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DEVELOPMENT AND APPLICATION OF A RESIN-BAG METHOD TO DETERMINE AVAILABLE NITROGEN IN FOREST SOILS

by

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SUMMARY

A resin-bag method was compared to a conventional KCl extraction procedure for determining available nitrogen in forest soil. Under controlled growth room conditions, both the amounts of ammonium and nitrate nitrogen adsorbed by resin-bags and extracted by 6% KCl correlated well with the uptake of nitrogen by birch seedlings from 6 widely differing soils. Also, resin-bags placed *in situ* under 5 differing forest stands and one upland grassland plot gave a close relationship between their adsorption of nitrogen and 6% KCl extractable nitrogen from the soil. This provides further evidence that the resin-bag method can be used to assay nitrogen availability in forest soils.

INTRODUCTION

Nitrogen is a constitutent of proteins, nucleic acids and other cell components and is possibly the most important nutrient for plant growth. In forest ecosystems, trees obtain nitrogen from the soil via their root systems, in the form of ammonium or nitrate ions (Russell 1973). Only a few tree species can fix atmospheric N, so if the soil is unable to supply sufficient nitrogen to support optimum tree growth, it is said to be nitrogen deficient (Russell 1973; Miller & Miller 1976). Consequently, there is a frequent need to test soils for available nitrogen.

Various methods have been used to estimate the availability of nitrogen in forest soils, but none is considered wholly satisfactory (Keeney 1980). Results of laboratory methods of incubation and of chemical extraction typically show a high degree of inter-correlation (Keeney & Bremner 1966; Webster 1978; Lea & Ballard 1982), but these methods do not allow for the spatial and seasonal variations in the available N in the soil. Incubations of soil bags buried in the field are sensitive to environmental temperature fluctuations (Eno 1960), but are time consuming and expensive.

In this study available nitrogen was determined in growth room and in field samples of forest soils using ion exchange resins in a manner similar to the method of Binkley and Matson (1983) but omitting mercurial lacing of the resin. The resin-bag method was compared with a conventional 6% KCl extraction of fresh soil for inorganic nitrogen and with nitrogen uptake by birch seedlings. The potential value of the method for determining available nitrogen is discussed.

MATERIALS AND METHODS

1 POT EXPERIMENT

Birch seedlings were pot grown in 6 sieved soils for 3½ months. During this period, cation and anion resin-bags were also buried in separate identical pots, except without plants. A randomized design of 3 replicate blocks was chosen, with the 3 treatments randomized within each block. The experiment was carried out in a growth room under controlled conditions of continuous light for 16 hours a day at 20°C. Soil moisture was adjusted each day to 60-70% of the water-holding capacity of the sieved soils, using a water-spray. Pots were watered to a standard weight, including an approximate allowance for changing plant weights. Mineralization of organic nitrogen is usually maximal at 50-60% of water-holding capacity (Gasser 1969), but soils in pots were adjusted slightly higher to allow for water loss during the day.

i) Soils

The soils (Table 1) were obtained from six different sites of the United Kingdom in March 1985. At each site, the L and F layers were removed and about 50 kg soil were collected from the top 5 cm layer. With the exception of the Moor House peat, all soils were sieved through a 1.2 cm (½") mesh mounted on an adapted concrete mixer (Benham & Harrison 1980). Each soil was then thoroughly mixed and nine 1.7 litre plastic pots (approximately 13 x 15 cm) were filled with the appropriate weight of soil (Table 1).

To ensure that plant growth was not limited by P or K deficiency, these elements were added to the soils in the form of KH_2PO_4 at the equivalent rates of 50 and 63 kg P ha^{-1}. The soil from each pot was spread in a plastic tray (30 x 40 cm) and 10 ml KH_2PO_4 solution (20 ml containing 0.77 g KH_2PO_4) were sprayed on to the soil using a spray nozzle attached to a Zippette. The soil was then mixed and a further 10 ml solution were added. After a second mixing the soil was returned to the pots. At the end of the experiment soil samples were collected from pots without plants and 6% KCl extractable N as well as total soil N were determined.

ii) Birch seedlings

The birch seed (Betula pendula Roth) was collected during the autumn of 1980 and 1981 from one tree which had been planted in 1975 at the Institute of Terrestrial Ecology, Bush Estate, Penicuik, Midlothian. The seed was germinated in potting compost in a seed tray and birch seedlings grown in the growth room for 5 weeks. Then 6 similar-sized seedlings were taken at random and transplanted into each planted pot, being gently firmed in and thoroughly watered.

The birch seedlings were grown at 20°C, 16 hours day-length, with an intensity of 8000 lx in a growth room for 14 weeks. After harvesting, the shoots and roots of seedlings in each pot were separated, air-dried and then weighed. The samples of roots and shoots from each pot were ground and analysed for total N.

iii) Resin-bags

Cation exchange resin for NH_4^+ (Dowex 50 W-X8) was used in the hydrogen form. It was regenerated by conversion to the potassium form using 2.5% KCl and returned to the hydrogen state using 3% HCl. The anion exchange resin for NO_3^- (Duolite A303) was used in the chloride form. Regeneration was achieved by conversion to a sulphate form using 3.2% H_2SO_4, then returning it to the chloride state using 2.5% KCl. Both resins were stored under deionised water in a cold room at 2°C.

15 cm lengths of dialysis tubing (viscose cellulose) were cut and placed in 1-2 litres of deionised water for 4 periods of 10 minutes to allow the glycerine present to diffuse out of the tubing. One end of the tube was sealed by folding the end over twice and closed with a staple. The tube was filled under water with wet resin and the top of the tube sealed as before. Cation and anion resin bags were stored in deionised water in a cold room and used as soon as possible after production.

Six weeks after transplanting the birch seedlings 2 resin-bags were placed in pots without plants every other week. They were inserted into each pot using a metal tube (1.2 cm diameter, 15 cm long) as a dibbet to make a hole in the soil. The soil round the resin-bag was compacted by spatula, with the top stapled end being just above the soil surface.

The resin-bags were removed from the soil a week later using a spatula and the position marked to avoid the subsequent resin-bags being placed in the same position.

After recovery, the resin was removed from each tube and eluted with 100 ml 6% KCl in 10 ml aliquots. Ammonium-N and nitrate-N contents of the eluates were determined. After elution, the resin was dried at 40°C for 2 hours and left to cool for 2 hours before being weighed.

2 FIELD EXPERIMENT

i) Study sites

Study sites were five 0.2 ha plots (*Quercus petraea*, *Alnus glutinosa*, *Pinus sylvestris*, *Picea abies* and *Picea sitchensis*) in a 30-year old experimental plantation and one open grassland plot in the Gisburn Forest, north-west England. The canopies of the *Quercus*, *Alnus* and *Pinus* stands were open and their forest floors possessed a variable ground flora. However, the canopies of both *Picea* species were closed and their forest floors were compact and largely free of vegetation. The type of soil at Gisburn is a peaty gley.

ii) Resin-bags

Five points were identified at random within each of the 6 plots. One of each type of resin-bag was buried in late June horizontally about 10 cm apart at 2-3 cm depth below the forest floor/mineral soil interface at each of these points and the soil lightly compacted about them. Any vegetation or litter, if present, was removed before placing the bags but replaced immediately afterwards. At the same time, fresh soil cores (0-5 cm) were collected for determining 6% KCl extractable nitrogen. The resin-bags were left in for a week, after which they were carefully removed, eluted and analysed as before.

3 ANALYTICAL METHODS

i) Total nitrogen

The total nitrogen content of soil and plant material was determined following a sulphuric acid-hydrogen peroxide digestion with a lithium salt and selenium catalyst (Allen *et al*. 1974) but taking only 0.2 g ground soil. The digest was then analysed for ammonium ions (see below).

ii) Ammonium-nitrogen

6% KCl was used to extract NH_4-N from fresh soil and also elute NH_4-N from a cation resin. NH_4-N was analysed by an indo-phenol blue colorimetric method using a Technicon Autoanalyzer system with the wavelength set at 660 nm (Rowland 1983).

iii) Nitrate and nitrite-nitrogen

6% KCl was used to extract NO_3+NO_2-N from fresh soil and elute both ions from an anion resin. Nitrate-nitrogen was reduced to NO_2-N with hydrazine and a naphthylamine-sulphonic acid procedure then used for development of the colour, which was measured using a Technicon Autoanalyzer system with the wavelength set at 535 nm (Henriksen 1965, modified by Rowland *et al*. 1984).

RESULTS AND DISCUSSION

GROWTH ROOM EXPERIMENT

There were varying amounts of total N and 6% KCl extractable N in the different soils examined and this was reflected by a considerable difference in the growth and nitrogen uptake by the birch seedlings (Table 2). The uptake of N by the seedlings was highly correlated with the total inorganic N absorbed from soil by the resin-bags and with the 6% KCl extractable total inorganic N (Table 3 and Figures 1 and 2), but not with total N in the soil. The amounts of ammonium-N and nitrate-N adsorbed by cation and anion resin-bags respectively were also highly correlated with 6% KCl extractable ammonium-N and nitrate-N in the soils (Table 3, Figures 3, 4 and 5). It was also clear that ammonium and nitrate-N accounts for a very small proportion of the total N content of these soils (Table 2).

Binkley and Matson (1983) used nylon stocking material to contain mixed-bed cation and anion resins. To restrict microbial interference, they included mercury ions at a rate equivalent to 5% of the exchange capacity of the cation-resin. However, nitrification appears to be influenced adversely by mercury which may interfere with this process in the immediate vicinity of the resin-bag used in their method (Binkley & Matson 1983; Van Faassen 1973). We have avoided the use of mercury by keeping the resin enclosed in a membrane which would prevent immediate contact between the resin and microbes. Data in Table 3 suggests that, in the absence of mercury, the recoveries of nitrate-N by the resin-bags were as good as those obtained for ammonium-N.

FIELD EXPERIMENT

The amounts of ammonium and nitrate-N adsorbed by resin-bags or extracted by 6% KCl from the soils under different tree species in the field were considerably less than recovered by both methods from the soils in the pot experiment in the growth room (Tables 2 and 4). Two possible reasons may account for this situation: (i) the mixing and sieving of the soils used for the pot experiment may have stimulated the mineralization rates of nitrogen in those soils and/or (ii) the available nitrogen in the field soils may have been extremely low due to very active root uptake of N by trees or the ground vegetation, where present. This may be compounded by a possibly low rate of mineralization of N due to the cool summer soil temperatures in 1985. Values for August 1981 were higher (Table 4) when the temperature was also higher, although the rainfall was similar. Nonetheless, there was still a strong correlation between the amounts of nitrate-N adsorbed by the anion resin-bags and 6% KCl extractable nitrate-N from soils (Figure 6). For ammonium-N the equivalent relationship was poor. However, when the ammonium-N and nitrate N results were combined the relationship between resin-bag adsorbed inorganic N and 6% KCl extractable inorganic N was a quadratic with $r^2 = 0.69$ (Figure 8) and was statistically significant ($P < 0.001$).

The results for this study suggest that nitrogen availability in upland or forest soils can be estimated using the resin-bag procedure, although it may be less sensitive for determining ammonium-N in soils where levels are particularly low. The sensitivity of the procedure described here may be partly limited by the burial time which had to be restricted to 7 days because the cellulose dialysis tubing used to contain the resin is liable to decomposition. This might lead to microbial interference in adsorption of N by the resins. However, it has been shown that no detectable change in the cellulose as monitored by its breaking strength (a more sensitive measure of decomposition than weight loss) occurs within 7 days of burial (Way & Harrison, personal communication). If a synthetic non-decomposable membrane with similar properties to viscose cellulose can be found then the burial time might be extended and thereby improve the method sensitivity.

The conventional 6% KCl extraction still gives satisfactory data but the strong preference for fresh samples makes it important to reduce any delay betrween sampling and analysis in order that changes in the inorganic N status of the soils may be minimal. In contrast a delay in transport to the laboratory and subsequent analysis is apparently not critical for the resin-bag method. If the burial time can be extended when desired beyond 7 days, then the resin-bags procedure as proposed above may have advantages over the 6% KCl extraction method.

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Table 1. Some characteristics of the experimental soils and their weight in a pot.

Soil type	Site	LOI (%)	Bulk density[b] ($g\ cm^{-3}$)	pH	Ext. Ca ($mg\ litre^{-1}$)	Total N ($g\ litre^{-1}$)	Fresh soil weight ($g\ pot^{-1}$)	Oven dry soil weight ($g\ pot^{-1}$)	WHC of sieved soil in pots (%)	Bulk density[c] of sieved soil in pots ($mg\ cm^{-3}$)
Brown podzol	Bogle Crag (woodland) SD 358929[a]	28	0.50	4.0	140	3.83	1700	945	114	0.44
Peaty gley	Gisburn (forest) SD 750585[a]	43	0.37	3.9	220	4.92	1530	583	207	0.25
Acid brown earth	Meathop (woodland) SD 435795[a]	11	0.80	4.6	430	2.40	2060	1356	68	0.60
Base rich brown earth	Merlewood (garden)[a] SD 408798	11	0.80	7.4	4740	3.42	2070	1468	57	0.63
Peat	Moor House (heather moor) NY 762340[a]	97	0.11	3.2	40	1.49	1600	281	460	0.16
Podzol (improved)	Wan Fell (improved grassland) NY 520345[a]	5	1.05	6.5	2890	1.89	2200	1754	33	0.79

[a] Grid References

[b] Estimated from the equation $y = 1.558-0.728\ (\log_{10}\ LOI\%)$ (Harrison & Bocock 1981)

[c] Measured value

Table 2. Total and available N content in soils together with plant biomass and its uptake of N (with standard error)

Soil Type	Total N in soil ($g\ litre^{-1}$)	Biomass dry weight g pot	Biomass N ($mg\ pot^{-1}$)	6% KCl ext. ($mg\ litre^{-1}$)	NH_4-N and NO_3-N % of total N
Brown podzol	3.82	18.14 (3.77)	200.4 (5.9)	77.8 (2.0)	2
Peaty gley	4.92	9.96 (0.17)	75.3 (1.6)	55.7 (4.1)	1
Acid brown earth	2.40	18.06 (0.78)	212.6 (5.1)	52.6 (2.0)	4
Base rich brown earth	3.42	8.49 (0.17)	61.2 (2.5)	24.8 (2.4)	0.7
Peat	1.49	3.16 (0.33)	26.8 (4.8)	14.3 (2.0)	0.9
Podzol (improved)	1.89	8.49 (0.18)	73.0 (2.4)	36.9 (2.6)	2

Table 3. Values of r^2 for relationships between plant uptake of N, resin bag adsorption of ammonium and nitrate-N and 6% KCl extractable ammonium and nitrate-N from six soils*

Methods	Biomass N	Resin bag NH_4-N + NO_3-N	Resin bag NH_4-N	Resin bag NO_3-N
Resin bag NH_4-N and NO_3-N	0.77***	-		
6% KCl ext. NH_4-N and NO_3-N	0.88***	0.82***	-	
6% KCl ext. NH_4-N	-	-	0.91***	-
6% KCl ext. NO_3-N	-	-	-	0.69***

* df = 1,16 *** significant at $P < 0.001$

r^2 x 100 = % variation accounted for

Table 4. Nitrogen availability index obtained by the resin bag and 6% KCl extraction methods in field (mean with standard error).

Tree species	Total inorganic N on resin bags ($mg.\ 2g^{-1}$) Late June 1985	Early Aug. 1981[a]	6% KCl extractable N(NH_4 & NO_3) from soil ($mg\ litre^{-1}$)
Quercus	0.003 (0.001)		2.88 (0.38)
Alnus	0.107 (0.035)	0.482 (0.08)	5.43 (0.47)
Pinus sylvestris	0.028 (0.011)	0.322 (0.10)	4.16 (1.14)
Picea abies	0.010 (0.003)	0.380 (0.09)	2.39 (0.35)
Picea sitchensis	0.147 (0.091)		5.83 (1.26)
Open plot (grass)	0.037 (0.013)		1.68 (0.18)

[a] Unpublished data from resin bags buried for two weeks (A F Harrison, ITE, Merlewood)

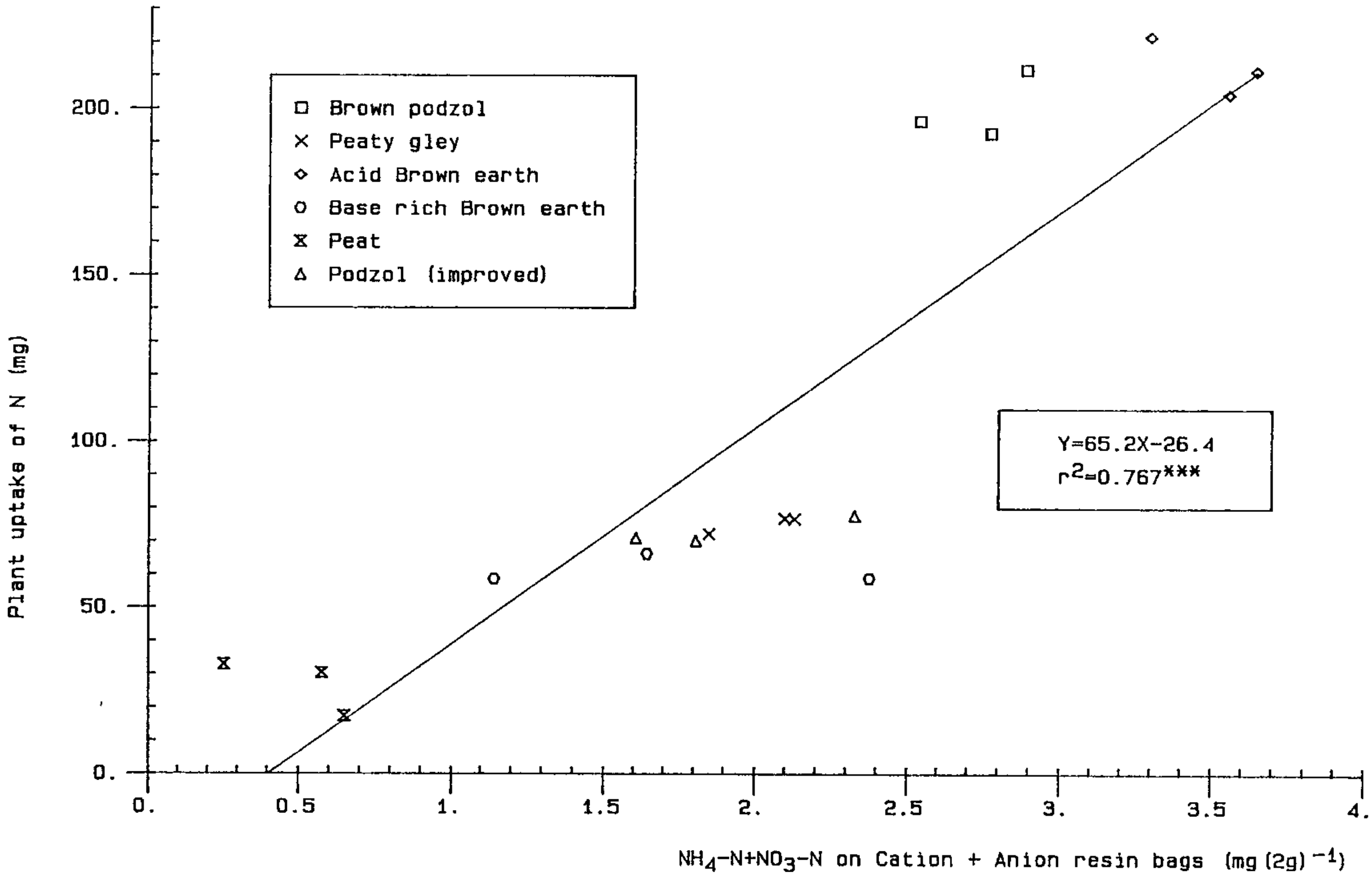

Figure 1. Relationship between plant and resin uptake of Nitrogen from six Cumbrian soils.

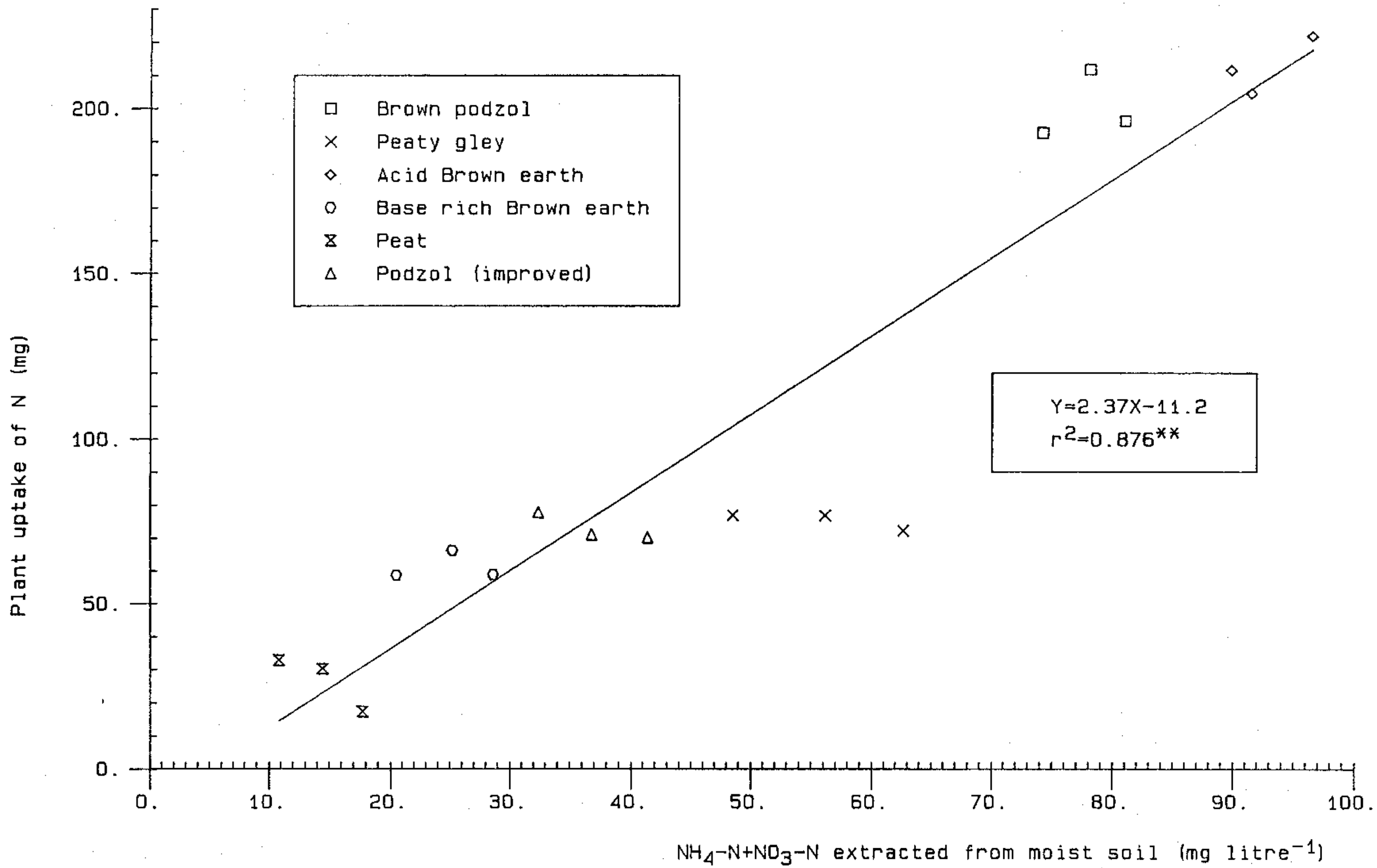

Figure 2. Relationship between plant uptake and 6% KCl extractable N from six Cumbrian soils.

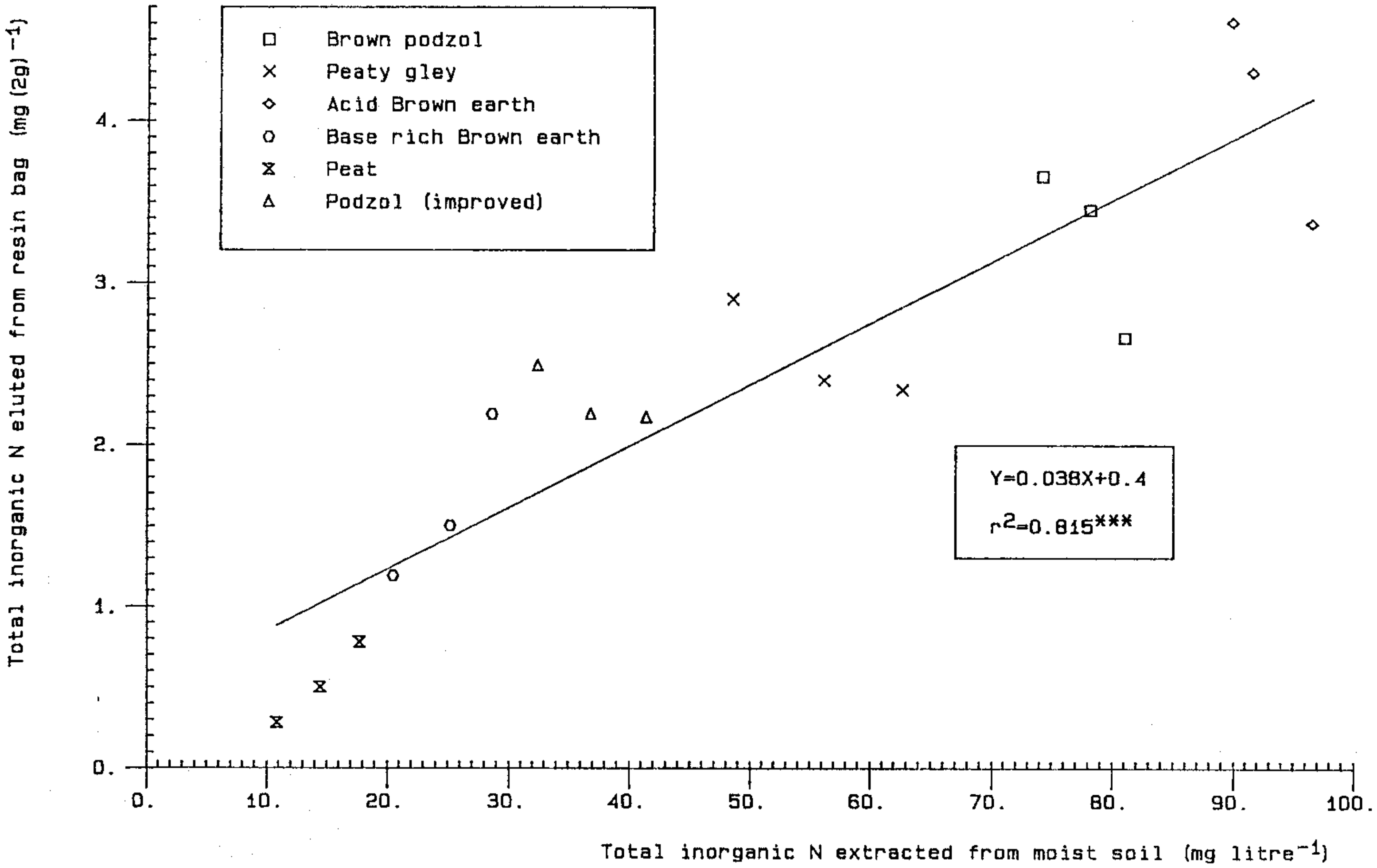

Figure 3. Relationship between Nitrogen adsorbed by resin and 6% KCl extractable N from six Cumbrian soils.

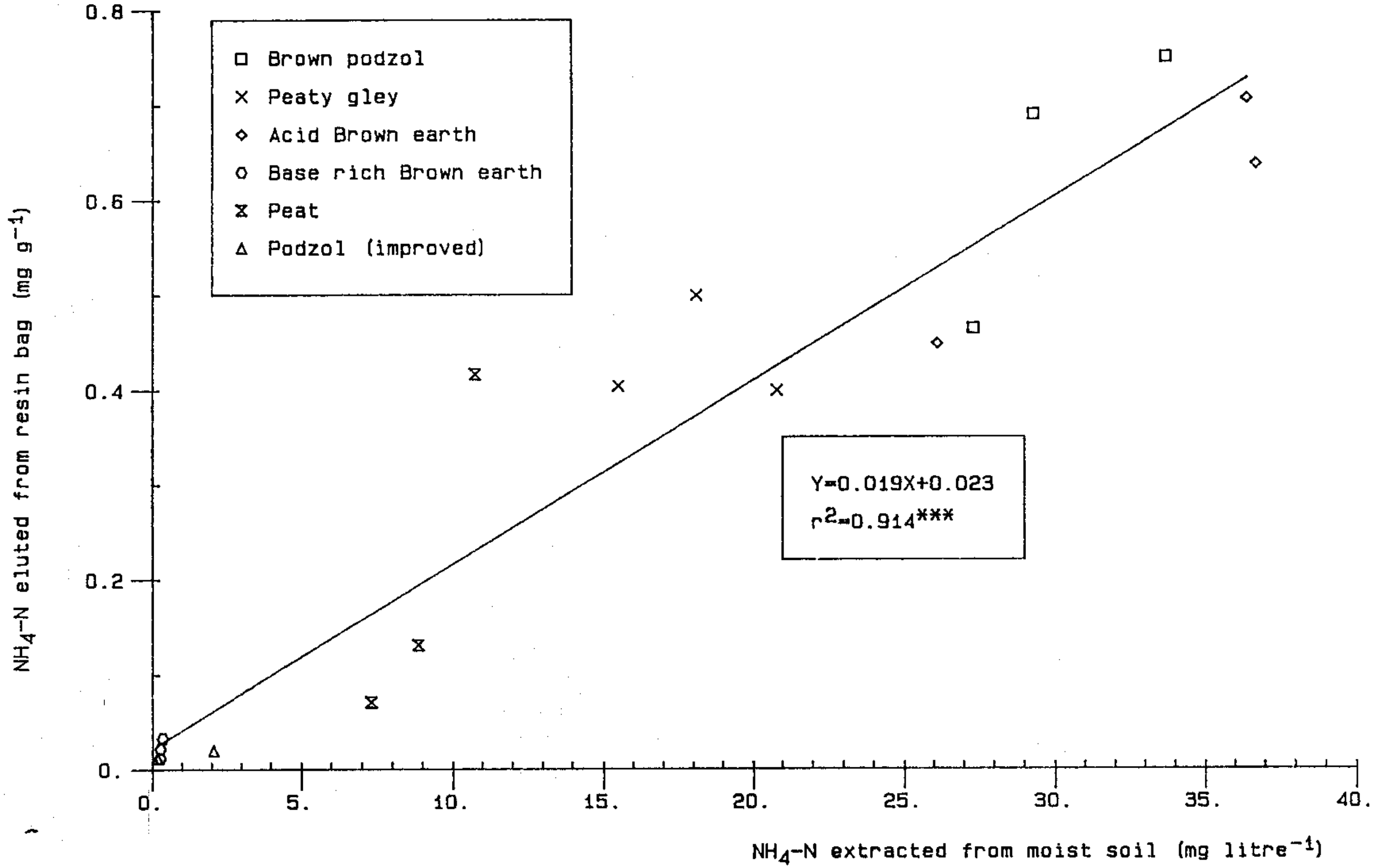

Figure 4. Relationship between NH_4-N adsorbed by resin and 6% KCl extractable NH_4-N from six Cumbrian soils.

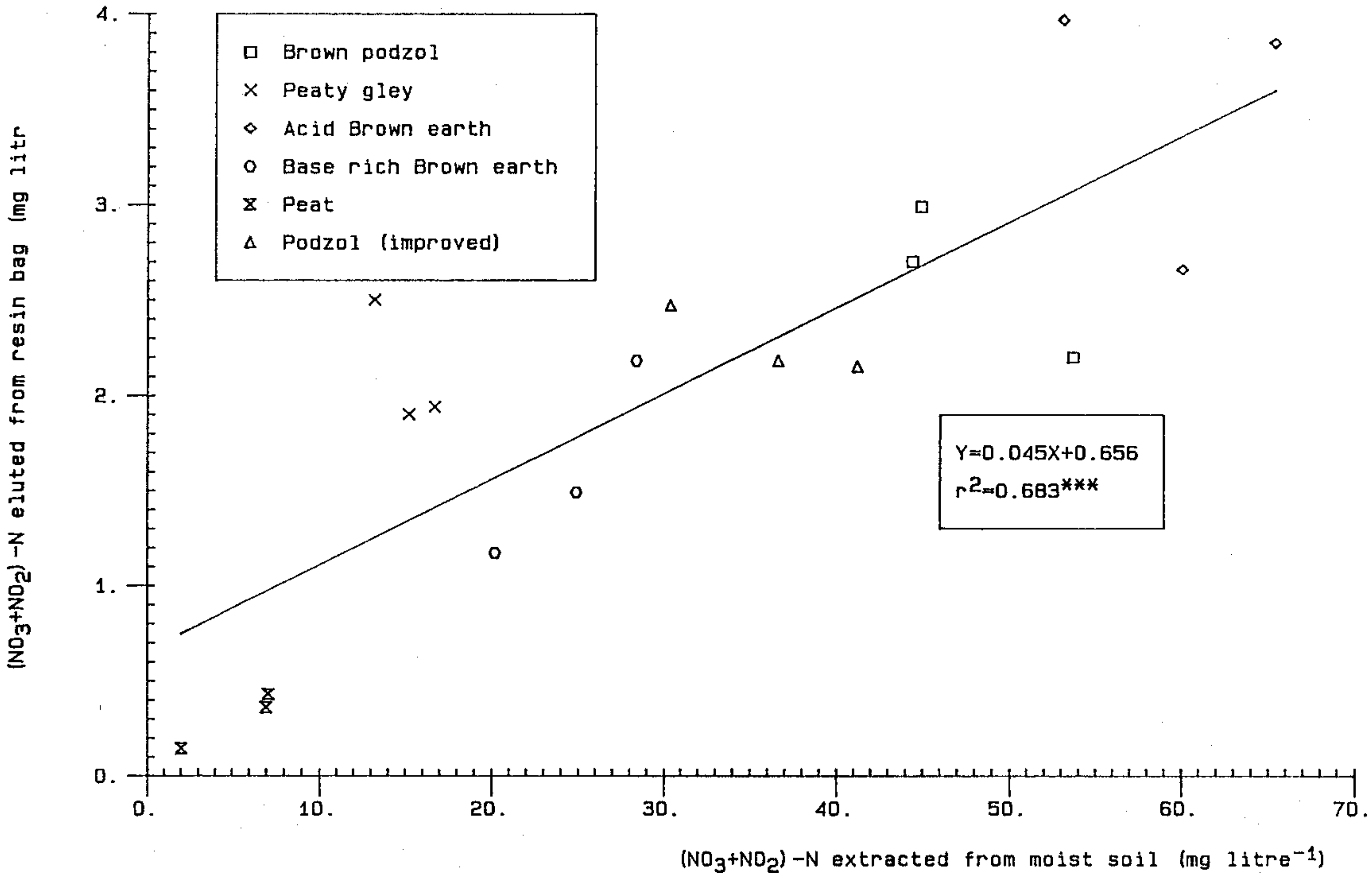

Figure 5. Relationship between (NO_3+NO_2)-N adsorbed by resin and extracted by 6% KCl from six Cumbrian soils.

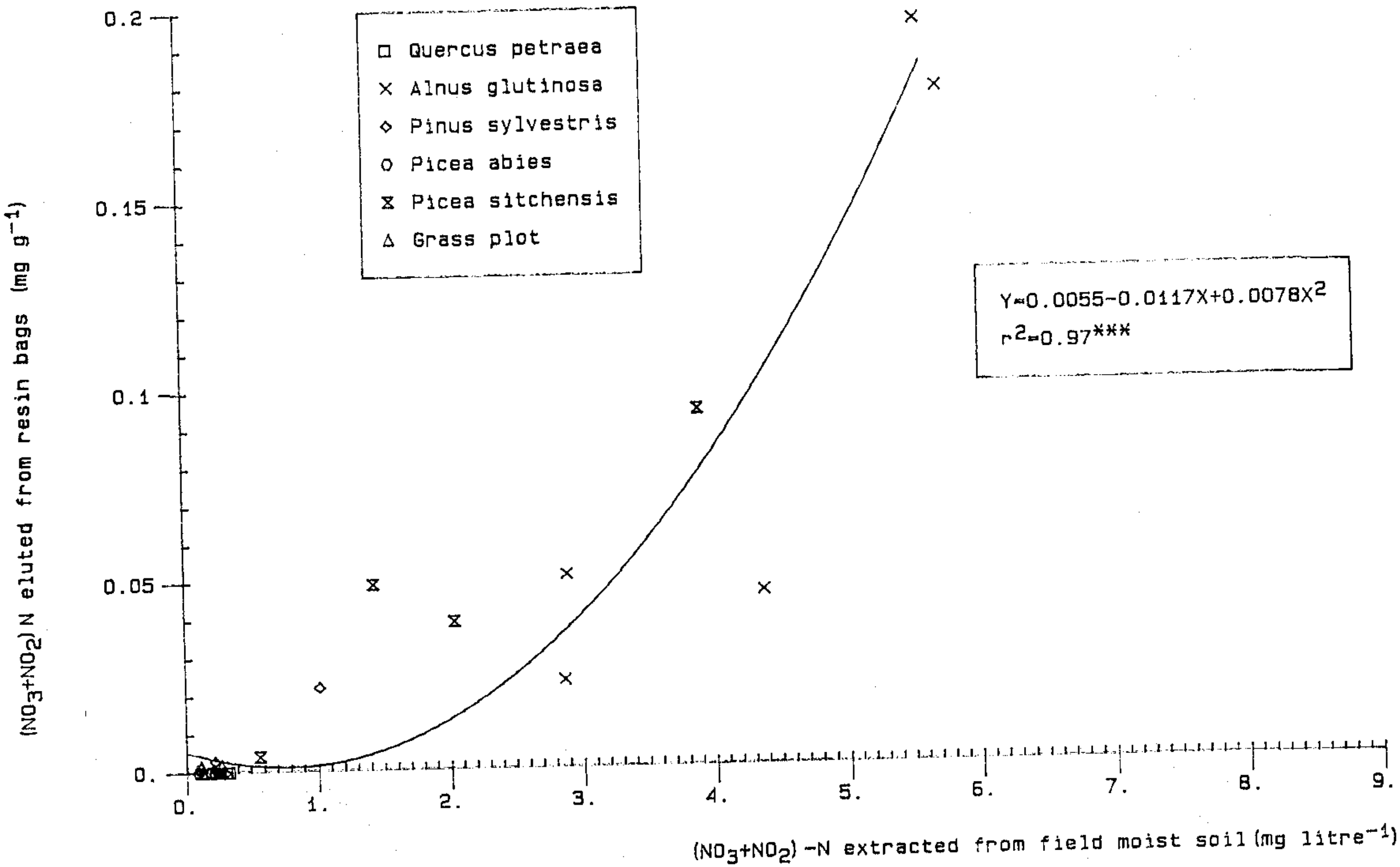

Figure 6. Relationship between (NO_3+NO_2)-N adsorbed by resin and extracted by 6% KCl from six field sites.

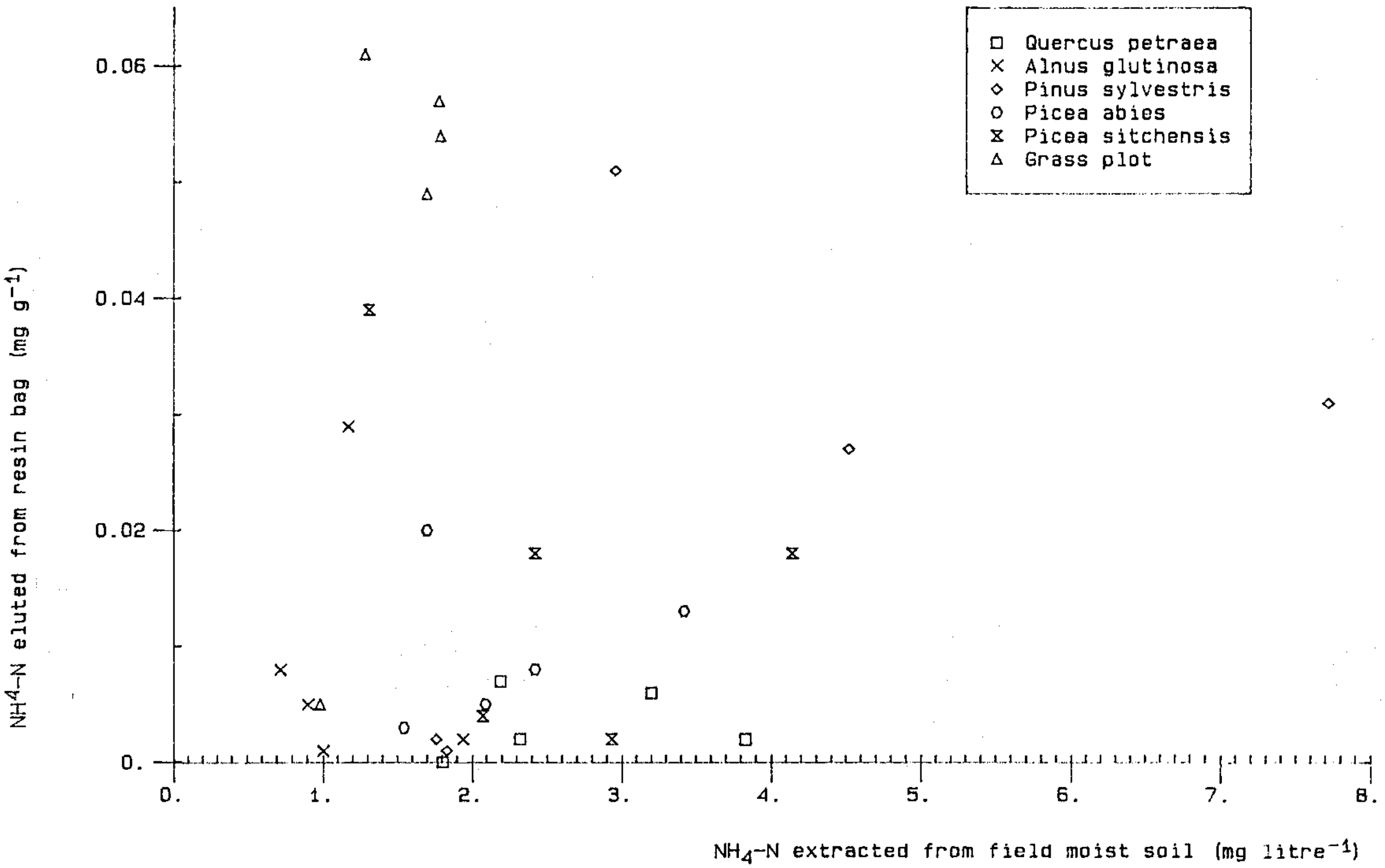

Figure 7. Relationship between NH_4-N adsorbed by resin and extracted by 6% KCl from six field sites.

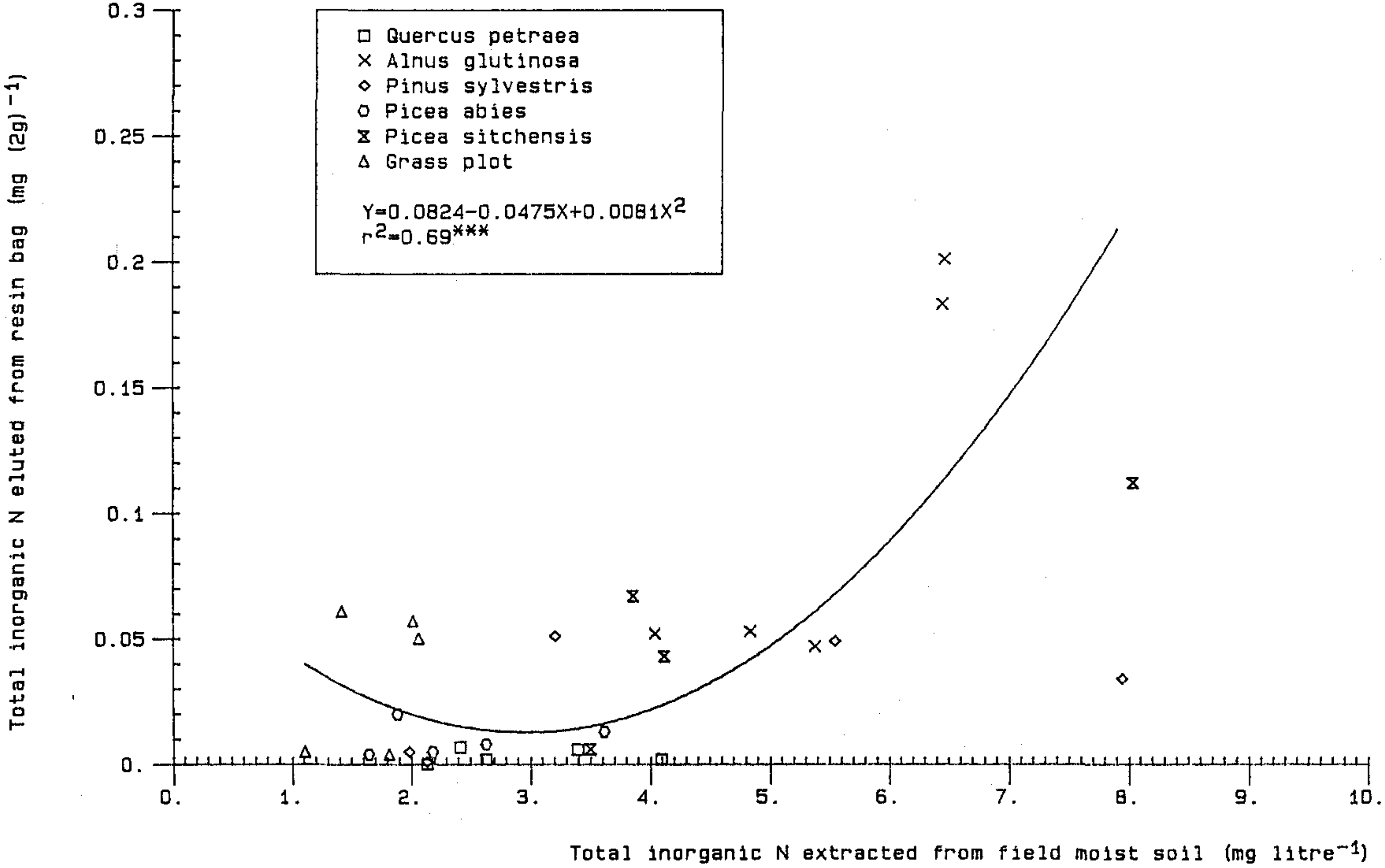

Figure 8. Relationship between Nitrogen adsorbed by resin and 6% KCl extractable N from six field sites.