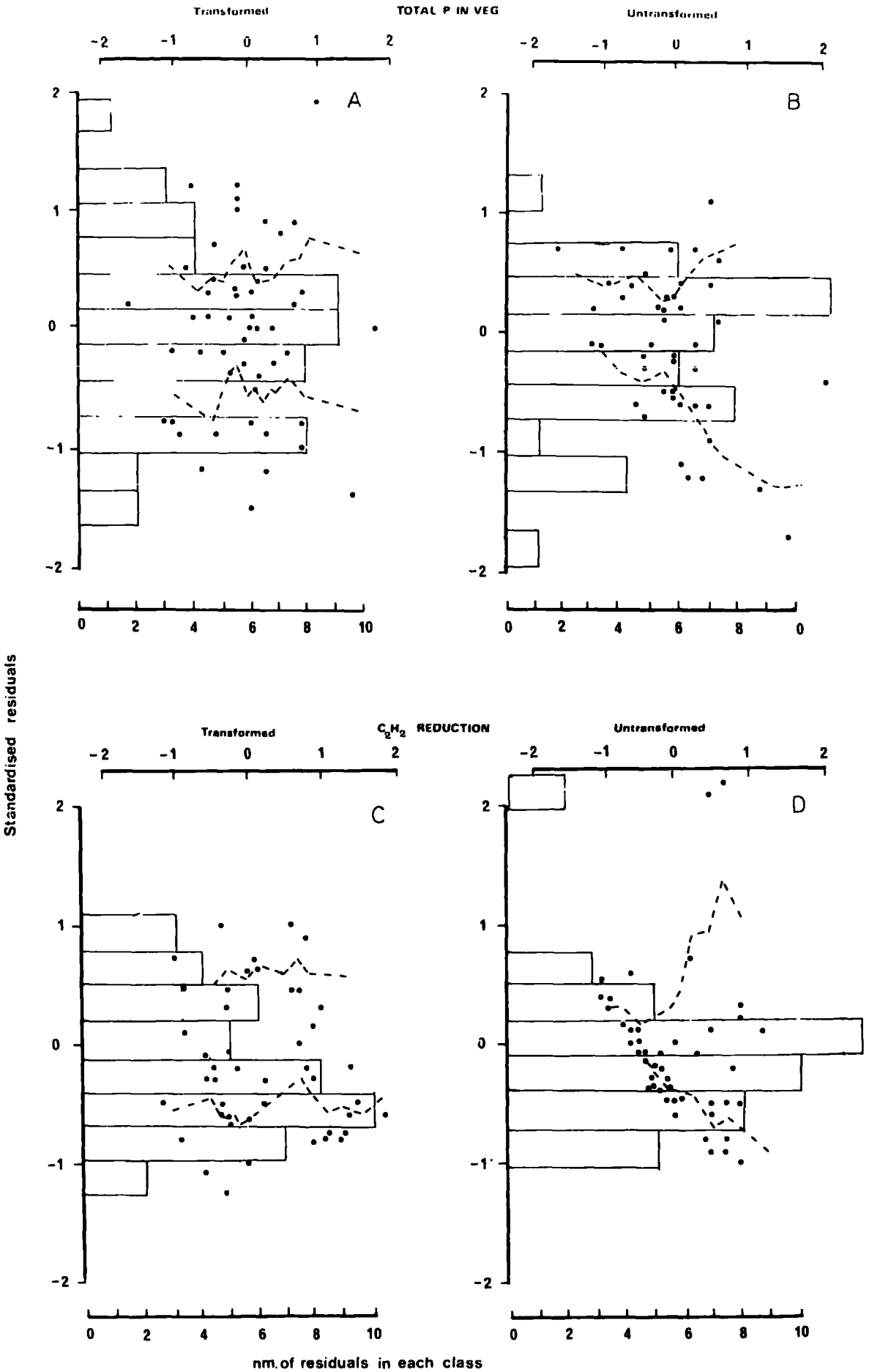


FIG. 55 PLOTS OF RESIDUALS (STANDARDISED) AGAINST PREDICTED DEPENDANT VARIABLE (STANDARDISED) FOR PHOSPHORUS IN VEGETATION AS PREDICTED BY SOLUBLE CHEMICAL FACTORS IN PEAT (A AND B), AND ACETYLENE REDUCTION AS PREDICTED BY SOLUBLE CHEMICAL FACTORS IN PEAT (C AND D), USING UNTRANSFORMED AND LOG TRANSFORMED DEPENDANT VARIABLES. THE DISTRIBUTION PATTERN OF RESIDUALS HAS BEEN EMPHASISED BY A HISTOGRAM, AND THE RELATIONSHIP BETWEEN THE SIZE OF RESIDUAL AND THE PREDICTED DEPENDANT VARIABLE BY A MOVING AVERAGE (BROKEN LINE).

Figure 55 illustrates problems frequently encountered during the analysis of ecological data, especially the point relating to stability of the variance. Three observations emerging from the above analysis might usefully be stated. (1) A preliminary plot of the figures for the dependent variable in histogram form, provides a useful indication of the distribution of the data. (2) Logarithmic transformation should, perhaps, be carried out more often prior to the analysis of ecological data, because it frequently improves the normality of the distribution, stabilises the variance, and gives rise to a more nearly linear relationship between the dependent and independent variables. (3) Values of statistical probability quoted in ecological reports involving data whose distribution has not been examined, should be treated with caution.

12(d) Factors influencing heterotrophic nitrogen fixation in peat

The results presented in Chapter 8 provide a strong indication that potassium may be an important chemical factor influencing the rate of bacterial nitrogen fixation throughout the entire peatland ecosystem. There are basically two ways in which this element might affect the rate of activity. (1) By satisfying some direct, or closely related requirement of the nitrogenase enzyme complex, or (2) by generally stimulating the metabolism of either or both the micro-organisms and the higher plant populations, hence controlling the availability of energy to the nitrogen fixing organisms.

A large potassium requirement has been demonstrated for Azotobacter, and the depletion of soil potassium by legumes is significantly greater than by other crops (Gukova and Bogomolova, 1963). Roberts and Olsen (1942) carried out trials on land fertilised with both potassium and phosphorus, and found that only potassium was effective in stimulating nitrogen fixing activity. Wilson and Wilson (1933) found that the growth

of Azotobacter in peat soils of New York State was stimulated by phosphorus, but only in the presence of large quantities of potassium.

Thus there is some evidence that nitrogen fixing organisms may have a specific potassium requirement. Results of the various multiple regression analyses which have been reported in the preceding chapters, repeatedly demonstrate the importance of potassium in ecosystem metabolism. A deficiency of potassium in soil is probably more widespread than is fully appreciated. Barber, et al. (1963) carried out a survey of the concentrations of elements in wheat grown in the North Central U.S.A., and found that the concentration of potassium was about 10 times greater than any of the other major nutrients. Barber (1962) concluded that the supply of potassium and phosphorus in soils is generally so low that these two elements are likely to be depleted in the zone immediately surrounding the roots, which is probably a region of intense nitrogen fixing activity. Figure 50 shows that potassium is the most mobile of all major elements in mire ecosystems, and Figure 49 shows it to be one of the elements most highly concentrated into vegetation. Nitrogen fixation is one, probably of many, metabolic activities whose rate is influenced by the relative scarcity of potassium in mire ecosystems.

Magnesium is much less important than potassum with regard to nitrogenase activity (Table 6), however it is interesting to note that magnesium does appear to have a small, but statistically significant influence in the poor mires. Jensen (1954) observed that Azotobacter required more magnesium when grown in the presence of high concentrations of inorganic phosphorus. In previous sections it has been shown that relatively high levels of soluble phosphorus are characteristic of poor mires (c.f. rheotrophic mires), so the general decline in magnesium towards the ombrotrophic end of the mire ecocline may give rise to some deficiency with respect to nitrogen fixing organisms, a deficiency

exacerbated by the soluble phosphorus.

Calcium is essential for all living organisms, however the statistical analysis (Table 6) indicates that this element exerts a negative influence on the rate of bacterial nitrogen fixation in peat. Calcium contributes to the explained variance in all sections of the analysis, but its main effect is clearly in rich mires, and this may account for the slight decline of nitrogenase activity in extreme rich fens (Figures 18-20), because these sites contain very high levels of exchangeable calcium (Figure 40B). The high F value for its partial regression coefficient suggest that at least part of the effect of calcium may be direct. A possible interaction with phosphorus was suspected, but statistical test provided no support for this idea (the simple r value for calcium on nitrogen fixation is 0.56, with the effect of phosphorus removed r = 0.55). The possibility that the effect of calcium was simply a reflection of the high ash content was similarly eliminated.

Azotobacter from non-saline soils are sensitive to high salt levels, and concentrations greater than about 1% are known to inhibit their development (Babak, 1966). Mire water contains much less than 1% calcium, but values for exchangeable amounts show that in the immediate vicinity of peat colloids, the concentration of calcium can be considerably greater than 1%. Wilson and Wilson (1933) found that large amounts of $CaCO_3$ in peat prevented growth of Azotobacter, although they attributed the effect to interaction with phosphorus.

In view of the evidence it may be concluded that the decline in nitrogenase activity towards the extreme rich fen part of the mire ecocline is due to high salt levels, of which calcium is the most abundant; furthermore it appears that much of the influence of calcium is through direct inhibition of the nitrogen fixing organisms. This does not preclude

the possibility that the influence of calcium on nitrogen fixation in peat also operates via other elements, but simply that any such interactions are beyond the limits of detection by the methods of analysis used.

The negative partial regression coefficient for the influence of NH_4 - N in poor mires (Table 6) has an F value which is just below the biologically acceptable level of statistical significance (in fact $p = 0.08$). In 1893 Winogradsky reported that the biological fixation of gaseous nitrogen was reduced by inclusion of an inorganic nitrogen source into the bacterial growth medium. Recently this effect has been attributed to prevention of enzyme production, rather than inhibition of enzyme activity per se (Daesch and Mortensen, 1968). Reduced nitrogenase activity in the presence of NH_4^+ - N has also been demonstrated using the acetylene reduction assay (Brouzes and Knowles, 1971). It has been estimated that the assimilation of gaseous nitrogen by bacteria ceases when the ratio of available carbon to available inorganic nitrogen is less than about 150:1 (Willis, 1934). The soluble amounts of carbon in the peat were not estimated during this project, however Kaila et al. (1954) measured the soluble organic content of some Finnish peats, and their figures can be utilised to estimate a soluble C:N ratio. They found an average of 2.8% water soluble organic matter in the peats, with no significant differences between fen and bog peats (o.c. Table 2). If it is assumed that all of the soluble organic matter was available carbon, then a combination of this information with that on soluble nitrogen presented in Chapter nine, indicates a C:N ratio ranging from about 700:1 in rich fen peats to about 140:1 in bog peats. (Not all of the soluble organic material is available carbon, therefore both of these estimates are probably too high). Taking all the above facts into consideration it can be concluded that the decline of nitrogenase activity towards the

ombrotrophic end of the mire ecocline might, in part, result from the relatively high levels of NH_4^+ - N which occur in these peats. If the rate of heterotrophic nitrogen fixation in ombrotrophic peats took place at a rate similar to that found in rheotrophic peats, then this, in combination with other aspects of the nitrogen flux discussed in Section 12(a), could give rise to toxic levels of nitrogen in the system. Thus the trends of soluble nitrogen and nitrogenase activity in relation to the ecocline may well reflect part of the evolved homeostastis of the system as a whole.

The accumulated information regarding the effect of phosphorus on nitrogen fixation in mineral soils, suggests that a strong relationship between these two factors might be expected to exist in peat soils. The data presented in Chapter 8 and 10 show that this is not the case. The few previous attempts to investigate the relationship between phosphorus and nitrogen fixation in peat, have reached much the same conclusion. Kaila (1954) found the presence or absence of Azotabacter in Finnish peat was not dependent upon the level of acetic acid soluble phosphorus. Wilson and Wilson (1933) did not achieve any improvement in the growth of Azotobacter by the addition of phosphorus, that could not also be achieved by treatment with dilute acids. Vandecaveye (1932) demonstrated that the addition of superphosphate to peat neither raised the general level of metabolic activity, nor increased the count of bacteria. Considering that the trend of increase in soluble phosphorus in the peatland ecosystem is, like soluble nitrogen, in the same direction as the general decrease in nitrogenase activity, it must be concluded that there is no general shortage of phosphorus with respect to nitrogen fixation in peat.

Wallace (1961) stated that the mobility of iron is reduced in the presence of phosphorus, and from a very detailed experimental study Dolar and Keeney (1971) obtained negative partial regression coefficients for

the influence of phosphorus on the availability of trace metals in soils. Dolar and Keeney concluded that soluble phosphorus caused a significant reduction in the availability of copper, zinc and manganese. The negative partial regression coefficients for the influence of phosphorus on nitrogen fixation are not statistically significant (Table 6), although the percentage variance explained by phosphorus in rich mires is fairly large (13%). Iron explains about 5% of the variance; the partial regression coefficients of this element are positive, and, like those of phosphorus, statistically insignificant. The simple correlation coefficient between iron and nitrogenase activity for the entire mire is 0.41, the partial correlation coefficient for this relationship with the influence of phosphorus removed is 0.26. These inferential statistics are merely an aid to understanding multivariate interactions, but as such, these figures do not preclude the possibility that the negative influence of phosphorus on heterotrophic nitrogen fixation in peat, is a result of interaction with iron, and other essential trace metals.

The importance of pH as a determinant of nitrogenase activity, is second only to potassium (Table 6), however the influence of pH is a feature of poor, rather than rich mires where it appears to be totally unimportant: the simple correlation coefficient between pH and nitrogenase activity in rheotrophic peats is only -0.18. The relative size of the F values for the pH partial regression coefficients indicates that a considerable proportion of the effect of pH is indirect, resulting from its influence on other variables, as might be expected. Several workers have suggested that in very acid mineral soils, aluminium might inhibit free living bacterial nitrogen fixation (e.g. Kaila, 1954; Gromyko, 1963). The analyses reported in Chapters 8 and 11 provide no support for this possibility in peats, in Figure 46B it can be seen that acetic acid soluble aluminium in peat does in fact decrease significantly in very acid

conditions.

12(e) Nitrogen fixation and hydrogen ion concentration

Bacterial nitrogen fixation was not detectable in the most acid peats, but considerable activity was measured in peats with reactions as low as pH 3.8, which is particularly interesting when it is realised that bacterial nitrogen fixation is considered to be uncommon in soils with pH values lower than about 5.5 (Mishustin and Shil'nikova, 1971). However, there are several reports which, together with the findings of this project, indicate that peat soils as a group are exceptions to this rule. Vandecaveye (1932) isolated Azotobacter from virgin peat soils with reactions of pH 4.3, and Valvulo (1958) found Azotobacter to be widespread in peat bogs of Bellorussia with pH values varying between 4.8 and 4.9; Boyd and Boyd (1962) isolated the acid tolerant A. indicus from arctic peat with a pH value of 4.1. Kaila (1954) failed to detect any aerobic bacteria in Finnish peats, but recorded significant nitrogen gains in aerobically incubated peat samples with pH values of 4.3. After investigating a wide range of peats from New York State, with reactions varying between pH 3.6 and pH 7.6, Wilson and Wilson (1933) concluded that the distribution of Azotobacter in these peats was not related to the pH value.

Clostridium spp. are considered to be generally more acid tolerant than Azotobacter, and there are numerous reports of this genus being isolated from organic soil (e.g. Boswell, 1955; Valvulo, 1958; Jurgensen and Davey, 1971; Granhall and Selander, 1973).

Certain observations on nitrogen fixing bacterial in relation to the water content of soil are particularly interesting in respect of acid tolerance. In acid mineral soils of Southern Australia it has been found that an increase in the moisture content of soil promotes improved

development of both Azotobacter and Clostridium (Swaby, 1939; Jensen, 1940) and similar results have been obtained for the acid soils of Queensland (McKnight, 1949). Meiklejohn (1962) was able to isolate Azotobacter from acid soils in Ghana, but only in the rainy season. Thus it is possible that the widespread occurrence of nitrogen fixing bacteria or bacterial associations in very acid peats, may be related to the moist nature of these environments.

12(f) Blue-green algae in peatlands

There are very few reports of blue-green algae in mires, and the tests reported in Chapter 4 provide no evidence for the possibility of light induced autotrophic nitrogen fixation in peat. Two extensive surveys of peat bog microbiology report bacteria, fungi and actinomycetes, but make no mention of blue-green algae (Valvulo, 1958; Burgeff, 1961). Recent reviews of nitrogen fixation conclude that blue-green algae are generally intolerant of acid conditions (Stewart, 1966; Jurgensen and Davey, 1970; Mishustin and Shil'nikova, 1971), and some investigators have concluded that blue-green algae are of little importance in soil nitrogen budgets (e.g. Lund, 1947; Beck, 1968).

Blue-green algae are important in many aquatic situations, and this group may be significant during certain phases of the hydrosere, as such their potential role in mires is probably worthy of closer examination. Certainly blue-green algae do occur in mires, this fact has been demonstrated by workers whose specific objective was the examination of algae, in contrast to the more general micro-biological surveys quoted above (e.g. Flensburg, 1967; Flensburg and Malmer, 1970; Dooley and Houghton, 1973; Granhall and Selander, 1973; Flensburg and Sparling, 1973). Dooley and Houghton (o.c.) concluded that nitrogen fixation by blue-green algae in low level Irish blanket bog was insignificant compared with bacterial

fixation; they reported the complete absence of blue-green algae below pH 5.4. Granhall and Selander (1973) who investigated a sub-arctic 'mixed mire', considered both heterotrophic nitrogenase activity in the drier parts of the mire, as well as nitrogen fixation by blue-green algae in the wet mineral rich depressions to be important; as might be expected, the photo-autotrophic activity decreased rapidly below the top 2-3 cm of the substratum. (A mixed mire describes a skeletal peatland comprised of a mosaic of ombrotrophic hummocks, and mineral rich depressions).

The environmental requirements of blue-green algae probably preclude any significant role in the nitrogen balance of peatland ecosystems as a whole, however they may be locally important in open pools, and skeletal mires.

12(g) Symbiotic nitrogen fixation in peatlands

Most of the investigations reported in this thesis were concerned with non-symbiotic nitrogen fixation, however symbiotic associations of higher plants with micro-organisms do occur, and are undoubtedly of local importance. Apart from the two well known associations involving Alnus and Myrica, micorrhizal relationships are widespread amongst the mire ericads. The possibility that these associations might have a nitrogen fixing function was investigated by Marthaler (1939), but his results were negative, as were those of a more recent study of the same topic by Burgeff (1961).

Less well known are the small legume like root nodules (usually referred to as swollen roots) which are found on many members of the Cyperaceae, including Cladium, Schoenus and Carex. Some of these nodules do appear to contain bacterial like inclusions (Davies et al., 1972). I have examined the root nodules of several of these species, but in all

instances the tests for nitrogen fixation were negative. Thus, although associations between micro-organisms and higher plants are very common in mires, only those associations involving Alnus and Myrica have been established as having a nitrogen fixing function. The distribution of these two genera within the mire ecosystem can now be examined in the light of general peatland chemistry.

Björkman (1942) found that nodulation of alder roots decreased with increasing application of NH_4^+ - N, this result has since been confirmed by other workers (Stewart and Bond, 1961; Zavitkovski and Newton, 1968). Several field investigations have demonstrated that the rate of nitrogen fixation by alder slows down as the stand matures. Newton et al. (1968) found that increments of soil nitrogen due to fixation by alder nodules ceased before the trees were 20 years old. Tarrant et al. (1969) concluded that the total nitrogen in soil beneath a 40 year old stand of red alder (A. rubra), could not be accounted for by the then current rates of nitrogen accumulation, and decided, therefore, that nodules must have been more active during the early years of stand development. Alder is an important component of the vegetation during the early phase of succession on glacial moraines during the period of nitrogen accumulation, but it is not abundent in the later phases (Crocker and Major, 1955).

Alder is restricted to rheotrophic mires, and the evidence presented in previous sections indicates that it is this part of the mire ecosystem where problems of nitrogen shortage are likely to be greatest. Voigt and Steucek (1969) have demonstrated a very strong positive relationship between nitrogen fixation in A. rugosa, and the amount of moisture in the soil; this may be connected with the fact that the optimum oxygen concentration for nitrogen fixation by nodules of this species is well below atmospheric levels (Chapter 2). Taking into consideration the moisture and nitrogen regimes of rheotrophic mires, it is clear that Alder will have considerable

advantages in such situations.

Nitrogen fixation by Myrica is also affected by the amount of soluble nitrogen in the growth medium. Stewart (1961) found a decrease in the dry weight of nodules when Myrica plants were grown in a medium containing 150 p.p.m. NH_4^+ - N. The quantity of nitrogen fixed per gram dry weight of nodules when the plants were grown in a solution containing 100 p.p.m. NH_4^+ - N was only about half the amount fixed by nodules of plants in 50 p.p.m. NH_4^+ - N (Stewart and Bond, 1961). These results clearly demonstrate that nitrogen fixing activities of Myrica are much reduced in environments containing high levels of soluble nitrogen.

Myrica is frequently said to occur on bogs, but in most parts of Northern Europe it is essentially a plant of poor fens, rather than ombrotrophic mires proper. One possible explanation for this distribution may be the relative abundance of soluble nitrogen in ombrotrophic locations. The arguments in this respect which were outlined for heterotrophic nitrogenase activity in peat earlier in this chapter, may be equally applicable to Myrica: not only would its nitrogen fixing ability afford no special advantage in ombrotrophic situations, but additional nitrogen might even prove detrimental.

Before this section on symbiotic nitrogen fixation is concluded, mention must be made of lichens and bryophytes. Nitrogen fixation has been confirmed in the lichens Peltigera, Nephroma and Stereocaulon, and it has been suggested that these genera may make a significant contribution to the nitrogen economy of peatlands in tundra regions (Kallio et al., 1972; Kallio, 1973; Granhall and Selander, 1973). Blue-green algal symbionts also occur in some bryophytes, and of these nitrogen fixation has been confirmed in Sphagnum (Stewart, 1966), Blasia pusilla (Bond and Scott, 1955), Cavicularia densa (Bond and Scott o.c.), and

Frullania (Bellamy and Waughman, unpublished). There is no evidence to suggest that nitrogen fixing bryophyte associations are important in peatland systems.

12(h) The amount of nitrogen fixed by heterotrophic activity

In Chapter 4 it was emphasised that in view of the effect of oxygen, and the nature of the acetylene reduction assay, estimates of the amount of atmospheric nitrogen fixed by the heterotrophic component of the peatland ecosystem are likely to be very inaccurate. But having emphasised this fact, it must also be noted that the acetylene reduction assay is still the best method currently available.

Before attempting to produce an estimate it is well to briefly reiterate the two main problems. Firstly, the theoretical conversion factor of three C_2H_4 to one N_2 has not been confirmed in all soil systems. Rice and Paul (1971), and Brouzes, et al. (1971) obtained ratios considerably greater than three, although the second group later reported that they had not confirmed their earlier results (Knowles, et al., 1973). In view of these facts a conversion factor of 6:1 will be used in the following calculations. The second important problem is that a mixture of aerobic and anaerobic conditions stimulate nitrogenase activity to levels higher than those occurring in the purely aerobic, or purely anaerobic condition (Rice et al., 1967; Magdoff and Bouldin, 1970; Brouzes et al., 1971, and Chapter 4). However the fluctuating water table near to the surface in mire ecosystems, and the exudation of oxygen by some mire plants (Armstrong, 1964) must create just such a mixture of aerobic and anaerobic conditions in the rooting zone. Burgeff (1961) observed that peat above the water table was well supplied with oxygen, he also noted that precipitation and evaporation gave rise to frequent fluctuations of the water table. The surveys of Havas (1961) and Persson (1962) also demonstrated that

the upper layers of peat in mire ecosystems are by no means devoid of oxygen. Thus it is reasonable to conclude that conditions of the assay may approximate conditions which are widespread in peatlands, and therefore extrapolation on the basis of figures reported in Chapter 8 might not be entirely unjustified.

The general survey reported here involved nearly one thousand assays, and the results show that the variation between similar mire types in different locations is not great: much less than one order (Figures 18, 19 and 20). The tests in Southern Germany were all carried out during the summer, but material from other locations was assayed at various times of the year, the evidence from these results indicates that fluctuations of nitrogenase activity due to temperature changes are probably greater than fluctuations due to intrinsic seasonal variation. Therefore, in the following estimate, seasonal changes of temperature are allowed for by utilising the annual temperature data for Wielenbach (near to Pfrule Moss) in Walter and Lieth (1967); Central Europe diagram Nm 483. This information is used in conjunction with the method for temperature correction described in Chapter 4 and 5.

The estimates are derived from the curve in Figure 20:

$$y = 0.64 + 0.038x + 0.00049x^2$$

where $y = \log_{\lambda} \frac{nmol}{C_2H_4}$/ml fresh peat/day at 20°C and x = the weighted index. The weighted indices inserted for each mire category are as follows: fen, 49; poor fen, 74; bog, 94. It is assumed that nitrogen fixation occurs throughout the top 20 cm of peat, which was the depth of the sample cores. The estimates are shown in Table 16.

Table 16

Estimates of atmospheric nitrogen fixed by heterotrophic nitrogenase activity in peat from Southern Germany

	Fen	Poor fen	Bog
estimated daily rate in summer	25	6	1
estimated annual accumulation	2100	530	70

all figures mg N/m^2

The only other reported estimation of nitrogen fixed by heterotrophic activity in peat utilising acetylene reduction is that by Granhall and Selander (1973). Their estimate for aerobic nitrogen fixation in the ombrotrophic part of a sub-arctic mire was 150 mg $N/m^2/y$, which is about double the estimate for the ombrotrophic sites in Southern Germany. This difference is unimportant as compared with the general similarity of these two independent estimates.

The amount of nitrogen deposited in Northern Europe by precipitation ranges between about 200 and 2000 mg N/m^2, with an average figure of about 800 mg N/m^2 (Eriksson, 1952; Holden, 1966). Thus it is apparent that biological nitrogen fixation makes an important, and probably essential, contribution to the nitrogen budget of mire ecosystems.

SUMMARY OF PARTS ONE AND TWO

CHAPTER ONE

A short description of the history of investigations into biological nitrogen fixation is presented, commencing with the first experimental evidence from the middle of the nineteenth century, through to the discovery of acetylene reduction in the late 1960s. Difficulties in measuring nitrogen are emphasised, and the relative merits of both acetylene reduction and isotopic nitrogen techniques are discussed.

Some previous reports suggest that nitrogen fixation is temperature sensitive, some suggest otherwise; this matter is of considerable importance with regard to the use of acetylene reduction in the field, and needs to be resolved.

Alnus and Myrica are known to fix atmospheric nitrogen, but information on other aspects of nitrogenase activity in peatlands is scanty.

CHAPTER TWO

Low oxygen concentrations inhibit nitrogenase activity in root nodules of both Alnus and Myrica. The rate of nitrogenase activity in both species is also very sensitive to temperature change, with activity decreasing rapidly at temperatures above about 20°C. Activation energies between 5 and 20°C were calculated to be 30 Kcal/mol for Myrica and 38 Kcal/mol for Alnus. Nitrogenase activity in root nodules of Alnus occurs only whilst the tree is in leaf.

CHAPTER THREE

The rate of nitrogenase activity in Plectonema (anaerobic with respect to nitrogen fixation) increases with increasing temperature between 5 and

25°C, in saturating light intensities, although sensitivity is lower above 20°C. The calculated activation energies are 30 and 12 Kcal/mol below and above 20° respectively. In sub-saturation light intensities, nitrogenase activity in Plectonema is not sensitive to temperature change below, and only slightly sensitive above 20°C. The effect of temperature change on nitrogenase activity in Anabaena (aerobic with respect to nitrogen fixation) is similar to the response of this enzyme in Plectonema, except that in atmospheric levels of oxygen concentration complete inhibition occurs above about 20°C; by contrast, in low oxygen concentrations activity could still be detected at 40°C. The implication of these results to field assays of blue-green algae is discussed.

CHAPTER FOUR

Acetylene reduction by peat continues for at least five days under assay conditions. Responses to glucose, oxygen, carbon monixide and temperature change, as well as the incorporation of isotopic nitrogen, all serve to confirm that the observed acetylene reduction by peat results from the activity of a living enzyme, as opposed to a non-living catalyst. The optimum temperature for heterotrophic nitrogenase activity in peat is about 30°C. There is no evidence for autotrophic nitrogenase activity in the bulk of the peat mass. Both Clostridium spp. and Azotobacter spp. were isolated from peat.

A mixture of aerobic and anaerobic conditions appears to stimulate acetylene reduction by the peat micro-flora, and it is suggested that microbial associations involving aerobic and anaerobic organisms may be of particular importance with regard to nitrogen fixation in peat.

CHAPTER FIVE

The responses of nitrogenase to temperature change are divided into

three categories: anaerobic, with a biphasic arrhenius plot; aerobic non-legume, with a simple arrhenius plot; and legume nitrogenase which is comparatively insensitive to temperature change. Some evolutionary aspects are briefly discussed, and it is pointed out that legume nitrogenase, which has the highest specific rates of activity, also has a temperature response which least resembles that of a simple chemical. A simple method of correcting the results of assays for differences in temperature is described.

CHAPTER SIX

Concepts of the mire nutrient ecocline, fen plant limit, community, and continuum, are discussed. Seasonal and daily precipitation changes, greatly influence the chemical constitution of mire water, which limits the usefulness of mire water chemistry with respect to understanding the nutrient ecocline, and vegetation chemistry provide a more reliable basis for this.

CHAPTER SEVEN

Methods of field sampling, preliminary laboratory procedures, and statistics used are described. Phytosociological methods relevant to this project are discussed, and results of certain aspects of the phytosociological analysis are presented. A coenocline corresponding to the nutrient gradient is abstracted from the floristic data.

CHAPTER EIGHT

Nitrogen fixation was detected and estimated in peat from all eleven mire complexes examined. The rate of nitrogenase activity increased along the ecocline from bog to rich fen, but exhibited a marked decrease in extreme rich fens. Acidity is the single factor which explains most of the

variability of nitrogenase activity in peat, but much of the effect of pH results from its influence on other factors. Potassium appears to exert a significant positive influence on the rate of activity in all parts of the mire. By contrast the influence of calcium is negative, and exerted only in rich mires.

CHAPTER NINE

Total nitrogen in mire vegetation and peat is lowest in poor mires, however the trends are not very marked. By contrast, NH_4^+ - N in peat is lowest in rich mires, and increases along the ecocline in the direction of poor mires; NO_2^- - N in peat is highest in extreme rich fens, but declines along the ecocline to become non-existent in poor fens; the trends of both NO_2^- and NH_4^+ are very marked. NO_3^- - N was not detected. The distributions of the various inorganic species of nitrogen are discussed in the light of redox and pH.

No form of nitrogen in peat correlates very well with nitrogen in mire vegetation: of these, nitrogen dissolved by acidified NaCl is best when only poor mires are under consideration, and total peat nitrogen in any other circumstances. Soluble nitrogen, soluble phosphorus, and pH all explain some of the variability in the nitrogen content of mire vegetation, although most of the influence of pH is indirect. Nitrogenase activity in peat is weakly related to the amount of nitrogen in vegetation, the highest correlations being found in rich mires.

CHAPTER TEN

The lowest phosphorus concentrations in peat and mire vegetation were found in poor mires. However easily soluble phosphorus in peat was found to be lowest in rich mires, increasing along the ecocline to highest levels in ombrotrophic situations. The lower concentrations of soluble phosphorus

in the peat of rich mires is attributed to bonding by iron and aluminium in fens, and to precipitation as insoluble calcium phosphates in rich, and extreme rich fens.

Total phosphorus in peat was found to be closely related to the phosphorus concentration of mire vegetation, explaining up to 71% of the variability if a quadratic term is included in the equation. Soluble aluminium in peat appears to be weakly related to the amount phosphorus in vegetation, whereas the potassium content of mire vegetation accounts for 46% of the variation in vegetation phosphorus. There is some statistical evidence to support the contention that much of the effect of potassium on the amount of phosphorus in mire vegetation results indirectly from its influence on manganese.

CHAPTER ELEVEN

The amounts of Ca, Mg, K, Na, Fe, Al, Mn, and Zn in peat and mire vegetation are described with reference to the ecocline. Some data are also presented on Cu, Cd, Pb, Ni and Co. Total amounts of calcium, potassium and magnesium are highest in the peat and vegetation of extreme rich fens, and decrease along the ecocline in the ombrotrophic direction. The trends of exchangeable amounts of calcium and magnesium in peat relative to the ecocline reflect the total amounts of these elements, but exchangeable potassium shows the reverse trend, having its highest concentrations in ombrotrophic peats, where it is the dominant exchangeable ion. In general, the heavy metal content of peat in poor mires is smaller than the amount in peat from rich mires, whereas with regard to mire vegetation the opposite is the case; however lead is an exception.

A Ca/Mg ratio of less than one is no criterion of ombrotrophy in the German peats studied. The trend of the Ca/K ratio in these peats suggest that this ratio may provide a more reliable index.

Potassium, nitrogen and phosphorus are the elements most highly concentrated into the vegetation, throughout the entire mire system, although the level of concentration declines from rich to poor mires; in poor mires, manganese is also highly concentrated into the vegetation. With regard to mineral cycling the mobility of nitrogen and phosphorus is low as compared with potassium, zinc, and sodium in rich mires, and with potassium and manganese in poor mires. Throughout the entire mire ecocline calcium appears to be the element which explains most of the variation in the coenocline, but in the fen to bog section, potassium appears to be the major chemical determinant.

CHAPTER TWELVE

There is no evidence that peatlands are deficient in the supply of nitrogen, particularly on bogs where the levels of up to 250 p.p.m. NH_4^+ - N in peat are much higher than in most mineral soils. The main demands on nitrogen supply are likely to occur in rich mires, thus the high level of biological nitrogen fixation in such situations makes perfectly good ecological sense: if intense nitrogen fixation occurred in poor mires it could give rise to toxic levels of soluble nitrogen.

The distribution of soluble phosphorus resembles that of NH_4^+ - N, and a close examination of the literature indicates that high levels of soluble phosphorus in poor mires, as compared with rich ones, is a widespread feature. The concentration of soluble phosphorus in peat from ombrotrophic mires also appears to be greater than in most mineral soils. It is suggested that the terms poor mire and oligotrophic are inappropriate with reference to the <u>supply</u> of nitrogen and phosphorus in ombrotrophic peats. Results in the literature regarding fertilisation experiments on cultivated peatlands suggests that these systems have a greater requirement for potassium and phosphorus than for nitrogen. The low concentration of

phosphorus in poor mire vegetation as compared with vegetation in rich mires is attributed to the lower level of metabolic activity in the former sites, this is discussed with reference to Lundergårdh's theory of anion respiration. Differences in the uptake of phosphorus and nitrogen into the vegetation of poor mires may be due to the fact that the latter element is in a cationic form.

The lower levels of heterotrophic nitrogen fixation in extreme rich fens relative to fens is attributed to the abundance of calcium in this part of the mire ecosystem. Evidence is examined for the possibility that the decrease in nitrogenase activity along the ecocline in the direction of ombrotrophic mires may be partly related to the increase of soluble nitrogen in the same direction. Nitrogen fixation in peat occurs in very acid conditions, this feature is discussed with reference to literature on the subject. A brief review of non-heterotrophic nitrogen fixation in peatlands is presented. On the basis of acetylene reduction it is estimated that the annual contribution of nitrogen to the mire ecosystem by heterotrophic fixation is as follows: fens 2100 $mg/m^2/y$; poor fens 530 $mg/m^2/y$; and bogs 70 $mg/m^2/y$. By comparison with the input from precipitation this is considered highly significant.

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APPENDIX A :

LOCATION DESCRIPTIONS

1. Achmelvich Mire, near Loch Dubh, Sutherland, Scotland. (58°16' N, 5°23' W)

This mire is 1½ k from the sea at the headwaters of a small stream draining into Loch Dubh. The system is roughly circular and consists of a peripheral lagg, with input and outflow streams encircling a partly floating sphagnum lawn; the surrounding water partings are covered by blanket mire. This was one of the sites used in the study of the distribution of nitrogenase activity in mire ecosystems.

2. Dun Moss, Forest of Alyth, Perthshire, Scotland. (56°41' N, 3°21' W

A small mire situated at the head of the Drumturn Burn. Many mire types are present either side of the small stream flowing through the blanket mire. The site was used to study the distribution of nitrogenase activity throughout a sphagnum hummock.

3. Tarn Moor, Westmoorland, England. (54°30' N, 2°20' W)

An area of undulating moorland developed on carboniferous limestone and glacial drift deposts, it is drained by Tarn Sike into Sunbiggin Tarn. A wide range of mire types are present from deep ombrotrophic peats to alkaline rheotrophic flushes. The site was used for the study of the distribution of nitrogenase activity in peat, and for physiological investigations.

4. Roydon Common, Norfolk, England. (52°45' N, 2°20' W)

A mire complex developed from a stream which drains across Roydon

Common. There is some acid drainage from the nearby heathlands; both transitional and rheotrophic mires are present. The area was used for the study of nitrogenase distribution.

5. Taufach Moss, near Beuren, South Germany. (47°47' N, 9°40' E)

This Moss occupies a basin lying adjacent to the main valley of the Eschach. Although small areas of ombotrophic mire are still in existence, the main interest of the complex are the extensive areas of rheotrophic and transitional mire showing a variety of stages of biological succession from pioneer to climax. The site was used for chemical and nitrogenase studies.

6. Wurzacher Ried, near Wurzach, South Germany. (47°44' N, 9°54' E)

Extensive areas of ombrotrophic and rheotrophic mire are present in a broad basin which is drained by a number of streams forming the headwaters of the Wurzacherach, which in turn drains to the south of the basin. Pioneer and climax stages for both ombrotrophic and rheotrophic areas are in evidence. The site was used for chemical and nitrogenase studies.

7. Pfrule Moss, Escheloe, near Mürnau, South Germany. (47°35' N, 10°45' E)

This site contains a wide range of mire types from extreme ombrotrophic ones in the centre of the Cupola, to very clacareous rheotrophic ones adjacent to the streams which drain the complex. The broad development of pioneer stages in this complex form a continuum, which is very convenient for studying the complex mire gradient. Pfrule Moss was the most important site utilised in this project for chemical and nitrogenase investigations.

8. Simanggang, Sarawak, East Malaysia. (1°15' N, 111°20' E)

An extensive area of tropical rain forest growing on onbrotrophic peat on the north bank of the Batang Lupar, 3 miles to the west of Simanggang. A small amount of rheotrophic mire in the form of mangrove swamp is present at the river margin. Estimates of nitrogenase activity were made on peat collected from a single transect cut 2 miles into the rain forest.

9. Nordkjosbotn, between Nordkjosbotn and Øvergård, North Norway. (64°14' N, 19°34' E)

A small rheotrophic mire with Scorpidion scorpiodes and Menianthes trifoliata. One of the most northerly sites in which peat was tested for nitrogenase activity.

10. Parry Sound, S. Ontario, Canada. (45°22' N, 80°0' E)

A small mire situated about 5 k to the south of the township of Parry Sound, just off the main highway to Toronto. Mostly Picea spp. with some open mire covered by Chamaedaphnae spp. and Kalmia anjustifolia. The area of fen peat is very small. The fen peat was tested for nitrogenase activity.

11. Cortina, Italy. (46°60' N, 12°20' E)

A small area of poor fen situated at about 2100 m in the moutains to the East of Cortina. The peat was tested for nitrogenase activity.

APPENDIX B

List of Data Tables

List of Data Tables (continued)

TABLE A1 **Effect of temperature on acetylene reduction by detached nodules of Alnus and Myrica. μmol C_2H_4/g/h.**

		Sample nm.						
	T°C	1	2	3	4	5	6	7
ALNUS	5	0.01	0.14	0.24	0.15	0.31	0.13	0.28
	10	0.10	0.90	0.40	1.50	0.60	0.70	1.20
	16	1.80	2.70	4.60	2.50	3.8	1.60	1.30
	20	11.50	3.60	2.50	8.10	8.9	1.80	2.50
	25	7.10	8.40	8.60	9.40	7.8	8.20	8.00
	31	0.10	0.80	3.70	0.50	0.5	0.90	
MYRICA	8	2.6	3.7	1.7	1.7	0.5		
	17	12.1	10.6	9.7	8.4	13.2		
	22	21.3	18.6	17.0	14.7	23.2		
	27	13.6	12.8	11.4	8.9	25.0		
	30	7.1	6.6	13.4	11.2	8.2		
	36	3.4	1.3	9.4	8.0	1.4		

TABLE A2 **Effect of different oxygen concentrations on acetylene reduction by detached root nodules of Alnus (A), and Myrica (M). μmol C_2H_4/g/h.**

pO_2 atm.	sample nm.	A	M	pO_2 atm.	sample nm.	A	M
0.01	1	0.03	0.5	0.15	1	0.88	17.0
0.01	2	0.02	0.4	0.15	2	1.75	10.0
0.01	3	0.00	0.1	0.15	3	2.63	1.0
0.05	1	0.22	3.8	0.20	1	1.25	29.0
0.05	2	0.36	2.5	0.20	2	1.63	19.0
0.05	3	0.58	0.2	0.20	3	1.25	3.2
0.10	1	0.51	12.0				
0.10	2	1.25	7.9				
0.10	3	1.95	0.4				

TABLE A3 Annual cycle of acetylene reduction by nodules of Alnus. μmol C_2H_4/g/h. A, leaves absent; B, buds opening; O, full leaf; F, leaves falling.

	sample nm.										
date	1	2	3	4	5	6	7	8	9	T°C	leaves
30/1	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	4	A
28/2	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	7	A
25/3	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	9	A
25/4	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	11	B
18/5	6.9	6.0	23.0	1.1	2.4	4.3	2.4	6.0	1.0	11	O
12/6	8.9	1.0	1.0	3.1	2.6	4.5	8.3	5.2	6.6	20	O
10/7	3.7	27.0	5.1	14.0	31.0	31.0	1.4	3.4	13.0	15	O
14/8	2.4	5.6	15.0	7.0		6.3	6.3	6.0		18	O
12/9	21.0	15.0	4.5	8.2	14.0	3.2	2.0	18.0	17.0	16	O
18/10	26.0	22.0	24.0	13.0	1.3	6.3	13.0	1.1	14.0	14	O
27/10	4.9	0.5	19.2	1.7	0.6	3.7	5.4	1.2	5.0	12	O
5/11	0.7	0.6	7.8	2.6	0.4	0.6	0.3	1.0	5.3	7	F
17/11	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	8	A
15/12	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	5	A

TABLE A4 Effect of temperature on acetylene reduction by Anabaena cylindrica nmol C_2H_4/sample/h.

	sample nm.				
T°C	1	2	3	4	5
5	8.0		2.7	7.3	3.3
10	16.0	14.0	12.0	13.0	10.5
15	16.0	20.0	11.0	25.0	12.0
20	38.0	44.0	25.0	25.0	51.0
25	64.0	62.0	35.0	67.0	43.0
30	81.0	76.0	49.0	48.0	57.0
35	13.0	35.0	38.0	46.0	54.0
40	1.7	0.5	1.0	0.7	2.4

TABLE A5 Effect of temperature on acetylene reduction by Anabaena cylindrica in pO_2 of 0.02 atm. nmol C_2H_4/sample/h.

Temp °C	sample nm. 1	2	3	4	5
5	30	31	29	34	30
10	29	37	42	41	40
20	33	45	46	39	47
25	49	105	80	72	30
30	42	144	48	83	80
35	77	124	84	69	86
40	33	14	21	28	17

TABLE A6 Effect of temperature on acetylene reduction by Anabaena cylindrica in reduced light. nmol C_2H_4/sample/h.

Temp °C	sample nm. 1	2	3
5	4.9	5.0	5.2
10	4.4	5.4	5.1
15	9.3	9.2	9.6
20	17.2	16.0	17.9
25	26.0	21.0	27.2
30	14.8	14.4	15.2
35	12.4	12.4	11.9
40	9.8	10.0	10.1

TABLE A7 Effect of temperature on acetylene reduction by Plectonema boryanum. nmol C_2H_4/sample/h.

Temp °C	sample nm. 1	2	3	4	5
5	20	10	8	19	32
10	27	55	44	—	42
15	70	95	101	80	96
20	163	155	199	166	172
25	192	88	326	237	266
30	355	272	351	381	380
35	422	249	176	138	79
40	115	155	165	195	96

TABLE A8 Effect of temperature on acetylene reduction by Plectonema boryanum in reduced light. nmol C_2H_4/sample/h.

Temp °C	sample nm. 1	2	3
5	8	7	7
10	7	6	11
15	16	35	24
20	26	38	34
25	57	56	60
30	24	13	19
35	14	24	20
40	0	0	0

TABLE A9 Effect of temperature on acetylene reduction by Anabaena cylindrica (A.c.) and Plectonema boryanum (P.b.): summary of additional experiments. nmol C_2H_4/sample/h.

Temp °C	A.c. experiment nm. 2	3	P.b. 2	3	4
5	141	3	9	8	1
10	249	12	20	10	8
15	1012	53	58	23	25
20	2274	201	115	65	51
25	3187	199	95		81
30	3851	237	129	192	129
35	1427	108	44	67	107
40	214	20	10	26	15

TABLE A10 Time course for acetylene reduction by different types of peat. nmol C_2H_4/ml peat.

peat type	hours from start 2	8	20	32	44	54	68	168
rheotrophic	0.2	0.3	0.8	1.6	2.4	3.4	4.5	7.6
transitional	0.1	0.3	0.2	1.4	0.6	1.0	1.7	7.6
ombrotrophic	0.1	0.1	0.2	0.4	0.3	0.2	0.6	0.6
blanket	0.0	0.1	0.0	0.0	0.0	0.0	0.0	0.0

TABLE A11 Effect of different pre-incubation temperatures on acetylene reduction by rheotrophic peat. nmol C_2H_4/ml peat/h.

Sample nm.	Temperature of pre-incubation °C					
	5	12	23	32	37	59
1	3.4	4.2	3.2	1.1	1.14	0.11
2	1.3	7.2	3.1	0.6	0.73	0.19
3	3.7	2.6	0.9	1.7	1.82	0.12
4	1.8	2.8	3.6	3.2	0.21	0.12
5	2.8	2.5	0.8	6.3	0.31	0.10
6	2.6	5.3	2.4	2.0	0.28	0.11
7	0.7	1.1	1.7	0.6	0.12	0.12
8	4.1	2.1	2.1	2.0	0.22	0.11
9	1.7	3.4	1.2	2.3	0.18	0.10
10	1.3	3.5	2.1	1.6	0.19	0.18

TABLE A12 Effect of temperature on acetylene reduction by rheotrophic peat. nmol C_2H_4/ml peat/h.

Sample nm.	Temperature °C					
	7	16	25	33	37	45
1	0.21	0.38	2.3	22.0	1.3	0.31
2	0.31	0.46	2.7	4.5	1.4	0.73
3	0.29	0.36	4.9	15.8	1.6	0.52
4	0.27	0.51	2.8	7.4	2.0	0.31
5	0.33	0.37	1.0	17.9	1.4	0.38
6	0.27	0.59	2.1	9.2	1.4	0.29
7	0.30	0.53	1.3	17.8	1.9	0.30
8	0.21	0.50	2.6	4.7	1.1	0.41
9	0.18	0.32	2.2	10.4	2.0	0.35
10	0.22	0.52	9.9	3.2	2.3	0.55
11	0.29	0.42	0.8	3.2	1.2	0.41
12	0.15	0.61	4.0	10.6	1.6	0.31
13	0.18	0.51	3.5	5.6	1.8	0.38
14	0.21	0.38	1.9	16.0	1.4	0.32
15	0.15	0.34	3.6	19.0	1.5	0.21
16	0.25	0.26	5.5	7.0	1.4	0.18
17	0.24	0.34	4.9	15.4	0.8	0.41
18	0.10	0.42	5.4	6.0	1.8	0.40
19	0.25	0.42	2.2	17.0	1.2	0.27
20	0.21	0.55	3.4	6.2	1.5	0.43

TABLE A13 The effect of different light and oxygen conditions on acetylene reduction by rheotrophic peat. nmol C_2H_4/ml peat/48h.

Sample nm.	light aerobic	light anaerobic	dark aerobic	dark anaerobic
1	64	38	64	21
2	44	12	30	6
3	19	15	115	16
4	19	20	83	83
5	35	35	34	10
6	10	22	40	25
7	62	9	10	29
8	64	57	19	7

TABLE A14 The effect of CO, high pO_2, and glucose on acetylene reduction by rheotrophic peat, full details in text. nmol C_2H_4/ml peat.

time after start	control	CO added	high pO_2	glucose added
3	5.1	6.2	4.5	4.1
3	2.8	8.6	4.1	4.6
3	5.7	15.5	4.5	4.0
6	10.6	13.5	10.2	5.7
6	7.1	16.8	6.7	6.0
6	11.6	41.0	10.0	6.0
9	9.4	20.3	15.4	7.8
9	11.2	27.7	11.5	7.0
9	15.4	55.0	14.4	7.7
18	28.7	59.4	53.0	18.2
18	24.4	72.8	25.0	15.4
18	29.9	92.3	32.2	17.5
20	37.1	117.0	2.9	30.0
20	36.2	122.0	2.5	24.1
20	41.4	154.0	2.3	19.9
23	17.4	6.5	1.6	6.6
23	11.7	14.5	0.0	8.7
23	16.5	9.8	0.0	7.6
27	63.2	47.2	6.9	34.8
27	36.8	58.7	7.8	28.8
27	89.8	34.7	5.2	31.8
32	119.0	55.5	14.0	194.0
32	87.6	92.7	14.5	231.0
32	127.0	76.9	9.2	213.0
42	288.0	65.2	76.6	884.0
42	287.0	103.0	97.3	1451.0
42	343.0	47.8	44.9	1168.0

TABLE A15 Effect of different initial oxygen concentrations on the time course of acetylene reduction by rheotrophic peat. nmol C_2H_4/ml fresh peat.

$\%O_2$ at start	Time from start in hours						
	3	8	14	22	30	38	47
0	3	16	23	30	32	43	43
0	6	15	27	31	32	34	46
0	17	29	41	5[illegible]	60	65	69
4	[illegible]	[illegible]	28	31	58	104	203
4	[illegible]	[illegible]	14	28	31	55	60
4	5	14	50	9[illegible]	187	304	453
16	9	17	28	43	61	104	175
16	0	1	12	[illegible]	32	49	74
16	1	5	14	[illegible]	64	95	189
40	2	2	6	14	16	22	41
40	0	11	24	44	80	150	264
40	0	5	21	[illegible]	92	129	290

TABLE A16 Results of attempts to detect Clostridia in peat by plating and araerobic incubation.

	Plate num.	surface colonies	sub surface colonies	colourless colonies	milky colonies	butyric acid smell
rheotrophic	1	+	+	+	+	+
	2	+	+	+	+	+
	3	+	+	+	+	+
	4	+	+	-	+	+
ombrotrophic	1	+	-	+	+	-
	2	+	-	-	+	-
	3	+	-	-	+	-
	4	+	-	-	+	-

TABLE A17 Plate counts of Azotobacter from rheotrophic peat (R), and ombrotrophic peat (O).

dilution		
1/1	300	15
1/0	300	10
1/0	300	21
1/10	300	4
1/10	300	5
1/10	300	8
1/100	300	4
1/100	300	3
1/100	300	2
1/1000	120	22
1/1000	150	4
1/1000	160	0
1/10,000	29	0
1/10,000	36	0
1/10,000	30	0

TABLE A18 Attempt to confirm nitrogenase activity in colonies on plates by sub-culturing into a liquid medium and testing by acetylene reduction under aerobic (A) and anaerobic (AN) conditions. nmol C_2H_4/bottle/h. N.D. not detectable.

sample nm.	A	AN
1	ND	ND
2	ND	ND
3	ND	1.35
4	ND	0.04
5	ND	ND
6	ND	1.90
7	ND	ND
8	ND	3.51
9	ND	ND

TABLE A19 Floristic data from German mires. Braun-Blanquet cover abundance values. P, Pfruhle Moss 1973; W, Wurzacher Ried 1973; T, Taufach Moss 1973; M, Pfruhle Moss 1972; G, Grundlen Ried 1972.

	site nm.
	1111111112222222222333333333344444444445
	1234567890123456789012345678901234567890123456789012345
	PPPPPPPPPPPPPPPPPPPPPWWWWWWWWWTTTTTMMMMMMMMMMGGGGG
JUNCUS SUBNODULOSUS	1122 + 2 +1
PHRAGMITES AUSTRALIS	11211+ + 11 +1
EPILOBIUM PALUSTRE	+ +
NASTURTIUM MICROPHYLLUM	+
BRYUM PSEUDO-TRIQUETRUM	1+32
CALTHA PALUSTRIS	+
CLADIUM MARISCUS	4 13
GALIUM PALUSTRE	+2+
MENTHA AQUATICA	+11 +
CAREX ELATA	+3 3 132 1 1+ 1 2 41 33 +
ERIOPHORUM ANGUSTIFOLIUM	1 1 2 221 +1 1+1
CRATONEURON COMMUTATUM	4+ +
DREPANOCLADUS REVOLVENS	+33 1 +
CAMPYLIUM STELLATUM	1 + 1
CTENIDIUM MOLLUSCUM	+2 21 +
ACROCLADIUM CUSPIDATUM	+2 11
SPHAGNUM PLUMULOSUM	1 1
CIRSIUM PALUSTRE	2+ +
EUPATORIUM CANNABINUM	++
CHARA SP.	3
CAREX PANICEA	2 1 21 +
VALERIANA DIOICA	+
LYSIMACHIA VULGARIS	2 1
SCORPIDIUM SCORPIOIDES	332 + + 1 22+ 1+
FISSIDENS ADIANTOIDES	+ + +
CAREX FLAVA	3 11 1
GALIUM ULIGINOSUM	1 1
PARNASSIA PALUSTRIS	+ + +
SUCCISA PRATENSIS	+ + +
CREPIS PALUDOSA	+ +

Continued/

TABLE A19 (Cont'd)

site nm.

```
                          1111111111222222222233333333334444444445
                 12345678901234567890123456789012345678901234567890
                 PPPPPPPPPPPPPPPPPPPPPWWWWWWWWTTTTTMMMMMMMMMMGGGGG
HOLINIA CAERULEA          2232  111221  +1  1 +1        21+  11 +  12
POTENTILLA ERECTA         2  + +11 +          ++     +      +   +   +
RHYNCHOSPORA ALBA        +  +      11 ++ 111    1                +1    1
HOLCUS LANATUS            21    3
CAREX LEPIDOCARPA          1 1                              1
CAREX LASIOCARPA           +  +2111  ++ 1    1+  1    4     +12 +1  1
VACCINIUM OXYCOCCUS        1 +  2221131+11  1   +3211  +11    2++  + 411
CAREX DAVALLIANA           +                               2
PINGUICULA VULGARIS                                        1
EQUISETUM VARIEGATUM                        +              +
DROSERA ROTUNDIFOLIA          +1 +++++ 1+++   + +++    ++    11 1   +1+
SCHOENUS FERRUGINEUS                       +2              11
MENYANTHES TRIFOLIATA      11133+3+  + +++          +++     1 1 ++  1
VIOLA PALUSTRIS             1+                     +      +
EQUISETUM FLUVIATILE       +111 21                         1+2
CAREX DIANDRA               1  +                           + +
ERIOPHORUM LATIFOLIUM      11              1 +                    2
SCUTELLARIA GALERICULATA +  +                                     +
CAREX PANICEA              1     +          +              1      1
CAREX LIMOSA                +1   1 +111211    1     111+  +  + 11  ++2
SCHEUCHZERIA PALUSTRIS         +11 11+1111    2      +        111  +++1
LYTHRUM SALICARIA               1                                 +
LYSIMACHIA THYRSIFLORA                                            +
POTENTILLA PALUSTRIS            1    +                +        +
ANDROMEDA POLIFOLIA        +  1 1+1++  1+ 1      1++1  1     1+  1   11
TOFIELDIA PUSILLA                          1
PARNASSIA PALUSTRIS        +
VERATRUM ALBUM             1
TRICHOPHORUM ALPINUM        +  3                          1   1  1   +
EQUISETUM PALUSTRE          +     +
CAREX PANICULATA            +
TRICHOPHORUM CAESPITOSUM    1         2                   3
TARAXACUM OFFICINALE        +
```

Continued/

TABLE A19 (Cont'd)

site nm.

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                          1111111111222222222233333333334444444444445
                 12345678901234567890123456789012345678901234567890123456789012345
                 PPPPPPPPPPPPPPPPPPPPPWWWWWWWWWTTTTTMMMMMMMMMMMMGGGGG

CAREX ELONGATA                    +
CAREX ROSTRATA                    11  11   2                      + 1
MNIUM SELIGERI                    + +
POLYTRICHUM STRICTUM                                  + 1     1+
SPHAGNUM PAPILLOSUM                  5  225:3 313      3 +   +       2233
CAREX ECHINATA                        1   +
ERIOPHORUM VAGINATUM                  2 +2+1+ 2+ 2    ++     2+     21  2  +11
ELEOCHARIS SP.                        +
MELAMPYRUM PRATENSE                   1                 +
THELYPTERIS PALUSTRIS                 +
DACTYLORHIZA INCARNATA                +
SPHAGNUM MAGELLANICUM                 3  4++3 44 3    2141   12      22  3  124
SPHAGNUM RECURVUM                 1   33+1?1511       41+   3       3       4
SPHAGNUM PALUSTRE                     1 3           +
SPHAGNUM FIMBRIATUM                  1
SPHAGNUM TENELLUM                     +                    + 1
CAREX NIGRA                           1
SPHAGNUM RUBELLUM                    21  2 31        321    23      11      1
DROSERA INTERMEDIA                          1                   1   21
DROSERA ANGLICA                             1              +
SPAGNUM CUSPIDADUM                         133          1          333    2
UTRICULARIA INTERMEDIA                                          1
CAREX PAUCIFLORA                                        2       +      1+
AULACOMNIUM PALUSTRE                                    :       +
CALLUNA VULGARIS                              1     1122      +         1
LYCOPODIUM INUNDATUM                                    :       +
DROSERA LONGIFOLIA                      ++
CAREX ACUTIFORMIS                                 +
SPHAGNUM FUSCUM                                     5

WEIGHTED          1 12343467668788788891  8789935788  33677798825799
INDEX             35669775314182306679424768962445604583810795963335
```

TABLE A20 Effect of log transformation on some chemical parameters studied in this project.

	Untransformed		Transformed	
	KURTOSIS	SKEWNESS	KURTOSIS	SKEWNESS
Weighted index	-1.11	-0.57	1.74	-1.60
Nitrogen fixation	10.50	3.04	-0.97	0.59
Ash in peat	1.53	1.62	-0.91	0.68
C in peat	0.11	-1.21	4.54	-2.13
N soluble in peat	1.55	1.55	-0.08	-0.69
P soluble in peat	0.10	0.89	-0.32	-0.71
N total in peat	1.52	1.28	-0.37	0.29
P total in peat	3.92	1.62	0.10	0.43
N in vegetation	0.05	0.05	1.13	-0.95
P in vegetation	10.08	2.48	-0.68	-0.26
Ca in peat	2.32	1.80	-0.68	0.88
K in peat	2.41	1.74	-0.60	0.53
Mg in peat	3.92	2.26	0.19	1.17
Fe in peat	5.43	2.13	-0.98	0.31
Co in peat	1.91	1.62	-1.14	0.32
Pb in peat	0.39	0.36	17.74	-3.57
Ca Exch.	16.45	4.04	3.14	1.39
K Exch.	0.23	1.12	-0.17	-0.64
Mg Exch.	7.29	2.50	0.37	0.61
Ca in vegetation	27.45	4.80	2.94	1.22
K in vegetation	-0.62	0.34	-0.28	-0.56
Mg in vegetation	26.77	4.00	2.90	1.37
Pb in vegetation	0.46	0.67	-0.63	-0.81

Level at which deviation from normal distribution is statistically significant (p 0.05): Skewness 0.53; Kurtosis upper limit 3.99; Kurtosis lower limit 2.15.

TABLE A21 Correlation coefficients between the mire ecocline and N and P in peat calculated on the basis of different units.

	Nitrogen		Phosphorus	
	total	available	total	available
Unit dry weight	-0.10	+0.72	-0.31	+0.46
Unit humus	-0.58	+0.42	-0.72	+0.24
Unit volume	-0.69	+0.13	-0.66	+0.09

TABLE A22 Equation parameters for curves drawn on figures.

fig nm.	intercept	X	X^2	standard error of estimate
19A	1.0	0.022	-0.00054	0.58
19B	0.29	0.035	-0.00043	0.44
19C	1.5	0.030	-0.00053	0.51
19D	2.5	-0.96		0.18
23	30.0	0.32	0.008	59.0
24	32.0	-0.47	0.019	45.0
25	0.21	0.0045		0.37
33	1.5	0.33	-0.002	7.1
34	0.17	0.02		1.3
35	4.4	-0.25	0.004	6.8
37	7.8	0.10		7.0
38	0.67	0.019	-0.00023	0.37
39	0.36	0.01	-0.00013	0.16
40A	0.90	-0.0067		0.19
40B	1.1	-0.009		0.16
40C	2.1	-0.019		0.34
41A	1.06	0.007	-0.00012	0.13
41B	-1.4	0.029		0.45
41C	-0.09	0.015	-0.0002	0.29
42A	0.48	-0.005		0.20
42B	-0.016	-0.004		0.29
42C	1.5	-0.018		0.45
43B	-1.01	0.0047		0.46
44A	-0.5	-0.009	0.0001	0.33
44B	-1.16	0.019	-0.00025	0.39
44C	0.44	0.017	-0.00025	0.30
45A	-1.51	0.022	-0.00015	0.32
45C	-1.31	0.0098	-0.00013	0.29
46A	-0.91	-0.015	0.0002	0.45
46B	-1.19	0.014	-0.00015	0.23
46C	0.65	0.013	-0.00025	0.36
47B	1.01	0.013	-0.00014	0.18
48A	201.0	-5.8	0.042	38.0
48B	2603	-78.0	0.56	246.0
48C	1.4	-0.036	0.0003	0.54
48D	18.0	-0.51	0.0038	3.2
48E	23.0	-0.52	0.0036	5.6
48F	3.2	-0.076	0.00061	0.57
49FE	0.61	-0.024	0.0003	0.45
49MN	0.156	0.012		0.27
49AL	0.56	-0.042	0.00045	0.43
49ZN	0.78	-0.022	0.00018	0.29
49CA	-0.19	0.0026		0.21
49K	2.6	-0.014		0.43
49MG	0.50	-0.012		0.32
49NA	0.51	-0.0067		0.71
49N	2.8	-0.009		0.26
49P	2.5	0.02	-0.0004	0.56
50N	0.092	-0.0003		0.19
50P	0.32	-0.0011		0.16
50CA	-1.01	0.013		0.39
50K	1.13	0.004		0.48
50MG	-0.85	0.014		0.46
50NA	1.00	-0.004		0.42
50FE	-0.80	-0.028	0.0004	0.40
50MN	0.14	0.0118		0.31
50AL	-1.16	-0.047	0.0006	0.55
50ZN	0.61	-0.033	0.0003	0.36
51	25.0	-0.27	0.0004	14.0
52N	2.8	-0.009		0.26
52P	2.5	0.02	-0.0004	0.56
54	13.0	0.32		6.4

TABLE A23. Species weightings used in calculation of weighted index for each aufname.

Weighting = 0

Juncus subnodulosus
Bryum pseudo-triquetrum
Cladium mariscus
Cratoneuron commutatum
Drepanocladus revolvens
Ctenidium molluscum
Cirsium palustre
Scorpidium scorpioides
Fissidens adianthoides
Carex davalliana
Schoenus ferrugineus
Tofieldia pusilla

Weighting = 25

Phragmites australis
Carex elata
Campylium stellatum
Carex panicea
Lysimachia vulgaris
Carex flava
Parnassia palustris
Potentilla erecta
Carex lepidocarpa
Equisetum variegatum

Weighting = 50

Sphagnum plumulosum
Molinia caerulea
Carex lasiocarpa
Menyanthes trifoliata
Equisetum fluviatile
Carex rostrata
Carex echinata
Drosera intermedia

Weighting = 75

Eriophorum angustifolium
Vaccinium oxycoccus
Drosera rotundifolia
Andromeda polifolia
Trichophorum alpinum
Polytrichum strictum
Sphagnum papillosum
Eriophorum vaginatum
Sphagnum recurvum
Aulacomnium palustre
Calluna vulgaris

Weighting = 100

Scheuchzeria palustris
Melampyrum pratense
Sphagnum magellanicum
Sphagnum rubellum
Sphagnum cuspidatum
Carex pauciflora
Sphagnum fuscum

TABLE A24 Total cation content of peat samples collected in 1972. All figures are the mean of triplicate analyses N.D. not detectable.

mg/g dr.wt.

site nm.	Ca	K	Mg	Na	Fe	Mn	Al
36	110.0	2.6	87.0	0.19	5.8	0.10	10.0
37	86.0	1.0	62.0	0.23	2.6	0.03	4.4
38	68.0	3.3	53.0	0.16	15.0	0.24	12.0
39	5.6	1.9	2.5	0.14	6.0	0.14	7.1
40	2.4	4.5	3.0	0.16	3.0	0.04	12.0
41	2.5	0.8	0.8	0.15	0.7	0.04	2.0
42	2.9	0.4	0.8	0.06	0.9	0.02	0.8
43	3.5	1.4	1.5	0.05	0.9	0.03	2.2
44	3.0	0.7	0.7	0.17	0.8	0.04	1.1
45	3.7	0.3	0.7	0.04	1.1	0.02	1.1
46	15.0	0.9	2.6	0.09	19.0	0.40	10.0
47	3.6	0.5	0.5	0.04	2.3	0.04	4.1
48	1.7	0.4	0.5	0.05	0.5	0.03	0.4
49	1.7	0.2	0.7	0.16	0.6	0.04	1.0
50	2.8	0.2	0.5	0.03	1.0	0.03	1.0

µg/g dr.wt.

site nm.	Co	Cd	Ni	Cu	Pb	Zn
36	7.0	2.4	18.0	12.0	42	1110
37	7.8	4.5	15.0	15.0	60	170
38	4.6	1.1	20.0	6.4	72	50
39	2.3	1.7	6.8	66.0	82	53
40	ND	2.2	9.7	114.0	90	93
41	ND	0.2	3.5	104.0	76	26
42	ND	0.7	5.4	53.0	79	21
43	ND	1.2	2.9	89.0	73	26
44	4.5	ND	4.0	45.0	74	190
45	ND	ND	2.9	37.0	93	300
46	2.6	0.4	11.0	12.0	59	40
47	ND	1.0	3.8	16.0	57	1150
48	ND	ND	2.8	69.0	46	—
49	ND	4.1	4.5	53.0	62	1200
50	ND	ND	ND	83.0	59	60

TABLE A25 Total cation content of vegetation samples collected during 1972. All figures are the mean of triplicate analyses. mg/g dr.wt. N.D. not detectable

site nm.	Ca	K	Mg	Na	Fe	Mn	Al
36	6.2	18.0	4.0	0.02	0.12	0.16	0.06
37	3.8	17.0	1.5	0.62	0.13	0.02	0.01
38	4.0	14.0	2.1	ND	0.28	0.06	ND
39	2.6	13.0	1.5	0.01	0.05	0.20	ND
40	2.2	19.0	1.7	0.05	0.05	0.14	0.35
41	2.1	7.3	1.0	0.02	0.32	0.10	0.14
42	1.8	9.3	1.4	0.06	0.25	0.15	0.51
43	2.5	7.1	0.9	0.08	0.80	0.03	1.40
44	2.5	5.6	1.2	0.55	0.71	0.06	0.27
45	2.4	7.3	1.1	0.16	0.98	0.26	1.10
46	5.3	10.0	2.2	0.10	0.24	0.14	0.55
47	2.8	11.0	2.0	0.14	0.17	0.66	0.17
48	0.8	9.0	1.0	0.79	0.42	0.24	0.28
49	1.2	5.2	0.8	0.28	0.50	0.17	0.24
50	2.0	5.1	1.0	0.18	0.63	0.10	0.52

TABLE A26 Cations extracted with N ammonium acetate from peat samples collected during 1972. All figures are the mean of triplicate analysis. mg/g dr.wt.

site nm.	Ca	K	Mg	Na
36	5.7	0.09	1.80	0.08
37	10.0	0.29	1.30	0.15
38	7.1	0.23	2.20	0.17
39	4.1	0.51	0.92	0.34
40	2.2	0.72	0.60	0.34
41	2.0	0.56	0.53	0.66
42	2.7	0.41	0.57	0.30
43	2.2	0.79	0.52	0.23
44	2.1	0.51	0.43	1.40
45	2.6	0.79	0.55	1.40
46	14.0	0.15	1.00	0.07
47	2.5	0.11	0.18	0.04
48	1.3	0.42	0.31	0.17
49	1.3	0.12	0.26	0.08
50	2.1	0.17	0.32	0.21

TABLE A27 Some chemical properties (excluding cations) of peat and vegetation samples collected in 1972. (1) % dr.wt. (2) mg/g dr.wt. (3) µg/g dr.wt. N.D. not detectable.

		In Peat						In Vegetation		
site nm.	pH	(1) C	(2) N	(3) soluble N	(2) P	(3) soluble P	(1) Ash	(1) Ash	(2) P	(2) N
36	7.2	8	8	64	0.50	0.1	78	10	1.49	20
37	7.2	18	10	35	0.81	0.6	66	10	0.88	15
38	6.5	18	10	51	0.51	ND	59	7	0.78	17
39	5.4	39	19	118	0.58	4.7	10	5	0.81	21
40	5.0	39	13	48	0.51	5.3	3	5	0.65	19
41	5.0	45	9	92	0.34	8.7	6	4	0.66	14
42	4.5	40	13	182	0.36	13.0	4	5	0.55	22
43	4.2	43	10	170	0.31	6.5	5	6	0.33	25
44	3.8	43	12	246	0.33	8.2	7	4	0.54	26
45	3.8	44	14	159	0.39	15.0	5	5	0.60	20
46	6.5			102	1.19	ND	27	8	1.10	
47	4.5			134	0.98	0.5	14	6	2.80	
48	4.4			276	0.50	22.0	4	5	1.20	
49	4.3			218	0.31	6.3	7	5	0.50	
50	3.9			183	0.37	19.0	4	4	0.90	

TABLE A28 Total cation content of peat samples collected in 1973. All figures are the mean of triplicate analyses. ND not detectable.

Site nm.	Ca	K	Mg	Na	Fe	Mn	Al	Co	Cd	Ni	Ca	Pb	Zn
	mg/g dr.wt.							μ g/g dr.wt.					
1	213.0	0.49	107.0	0.13	1.5	0.03	2.0	22	1.9	ND	4.2	100	18
2	97.0	0.67	48.0	0.16	1.4	0.05	2.0	11	2.0	7	9.1	46	48
3	190.0	0.63	101.0	0.18	1.4	0.02	3.6	16	2.3	4	9.3	90	30
4	29.0	2.20	7.9	0.08	9.4	0.07	15.0	7	0.7	14	9.6	47	68
5	9.1	0.59	1.5	0.01	3.9	0.15	1.1	1	2.1	3	6.4	93	291
6	16.0	0.98	2.4	0.14	3.9	0.13	3.6	5	1.5	5	5.0	69	122
7	143.0	2.10	79.0	0.11	4.6	0.18	7.6	17	2.1	7	8.9	47	103
8	107.0	2.10	60.0	0.16	5.6	0.05	11.0	16	2.4	14	10.0	42	100
9	20.0	4.60	11.0	0.15	3.7	0.03	16.0	6	2.6	18	11.0	89	241
10	8.4	0.60	1.2	0.08	8.9	0.13	21.0	6	2.4	6	9.9	81	173
11	11.0	0.24	1.9	0.09	2.2	0.02	0.8	1	1.1	2	6.7	43	124
12	17.0	0.68	3.8	0.40	9.8	0.08	0.9	4	1.1	6	7.7	56	329
13	3.8	4.40	3.1	0.17	2.5	0.02	2.0	5	3.2	10	17.0	55	203
14	2.0	3.90	1.1	0.17	3.2	0.03	2.5	1	7.9	5	7.5	70	112
15	2.0	0.60	0.7	0.09	0.9	0.03	0.9	1	0.7	5	6.3	56	114
16	2.9	1.10	0.9	0.11	1.0	0.03	1.8	2	9.9	4	9.4	110	92
17	3.6	0.50	1.0	0.06	2.0	0.06	0.7	3	1.4	3	8.7	124	170
1	2C	0C33	0C6	0C05	1C1	0C02	0C5	ND	18C0	5	60C	133	135
19	4.2	0.34	1.0	0.14	1.7	0.05	0.3	1	14.0	3	5.7	131	132
20	2.8	0.33	0.6	0.05	1.1	0.03	0.4	1	ND	6	11.0	82	110
21	4.7	0.45	1.9	0.24	0.6	0.03	1.0	4	2.0	5	12.0	52	94
22	3.9	0.48	1.4	0.15	0.9	0.03	0.8	2	4.0	4	7.8	100	78
23	125.0	0.41	2.7	0.05	2.3	0.02	2.1	9	2.9	2	8.8	33	53
24	208.0	0.60	4.3	0.06	5.2	0.08	9.9	18	3.7	5	12.0	27	50
25	164.0	1.40	7.1	0.08	7.1	0.07	14.0	15	4.1	12	20.0	33	50
26	5.8	0.74	2.6	0.30	0.7	0.02	0.7	2	ND	3	6.8	32	69
27	4.0	0.29	1.1	0.10	1.1	0.05	0.8	3	ND	4	5.6	53	93
28	2.9	0.29	0.7	0.20	0.6	0.05	0.7	2	14ND	3	7.0	97	95
29	2.7	0.31	0.6	0.02	0.5	0.03	0.7	3	ND	3	4.0	45	82
30	2.1	0.31	0.6	0.06	0.6	0.05	0.7	1	ND	3	5.2	53	94
31	8.5	2.50	4.2	0.22	8.7	0.08	18.0	10	3.6	28	24.0	71	126
32	7.4	1.60	3.2	0.28	6.0	0.04	14.0	7	ND	14	12.0	52	68
34	4.9	0.54	0.9	0.20	7.0	0.13	2.8	4	4ND	4	9.4	86	76
35	5.8	0.48	0.7	0.08	3.1	0.08	3.5	3	1ND	5	9.9	75	59

TABLE A29 Cations extracted with N ammonium acetate from peat samples collected in 1973. All figures are the mean of triplicate analyses. ND not detectable. mg/g dr.wt.

Site num.	Ca	K	Mg	Na
1	10.0	0.17	0.30	0.04
2	9.6	0.09	1.30	0.07
3	7.0	0.01	1.30	0.13
4	9.7	0.01	1.20	0.01
5	4.7	0.03	0.66	0.15
6	5.0	0.03	0.60	1.50
7	5.5	0.07	1.90	ND
8	7.1	0.06	2.70	0.89
9	9.2	0.04	3.80	0.90
10	3.7	0.08	0.41	0.07
11	5.6	0.05	1.40	0.14
12	5.5	0.16	0.60	0.26
13	2.5	0.64	0.26	0.11
14	2.3	0.30	0.91	0.22
15	2.6	0.20	0.51	0.41
16	1.9	0.68	0.45	0.26
17	2.4	0.15	0.60	0.11
18	2.2	0.32	0.50	0.16
19	2.0	0.26	0.49	0.11
20	2.0	0.15	0.45	0.23
21	1.8	0.15	0.50	0.23
22	1.6	0.56	0.51	0.22
23	19.0	0.39	0.40	0.75
24	15.0	0.01	0.31	0.17
25	17.0	0.01	0.36	0.09
26	1.2	0.40	0.24	0.85
27	1.5	0.06	0.40	0.20
28	1.2	0.21	0.31	0.36
29	1.7	0.19	0.32	0.53
30	1.5	0.10	0.35	0.14
31	3.4	0.02	0.27	0.09
32	2.9	0.07	0.21	0.17
33	5.0	0.17	0.10	0.03
34	2.3	0.05	0.34	0.27
35	3.2	0.16	0.35	0.20

TABLE A30 Cations extracted with N acetic acid from peat samples collected in 1973. ND not detectable. All figures are the mean of triplicate analyses. mg/g dr.wt.

Site nm.	Ca	K	Mg	Na	Fe	Mn	Al	Zn
1	62.0	0.33	24.00	0.28	0.12	0.02	0.14	0.01
2	53.0	0.26	27.00	0.35	0.20	0.05	0.10	0.01
3	32.0	0.21	16.00	0.07	0.30	ND	0.05	ND
4	12.0	0.08	1.80	0.37	0.20	0.02	0.17	0.02
5	5.8	0.22	0.93	0.12	0.12	0.06	0.05	0.03
6	6.1	0.19	0.88	0.94	0.30	0.06	0.07	0.04
7	5.3	0.25	31.00	0.80	0.11	0.10	0.14	0.04
8	84.0	0.46	53.00	0.15	0.47	0.04	0.34	0.04
9	12.0	0.50	5.00	0.01	0.23	0.01	0.26	0.05
10	4.1	0.30	0.46	0.01	0.16	0.03	0.10	0.08
11	7.9	0.19	2.90	0.31	0.07	0.01	0.09	0.04
12	6.3	0.42	0.82	0.31	0.70	0.04	0.08	0.13
13	1.5	0.23	0.32	0.05	0.05	ND	0.16	0.03
14	3.9	1.40	2.00	0.65	0.11	0.01	0.10	0.03
15	2.9	0.52	0.62	0.27	0.02	0.02	0.06	0.05
16	2.1	0.57	0.69	0.09	0.04	0.02	0.07	0.05
17	3.7	0.41	1.50	0.34	0.05	0.03	0.08	0.06
18	2.3	0.43	0.48	0.05	0.10	0.01	0.05	0.06
19	2.0	0.43	0.57	0.13	0.05	0.02	0.09	0.06
20	2.0	0.51	0.49	0.16	0.08	0.02	0.10	0.05
21	1.9	0.20	0.66	0.56	0.03	0.01	0.06	0.03
22	1.6	0.29	0.48	0.21	0.02	0.01	0.08	0.04
23	86.0	0.26	1.10	0.73	0.05	0.01	0.07	0.02
24	99.0	0.13	0.86	0.11	0.03	0.03	0.05	0.01
25	76.0	0.26	0.84	0.03	0.03	0.02	0.06	0.03
26	1.5	1.96	0.46	0.19	0.03	0.01	0.05	0.04
27	2.8	0.35	1.20	0.36	0.01	0.03	0.05	0.04
28	1.0	0.33	0.26	0.27	0.02	0.02	0.04	0.03
29	2.2	0.32	0.47	0.25	0.02	0.02	0.06	0.04
30	5.3	0.23	0.68	0.60	0.02	0.03	0.12	0.05
31	4.8	0.78	0.43	1.79	0.08	0.04	0.20	0.03
32	4.2	0.31	0.37	0.18	0.04	0.01	0.38	0.02
33	5.1	0.20	0.65	0.05	0.06	ND	0.17	0.02
34	2.4	0.24	0.18	0.11	0.10	0.07	0.17	0.03
35	2.9	0.48	0.22	0.28	0.40	0.02	0.14	0.03

TABLE A31 Total cation content of vegetation samples collected in 1973. All figures are the mean of triplicate analyses. (1) mg/g dr.wt. (2) µg/g dr.wt.

Site Nm.	(1) Ca	(1) K	(1) Mg	(1) Na	(1) Fe	(1) Mn	(1) Al	(2) Cu	(2) Zn	(2) Pb
1	33.0	12	14.0	1.20	1.20	0.05	0.77	3.2	240	74
2	4.9	10	1.6	0.81	0.06	0.05	0.03	1.8	21	5
3	14.0	21	4.3	1.30	0.25	0.02	0.20	192.0	180	32
4	8.7	9	3.7	0.91	0.47	0.18	0.28	152.0	140	24
5	3.1	10	1.5	0.26	0.31	0.13	0.10	2.0	43	4
6	4.2	15	2.2	0.43	0.15	0.18	0.05	76.0	115	72
7	8.3	13	4.1	0.37	0.18	0.10	0.25	2.7	34	10
8	4.4	12	3.1	0.52	0.17	0.05	0.03	2.0	26	2
9	5.9	17	3.2	0.28	0.14	0.11	0.04	52.0	91	89
10	3.7	10	1.4	0.21	0.17	0.35	0.08	4.5	58	20
11	2.5	9	1.4	0.51	0.20	0.24	0.09	11.0	72	22
12	8.4	16	3.2	1.00	3.10	0.24	0.11	323.0	328	62
13	3.2	14	1.9	0.22	0.11	0.16	0.03	1.0	50	2
14	2.1	15	1.5	0.06	0.14	0.20	0.02	4.0	48	3
15	2.5	9	1.3	0.30	0.18	0.37	0.12	5.0	65	36
16	1.9	9	1.1	0.12	0.15	0.16	0.09	3.0	64	20
17	2.0	7	1.0	0.12	0.23	0.40	0.17	5.0	87	51
18	2.9	11	1.3	0.15	0.20	0.21	0.11	4.0	76	25
19	2.2	4	0.9	0.18	0.41	0.15	0.21	4.0	84	80
20	1.7	5	1.0	0.20	0.34	0.14	0.19	4.1	61	34
21	2.1	9	1.3	0.48	0.48	0.09	0.14	2.0	82	34
22	2.1	5	0.9	0.21	0.32	0.20	0.21	6.0	61	43
23	3.8	15	1.1	0.51	0.11	0.03	0.02	4.0	20	2
24	5.4	13	1.0	0.72	0.16	0.04	0.14	5.0	32	8
25	7.1	9	1.1	0.36	0.18	0.03	0.11	5.9	26	15
26	1.6	14	0.9	0.55	0.25	0.08	0.16	4.9	75	34
27	4.7	5	0.9	0.48	0.19	0.59	0.13	5.2	56	25
28	2.3	3	0.6	0.37	0.54	0.40	0.29	7.2	56	51
29	2.5	3	0.6	0.61	0.25	0.49	0.26	3.6	59	59
30	2.2	3	0.6	0.82	0.42	0.44	0.39	5.9	55	59
31	2.7	18	1.4	0.71	0.93	0.11	0.03	4.4	29	1
32	2.8	14	1.4	0.43	0.25	0.12	0.08	5.2	49	2
33	2.6	10	2.1	0.25	0.12	0.20	0.02	5.0	50	1
34	2.4	7	1.0	0.18	0.15	1.10	0.12	10.0	59	27
35	2.3	8	1.0	0.17	0.31	0.46	0.14	131.0	119	36

TABLE A32 Phosphorus in peat and vegetation samples collected in 1973. All figures are the mean of triplicate analyses. (1) μg/g dr.wt. (2) mg/g dr.wt. ND not detectable. Extractant indicated.

Site nm.	(1) NH_4Cl	(1) NH_4 Acetate	(1) Acetic Acid	(1) 0.002N H_2SO_4	(1) 0.02N H_2SO_4	(1) 0.5N H_2SO_4	(2) Total in peat	(2) Total in veg
1	4.7	5.0	14.0	0.6	5.1	30	0.28	1.00
2	5.3	30.0	19.0	0.9	12.0	45	0.41	0.70
3	2.0	2.5	6.3	0.5	6.7	21	0.27	0.75
4	2.9	ND	6.8	0.3	14.0	16	0.52	0.74
5	2.0	9.5	11.0	1.1	20.0	27	0.44	0.70
6	0.2	2.5	9.1	1.0	28.0	28	0.63	1.10
7	3.0	ND	13.0	0.3	3.0	59	0.63	0.94
8	0.3	0.1	18.0	0.2	3.9	19	0.47	0.79
9	3.2	6.0	7.6	1.3	16.0	24	0.42	0.81
10	0.7	2.5	3.8	1.2	14.0	36	0.62	0.67
11	7.0	14.0	21.0	2.0	14.0	17	0.35	0.93
12	1.3	0.2	5.7	0.7	18.0	56	0.70	1.60
13	2.2	0.1	6.5	1.0	8.8	16	0.35	0.85
14	5.4	19.0	15.0	1.6	11.0	12	0.48	1.10
15	6.0	46.0	27.0	1.4	19.0		0.33	0.81
16	5.4	11.0	30.0	1.6	12.0	31	0.34	0.79
17	17.0	9.0	17.0	2.2	22.0		0.31	0.40
18	19.0	12.0	17.0	5.9	28.0	28	0.34	0.40
19	8.0	4.0	9.5	1.6	7.9	26	0.25	0.40
20	21.0	5.4	24.0	2.1	7.7	27	0.29	0.64
21	22.0	2.5	11.0	1.5	30.0	28	0.35	0.74
22	26.0	15.0	2.9	2.6	7.3	12	0.34	0.32
23	0.4	11.0	11.0	0.4	0.6	23	0.29	0.57
24	2.3	0.2	3.8	0.3	1.3	24	0.35	0.52
25	0.2	2.5	3.8	0.3	0.3	36	0.32	0.60
26	14.0	12.0	9.5	1.2	11.0	25	0.20	0.84
27	23.0	3.2	30.0	7.7	7.2	21	0.48	0.61
28	33.0	10.0	17.0	1.1	5.9	48	0.32	0.43
29	7.6	15.0	28.0	0.8	7.5	22	0.29	0.33
30	4.8	12.0	19.0	0.7	14.0	21	0.20	0.31
31	0.2	ND	11.0	0.1	14.0	26	0.60	0.20
32	0.5	ND	11.0	0.1	16.0	38	0.59	1.30
33	1.3	0.2	10.0	0.7	11.0	29	0.56	1.20
34	3.8	5.0	12.0	0.7	14.0	33	0.65	0.74
35	4.3	2.5	19.0	1.2	22.0	34	0.63	0.47

TABLE A33 Nitrogen in peat and vegetation samples collected in 1973. ND not detectable. All figures are the mean of triplicate analyses. Extractant as indicated. (1) μg/g dr.wt. (2) mg/g dr.wt.

Site nm.	(1) NO_2^--N neutral NaCl	(1) NH_4^+-N neutral NaCl	(1) NH_4^+-N acid NaCl	(2) total in peat	(2) total in veg
1	0.26	34	22	12	17
2	0.18	5	10	13	14
3	0.10	37	5	14	18
4	0.03	23	38	14	18
5	0.16	52	63	17	19
6	ND	39	63	28	22
7	0.14	45	67	10	23
8	0.07	23	35	7	18
9	0.05	42	55	21	17
10	ND	54	102	22	16
11	ND	89	80	17	16
12	ND	94	85	22	23
13	ND	57	30	14	14
14	ND	110	70	14	19
15	ND	161	97	12	17
16	ND	85	225	14	16
17	ND	64	140	11	22
18	ND	82	100	7	21
19	ND	40	81	9	13
20	ND	75	240	19	29
21	ND	64	200	20	30
22	ND	200	270	13	25
23	0.47	21	25	15	19
24	0.13	8	57	12	13
25	0.13	36	24	8	13
26	ND	94	105	6	12
27	ND	55	120	10	10
28	ND	66	135	8	7
29	ND	20	88	7	7
30	ND	26	88	7	7
31	ND	6	11	12	20
32	ND	5	23	20	24
33	ND	16	35	33	19
34	ND	210	100	25	13
35	ND	64	80	34	13

TABLE A34 Some properties of peat collected in 1973.

Site nm.	pH	density g/l	Ash %
1	7.6	517	88
2	6.7	128	44
3	7.1	390	78
4	7.1	194	29
5	6.7	93	11
6	5.8	110	10
7	6.7	294	66
8	6.7	220	54
9	6.5	111	29
10	5.7	94	7
11	4.5	76	6
12	4.9	82	2
13	4.5	87	3
14	4.2	75	6
15	4.4	76	3
16	3.8	75	5
17	3.8	65	4
18	3.5	72	7
19	3.5	65	5
20	3.5	68	4
21	3.6	76	7
22	3.4	86	3
23	7.1	112	39
24	7.0	314	29
25	7.0	377	22
26	3.6	54	6
27	3.3	72	5
28	3.0	60	4
29	3.6	57	5
30	3.3	50	7
31	6.4	147	39
32	5.4	119	22
33	4.9	192	9
34	4.4	96	8
35	4.4	116	8

TABLE A35 Nitrogenase activity in peat collected from United Kingdom sites. All figures adjusted to 20°C. nmol C_2H_4/ml/day. T, Tarn Moor; R, Roydon Common; A, Achmelvich; D, Dun Moss.

	date	1/72	1/72	1/72	1/72	6/72	6/72	6/72	6/72
	location	T	T	T	T	R	R	R	R
	pH	4.6	5.0	6.9	7.9	4.8	5.4	5.7	7.3
replicate	1	0.02	50	85	2	1.0	1	24	0.2
	2	0.01	2	15	2	39.0	1	5	7.7
	3	0.66	60	39	9	1.3	11	29	7.0
	4	0.67	6	91	24	22.0	88	24	7.5
	5	0.38	11	145	10	4.7	13	38	13.0
	6	0.65	71	14	12	1.4	13	10	14.0
	7	1.40	2	83	16	3.7	28	56	21.0
	8	0.97	117	119	8	11.0	4	1	5.7
	9	0.01	1	51	118	7.0	64	3	3.4
	10					0.2	14	11	6.4
	11					11.0	31	21	37.0
	12					39.0	10	14	8.4
	13					0.3	21	15	9.3
	14					9.3	7	90	16.0
	15					6.7	19	32	6.4
	x̄	0.53	36	71	22	10.5	21	25	11

	date	4/71	4/71	4/71	4/71	4/71	5/71	5/71	5/71	5/71
	location	A	A	A	A	A	D	D	D	D
	pH	3.7	4.8	6.4	6.4	7.4	4.9	5.7	5.9	6.7
replicate	1	0	2.7	14.0	54.0	14.0	0	1.0	5.7	6.8
	2	0	2.5	1.4	8.6	6.6	0	1.3	3.8	3.9
	3	0	3.0	4.3	8.6	12.0	0	5.6	1.0	4.1
	4	0	6.2	3.0	8.4	13.0	0	1.3	1.3	5.2
	5	0	2.1	2.5	11.0	14.0	0	0.7	1.4	5.6
	6	0	1.5	4.3	16.0	12.0	0	0.4	1.9	6.5
	7	0	1.1	2.5	44.0	6.6	0	0.9	2.3	10.0
	8	0	2.3	4.4	5.5	15.0	0	0.4	1.0	4.7
	9	0	2.5	2.1	2.1	3.2				
	10	0	2.1	4.0	26.0	12.0				
	x̄	0	2.6	3.9	18.4	11.0	0	0.9	2.3	5.8

TABLE A36 Nitrogenase activity in peat from sites outside of the United Kingdom, but excluding Germany. nmol C_2H_4/ml/day. P, Parry Sound; S, Simmangang; O, Øvergård; C, Cortina. All figures adjusted to 20°C.

	date	6/74	8/72	8/72	6/72	7/72
	location	P	S	S	O	C
	pH	5.0	3.0	7.1	6.0	-
replicates	1	4.5	0	65	6.2	129
	2	5.9	0	152	1.0	12
	3	12.0	0	91	0.4	30
	4	9.6	0	22	1.2	8
	5	13.0	0	184	1.6	4
	6	6.7	0	126	1.2	39
	7	1.3	0	72	2.2	
	8	10.0	0	41	0.0	
	9	11.0	0	10	1.4	
	10		0	242	0.4	
	11		0	84	0.0	
	12		0	147		
	13		0	159		
	14		0	121		
	15		0	97		
	16		0	36		
	17		0	101		
	18		0	22		
	19		0	98		
	20		0	114		
	x	8.2	0	107	2.1	37

TABLE A37 Nitrogenase activity in peat collected at Pfrule Moss 1972. nmol C_2H_4/ml/day. All figures adjusted to 20°C.

replicate	site nm. 36	37	38	39	40	41	42	43	44	45
1	79	23	81	655	43	2.7	1.1	1.3	0.0	0.0
2	74	30	130	491	10	0.1	0.0	2.1	1.5	0.0
3	9	11	491	1226	88	14.0	0.0	6.5	0.0	0.9
4	74	9	256	273	12	0.0	0.0	13.0	0.0	0.0
5	68	10	518	109	15	1.2	2.7	23.0	0.0	0.0
6	163	23	682	27	103	1.2	1.7	2.1	0.0	0.0
7	496	45	477	20	47	0.0	0.0	0.4	1.2	0.0
8	461	13	750	163	86	0.0	0.0	8.1	0.0	1.7
9	54	5	327	104	122	0.9	0.0	2.9	0.0	4.9
10	19	12	201	262	81	0.0	0.0	2.0	0.0	1.0
11	15	10	265	327	36	1.2	1.0	3.4	0.0	0.0
12	47	17	174	129	45	0.0	0.0	23.0	0.0	0.0
13	51	8	348	73	68	0.0	0.0	12.0	0.0	0.0
14	50	50	273	76	49	0.0	0.0	17.0	0.0	0.0
15	32	85	296	92	8	0.0	0.0	3.1	0.0	0.0
$\bar{x}$	113	23	353	268	54	1.4	1.0	8.0	0.2	1.0

TABLE A38 Nitrogenase activity in peat collected at Wurzacher Ried 1973. nmol C_2H_4/ml/day. All figures adjusted to 20°C.

replicate	site nm. 23	24	25	26	27	28	29	30
1	39	1.9	0.4	0.0	0.0	0.0	0.0	0.0
2	15	0.1	0.4	0.0	0.0	0.0	0.0	0.0
3	101	1.2	0.4	0.0	0.0	0.0	0.0	0.0
4	16	1.0	0.0	0.0	0.0	0.0	0.0	0.0
5	16	1.9	0.0	0.0	0.0	0.0	0.0	0.0
6	25	1.0	0.4	0.0	0.0	0.0	0.0	0.0
7	28	1.0	0.0	0.0	0.0	0.0	0.0	0.0
$\bar{x}$	34	1.2	0.2	0.0	0.0	0.0	0.0	0.0

TABLE A39 Nitrogenase activity in peat collected at Taufach Moss 1973. nmol C_2H_4/ml/day. All figures adjusted to 20°C.

replicate	site nm. 31	32	33	34	35
1	9	37	0.8	1.1	2.2
2	5	5	3.6	1.0	0.2
3	14	60	6.7	1.1	0.7
4	198	24	1.3	1.2	0.0
5	102	7	0.3	1.8	3.0
6	17	14	0.3	1.3	1.0
7	99	77	17.0	1.1	4.5
$\bar{x}$	63	31	4.5	1.2	1.7

TABLE A40 Nitrogenase activity in peat collected at Pfrule Moss 1973. nmol C_2H_4/ml/day. All figures adjusted to 20°C.

replicate	site nm. 1	2	3	4	5	6	7	8	9	10	11
1	5.4	2.5	128	17	35	96	10	246	124	1.2	0.3
2	1.1	1.4	110	8	6	7	436	57	1	11.0	0.6
3	7.3	1.6	198	2!	99	90	260	51	56	14.0	0.5
4	2.0	0.4	51	1	63	5	30	59	9	3.4	9.6
5	27.0	1.6	163	4	56	15	15	93	0	2.0	0.5
6	0.9	3.7	110	2	73	13	20	21	18	3.5	0.5
7	53.0	1.8	180	1	62	4	88	126	1	1.5	0.6
$\bar{x}$	14.0	1.9	134	8	56	33	122	83	30	5.2	1.8

replicate	site nm. 12	13	14	15	16	17	18	19	20	21	22
1	14	80	26	0.1	139	0.4	0.9	0.5	2.9	0.0	0.0
2	6	115	125	0.5	50	0.2	0.6	0.2	0.9	0.0	0.1
3	201	103	82	0.9	1	4.3	2.4	0.0	8.4	0.3	0.3
4	27	42	149	0.6	0	0.2	18.0	0.3	12.0	1.5	0.0
5	2	32	35	0.6	0	0.5	0.6	0.5	0.6	0.0	0.3
6	10	159	215	0.3	51	2.8	0.6	11.0	0.1	0.2	0.3
7	10	41	50	6.9	2	0.4	0.1	0.0	0.3	0.4	0.0
$\bar{x}$	39	79	98	5.9	35	1.2	3.3	1.8	3.6	0.3	0.2

TABLE A41 Standing crops of some plant communities in German mires.

site nm.	dominant species	nm. of samples cropped	mean g dr.wt. /m^2	95% limits
2	Cladium mariscus	9	506	295-717
7	Carex davalliana	6	203	80-326
8	Carex elata	10	197	165-228
13	Eriophorum angustifolium	10	291	55-526
14	Molinia caerulea	10	518	405-631
16	Carex rostrata	10	564	319-808
17	Trichophorum caespitosum	10	597	444-752
19	Sphagnum magellanicum	9	690	414-966
20	Rhynchospora alba	10	534	308-766
21	Carex limosa	8	133	9-257
23	Juncus subnodulosus	9	121	68-174
24	Cladium mariscus	10	458	247-668
25	Schoenus ferrugineus	8	135	79-191
26	Rhynchosphora alba	10	516	445-587
27	Sphagnum recurvum	8	439	214-664
28	Sphagnum papillosum	10	632	444-820
29	Sphagnum magellanicum	5	554	270-858
30	Sphagnum fuscum	7	632	444-820
31	Carex elata	8	354	235-437
32	Carex lasiocarpa	9	204	142-266
33	Carex limosa	4	93	0-206
34	Eriophorum angustifolium	8	401	242-643
35	Trichophorum caespitosum	5	457	316-598