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1 **Resilience of upland soils to long term environmental changes**

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14

15 **ABSTRACT**

16

17

18 The effect of long-term changes in land-use, pollution deposition and climate change
19 on upland soils was evaluated by resurveying a large set of sites in a mountain
20 landscape in the UK, which were initially sampled forty years ago. Unexpectedly,
21 despite the length of time between sampling dates, no significant changes in pH, soil
22 exchangeable base cations or C and N percentage content by weight were observed
23 across a range of soil type and parent material. This suggests that the soils have been
24 relatively resistant to the large changes in the environmental pressures experienced in
25 the past forty years, which include a 1.5 °C increase in mean temperature; the peak of
26 UK sulphur deposition in around 1970, followed by ~90% deposition reduction; long-
27 term increases in nitrogen deposition; and major changes in grazing intensity. These
28 results suggest that upland soils may be considerably more resilient to the future
29 environmental changes than many previous assessments have suggested.

30 *Keywords:*

31 Climate change

32 Nitrogen

33 Montane

34 Grazing

35 Land use

36 Atmospheric pollution

37 Soil cores

38 Soil chemistry

39

40 **1. Introduction**

41

42 Mountain ecosystems are considered particularly sensitive to environmental
43 changes (Thuiller, 2007) and may provide effective systems in which to detect and
44 assess the ecological impacts of climate change (Beniston, 2003). Some of the
45 greatest changes in land-use, and strongest climatic warming, have been experienced
46 in Central and Northern European mountain ecosystems due to the complex
47 variability of snow, ice and temperature extremes (Hagedorn et al., 2010). However,
48 the impact of these changes on soils, and their role in cycling of carbon and nutrients,
49 is largely unknown (Hagedorn et al., 2010).

50 Soil biogeochemistry can also be significantly affected by acidifying inputs of
51 sulphur (S) and nitrogen (N) (Morecroft et al., 2009). Because deposition rates vary
52 with topography, upland areas receive disproportionately more S and N deposition
53 compared with lowlands (Kirkham, 2001). These inputs can cause reductions in soil
54 pH and a loss of base cations (Reuss and Johnson, 1986), ultimately resulting in shifts
55 in plant species composition (Smart *et al.*, 2005; Horswill et al., 2008; Maskell et al.,
56 2010; Stevens et al., 2010). Sulphur emissions in the UK peaked around 1970, and
57 subsequently declined, by 80% between 1986 and 2007 (RoTAP, 2012). Soil
58 resurveys and a small number of soil solution monitoring sites show that decreasing
59 acid deposition has resulted in an increase in soil pH across the UK (Kirby et al., 2005;
60 Morecroft et al., 2009; Emmett et al., 2010; Kirk et al., 2010). This trend is also
61 evident in higher-frequency long-term monitoring data from surface waters in upland
62 semi-natural ecosystems (e.g. Davies et al., 2005; RoTAP, 2012).

63 Humans have greatly impacted the rates of supply of many of the major
64 nutrients that constrain the productivity, composition and diversity of terrestrial

65 ecosystems, and as such are causing rapid environmental changes (Tilman and
66 Lehman, 2001). The formation of reactive N has increased globally by 120% and
67 continues to increase each year (Galloway et al., 2008). Within the UK, acidic and
68 calcareous grasslands are threatened by increases in N deposition (Lee and Caporn,
69 1998), particularly where vegetation is adapted to low soil fertility (Willems et al.,
70 1993). The fate of pollutant N within a system and its effects on N budgets is not
71 fully understood at present. If these systems are at least partially phosphorus (P)
72 limited they are likely to become more readily N saturated (Phoenix et al., 2004),
73 resulting in increased leaching of pollutant N, or they may accumulate much of the N
74 in biomass and soil organic matter (Aber et al., 1989; Phoenix et al., 2004). In general,
75 carbon-poor ecosystems such as montane habitats on thin soils are more susceptible to
76 N saturation, because they have a low capacity to store deposited N in organic matter
77 (Evans et al., 2006a). In the UK, N deposition over the last 20 years has remained
78 fairly stable (RoTAP, 2012), and there is little clear evidence of progressive N
79 saturation of terrestrial or freshwater ecosystems during this time (Curtis et al., 2005;
80 Emmett et al., 2010). However, N inputs remain far above background levels, and
81 budget studies suggest that much of this N is accumulating in soils (Morecroft et al.,
82 2009). This may have considerable consequences for the recovery of the ecosystem
83 in the future if deposition levels were to decline (Phoenix et al., 2004).

84 Increased N deposition may also impact on other nutrients, particularly carbon
85 (C). In most regions of the world, including the UK, the largest terrestrial C stocks lie
86 below ground (Bradley et al., 2005). N addition has been found to be positively
87 correlated with increases in soil organic matter in both moorland and forest
88 ecosystems (Evans et al., 2006b; de Vries et al., 2009) and Kirby et al. (2005)
89 observed a positive correlation between N deposition and soil organic matter across

90 the UK. The increased rate of accumulation is thought to occur through two
91 mechanisms: firstly, through an increase in plant biomass and increased litter
92 production, and secondly, through a reduction in the long-term decomposition rate of
93 organic matter (e.g. Waldrop et al., 2004; Reay et al., 2008; Janssens et al., 2010).
94 Additional C is not fixed however, if the additional N inputs are directly immobilised
95 within the soil in a form which is inaccessible to plants (De Vries et al., 2006), or if
96 systems are N saturated, so that additional N is leached to surface waters or lost via
97 denitrification. Understanding whether soils will become long term sinks or sources
98 of C is dependent on understanding the N cycle within semi-natural ecosystems (De
99 Vries et al., 2006). A resurvey of UK soils reported a loss of topsoil C between 1978
100 and 2003, and attributed this to climate change (Bellamy et al., 2005). However, the
101 attribution of the decrease to climate change has been questioned (Smith et al., 2007),
102 and later analyses of these data suggest that only 10% of the observed changes could
103 be attributed to climate, with the majority of soil C loss attributed to changes in land
104 use and management (Smith et al., 2007; Kirk and Bellamy, 2010), possibly
105 augmented by reducing soil acidity in the uplands (Evans et al., 2007). Land use
106 change has also been identified as responsible for the greatest changes in soil C
107 (Stevens and Wesemael, 2008). Furthermore, other long term soil resurvey studies in
108 the UK have observed either a slight increase (Kirby et al., 2005), or no significant
109 change in soil organic matter over similar time periods (McGovern et al., 2011;
110 Emmett et al., 2010).

111 The Snowdon area of North Wales has the highest elevation within the
112 southern UK, and a wealth of historic and current environmental data are available.
113 Snowdon itself has an iconic status as the highest mountain in Wales, and provides a
114 diverse range of ecosystem services, ranging from provision of drinking water, food

115 from livestock grazing and energy from hydropower, to recreation and culturally
116 significant biodiversity (Dick et al., 2011). Ball et al. (1969) provided detailed
117 information on the distribution of soil types across Snowdon and their chemical
118 composition in 1968. Concurrently, the International Biological Programme (IBP)
119 completed an assessment of land-use (Brasher and Perkins, 1978) and climatic
120 measurements (Perkins, 1978) in the area. Additional land use measurements on the
121 intensity of grazing were also carried out (Dale and Hughes, 1978). More recently,
122 part of the Snowdon massif became a site within the Environmental Change Network
123 (ECN), which monitors environmental drivers, particularly climate, pollution and
124 land-use, and the biological responses of habitats and key taxa (Morecroft et al.,
125 2009). ECN protocols for plant, animal, soil and water monitoring (Sykes and Lane,
126 1996) have been conducted since 1995, along with measurements using the original
127 IBP meteorological equipment to enable comparison with the original IBP data. This
128 monitoring has revealed significant shifts in key drivers of soil change, including
129 reductions in grazing intensity following their 1990s peak, increases in temperature
130 since the 1970s, and decreases in atmospheric SO₂ and NO₂ (Lloyd et al., 2012).
131 These changes are compared to longer-term national- or regional- scale trends in these
132 environmental drivers in Fig. 1, which illustrates the magnitude of changes that have
133 occurred since the original soil survey.

134 In this study, we present the results of a resurvey of soil chemistry at a large
135 set of sites first visited in 1968 as part of a survey by Ball et al. (1969). The aims of
136 the study were: i) to quantify any change in the soil chemical composition that has
137 occurred on Snowdon during this 40 year period; and ii) to identify the main drivers
138 of these changes based on analysis of the detailed environmental records for the site.

139

140 **2. Materials and Methods**

141

142 *2.1. Sample site*

143 Sample sites, located across the Snowdon massif, were relocated in autumn
144 2008 using original GB six figure Ordnance Survey grid references. Precise sampling
145 sites were further identified by detailed location, relief, altitude and soil descriptions
146 in the original report. To account for any error in exact site relocation, three replicates
147 were taken at each site, spaced one metre apart. Each replicate consisted of three 5
148 cm diameter cores, taken to a depth of 15 cm and spaced 30 cms apart. After
149 collection, cores were split into horizons and bulked, to provide the three individual
150 replicates per original sampling site. Each sample site was categorised by underlying
151 geology and soil type so that the investigation could also test whether the response of
152 soil chemistry to environmental drivers was consistent.

153

154 *2.2. Chemical analyses*

155

156 Chemical analyses were carried out following the methods originally used by
157 Ball et al. (1969), to minimise any systematic errors that might result from changes in
158 analytical methods since the original survey was undertaken. Briefly, analysis was
159 completed on soil that passed through a 2mm sieve after air drying (35°C) and light
160 grinding. pH was measured using a Hanna instruments pH 209 pH meter in a solution
161 of 1:2.5 soil to water ratio. C was calculated from loss-on-ignition (Ball, 1964).
162 Total nitrogen was determined using the Kjeldahl method on oven-dry soil (105°C)
163 using a Foss 2300 Kjelttec analyzer unit. Exchangeable cations were extracted using a
164 1:40 soil to neutral 1M ammonium acetate ratio and analysed using a Perkin Elmer

165 AAnalyst 400 Atomic Absorption Spectrometer. P was extracted using a 1:40 soil to
166 0.5M acetic acid ratio and analysed using the molybdate blue colorimetric method.

167

168 *2.3 Statistical analysis*

169

170 To assess any change between sampling years, analysis was carried out in R
171 (R Development Core Team, 2010) using paired t-tests. To test if underlying geology
172 or soil type influenced soil chemical changes, the difference between the two years
173 was calculated and analysed using an ANOVA. Data were transformed where
174 necessary to meet assumptions of normality and homogeneity of variance.

175

176 **3. Results**

177

178 Results of the soil resurvey indicate that there have been only small changes in
179 the measured chemical soil properties on Snowdon during the last forty years (Table
180 1). Between the two sampling dates (1968 and 2008) there was a slight decline in pH,
181 by an average of 0.08 units. Similarly, there was a small decrease in total soil
182 exchangeable base cations. These results both suggest some acidification of the soil
183 by deposition of acidifying compounds during the intervening period, rather than any
184 recovery in response to falling S deposition levels since the 1970s, although changes
185 were not statistically significant. Individual responses of soil types varied with no
186 consistent trends in exchangeable base cations found.

187 The direction of change in pH appears to be dependent on the original pH
188 value of the soil. Acid soils have increased in pH and are now less acidic than initial
189 values; whereas the previously less acidic soils have shown a decrease in pH (Fig. 2).

190 Similarly contrasting results were found for base cation concentrations, with increases
191 in the previously more acidic soils and decreases in the less acidic soils.

192 The C and N content of the soil appears to have increased, particularly in
193 brown podzolic soils. On the other hand, the C:N ratio has shown little change. An
194 increase in extractable P was also found. However, none of these results were
195 statistically significant. These changes all suggest a small increase in soil total
196 nutrient stocks, consistent with the long-term accumulation of organic matter.
197 However, the absence of a reduction in C:N ratio implies that this may not necessarily
198 equate to an increase in soil fertility.

199 There was little difference in the amount of within-year variation between the
200 two surveys for most of the soil chemical variables measured; only Potassium (K)
201 showed a large increase in the within-survey variation in 2008. Because K comprises
202 only a small part of the exchangeable base cation pool, which is dominated by Ca
203 (Table 1), total soil exchangeable base cations showed little difference in variation
204 between years.

205 To investigate if there were significant differences between individual soil
206 types or soils on the same underlying geology, the percentage change in the variables
207 measured relative to their concentration in 1968 were calculated. Groups were only
208 included within the analysis if they contained three or more replicates. Snowdon has
209 a highly complex geological history, and as such many geological groups were found.
210 Few, however, contained three or more replicates and so most were discarded from
211 the analysis.

212 There was large variation in the response of the soils, even within the same
213 soil type (Fig. 3). No significant differences were found in any of the soil chemical

214 variables measured although there was some variation between soil types. No
215 significant difference was found between the years within individual soil categories.

216 Similar results were found for underlying geology as were found for soil type.
217 No significant differences existed between geological groups in any soil chemical
218 variable measured (Fig. 4). One group however, the rhyolitic and bedded pyroclastic
219 series, did show a significant increase in N content between 1968 and 2008 ($p =$
220 0.018).

221

222 **4. Discussion**

223

224 Despite large-scale changes in the environmental conditions on Snowdon, this
225 study indicates that the soils are in a similar condition to that of forty years ago. A
226 number of long term monitoring schemes have identified a recovery in pH of the soil
227 across the UK and attributed this to the reductions in the deposition of acidifying
228 compounds (Kirby et al., 2005; Morecroft et al., 2009; Emmett et al., 2010; Kirk et al.,
229 2010). This study identified an overall reduction in soil pH (albeit very small), in
230 contrast to these studies, and to a similar study completed on Snowdon based on a
231 different dataset covering a smaller area (McGovern, et al., 2011). This apparent
232 contradiction may not be as surprising as it first seems. The peak in deposition of
233 acidifying compounds, namely S, occurred around 1970, just after our original survey
234 took place. By the time the initial samples from the other, UK-wide studies were
235 collected, in the late 1970s, there had already been a drop in S emissions of
236 approximately 20% (NEGTAP, 2001). In general, there is a lag between deposition
237 and environmental effects (Monteith and Evans, 2005), and this is reflected in process
238 models of acidification which show a delay between increasing deposition and
239 ecosystem damage, and reducing deposition and ecosystem recovery (e.g. Hettelingh

240 et al., 2007). As the first Snowdon survey was undertaken during a period of rising
241 acid deposition, it is likely that the full extent of soil acidification had not yet occurred.
242 Similarly, as deposition levels are now falling, soil pH recovery may also be lagged.
243 Furthermore, since deposition levels remain above critical loads for acidification in
244 much of Wales (Hall et al., 2004), only a partial recovery in pH and base saturation
245 can be expected (e.g. Evans, 2005). The initial samples in the UK wide studies may
246 have captured the peak in acidification exhibited by UK soils, whereas this study pre-
247 dates it. This may be why the soils appear to have acidified further rather than
248 indicated a recovery.

249 Looking more closely at the response of soil pH, it appears that the previously
250 less acidic soils show larger changes in pH. The initially more acidic soils showed an
251 increase in soil pH, suggestive of recovery from acidification while the previously less
252 acidic soils are still showing a decrease in soil pH. The reduction in soil pH may have
253 implications for the plant communities on Snowdon and a number of studies have
254 found links between reductions in soil pH and a loss of species richness (Maskell et
255 al., 2010; Stevens et al., 2010). The buffering capacity of acid and alkaline soils
256 differs, with poorly buffered acid soils tending to respond relatively quickly to
257 changes in deposition, whereas the larger buffering capacity of alkaline soils will lead
258 to lags between the deposition peak and the peak of acidification ('Damage Delay
259 Time'; Hettelingh et al., 2007). This could explain the contrasting changes in pH of
260 acid and alkaline soils on Snowdon during a period in which S deposition has peaked,
261 and then fallen, but still remains above the critical load. The absence of consistent
262 recovery in soil pH across Snowdon, despite substantial reductions in the UK
263 deposition acidifying compounds (Figure 2), highlights the considerable length of

264 time it may take for ecosystems to recover from the large historic perturbations to
265 their chemical cycles.

266 Acid deposition affects not only soil pH but also soil exchangeable base cation
267 concentrations. A small decrease was observed in soil exchangeable base cation
268 concentrations, although there was high variation and no clear trends emerged,
269 consistent with the results for soil pH. The extent of base cation depletion is largely
270 determined by the type of soil and the rate of weathering of the underlying geology.
271 In coastal areas, deposition of base cations from sea-salt can occur, resulting in the
272 alteration of cation exchange equilibria within the soil (e.g. Evans et al., 2001), with
273 greater deposition occurring at times of increased storm events, which tend to
274 coincide with large positive values of the winter North Atlantic Oscillation (NAO)
275 index (Hurrell, 1995;). Despite a higher winter NAO index in 2008 compared to
276 1968 (December-March means derived from
277 <http://www.cru.uea.ac.uk/~timo/datapages/naoi.htm>), which might indicate greater
278 seasalt inputs prior to the recent survey, the Na content of the soil was actually higher
279 in 1968. Therefore there was no evidence to suggest that frequency of antecedent
280 storm occurrence had a major impact on the soil chemistry within the sampling years.
281 Few other long term studies have reported data for exchangeable base cation
282 concentrations, so there is little measurement data against which to evaluate these
283 results. However modelling studies conducted in similar locations have suggested
284 that recovery in soil pH will be more rapid than recovery in base saturation (e.g.
285 Evans, 2005). A recent study on Snowdon incorporating only brown earth soils above
286 pumice-tuffs of relatively high base status found significant reductions in soil
287 exchangeable base cation concentrations, concurrent with an increase in pH,
288 suggesting that recovery from acidification following S deposition reductions may not

289 yet have led to the effective recharge of soil base cation status (McGovern et al.,
290 2011). The underlying geology was not recorded as part of the current study, but it is
291 likely that the greater variability of soil and bedrock type across the study area
292 contributed to the observed variability of base cation changes.

293 As was found in the Countryside Survey 2007 (Emmett et al., 2010) and
294 previous Snowdon surveys (McGovern et al., 2011), this study has shown no
295 significant change in the C content of the soil, although there was a small increase in
296 percentage C content . It has been suggested previously that warming (Bellamy et al.,
297 2005) and changes in land-use and management can affect soil C (Smith et al., 2007;
298 Kirk and Bellamy, 2010). Soils were sampled from a wide area of Snowdon, so it is
299 not possible to ascertain the exact changes in grazing density for each site, but it is
300 likely that the broad trend of decreasing grazing density has occurred throughout the
301 area. Increasing grazing has been linked to a loss of soil C and N, as nutrient retention
302 in high disturbance habitats is lowered (Klumpp et al., 2009). Therefore the recent
303 reduction in grazing levels may have contributed to the increase in soil C and N
304 identified in this study. Alternatively, the increases in soil C and N may be as a result
305 of increased N deposition, and associated organic matter accumulation within the soil
306 (e.g. De Vries et al., 2009). Kirby et al. (2005) found a correlation between increases
307 in soil organic matter and N deposition, and Morecroft et al. (2009) found evidence of
308 N accumulation in soil. The results from this study are in agreement with this,
309 although distinguishing between the two mechanisms of soil C and N increases is
310 difficult.

311 Overall, the soils on Snowdon appear to have been remarkably robust through
312 a period of major environmental change. Despite a temperature increase of around 1.5
313 °C, no significant changes attributable to climate change could be detected. As there

314 are currently only two time points of data, it is not possible to identify whether the
315 rate of change of soil chemistry has varied throughout that period. With snapshot
316 studies such as this, it is also possible that fluctuations in values could occur between
317 collection times but remain undetected. Repeating this study in the future would
318 potentially allow identification of patterns of change, albeit at a coarse resolution.
319 More intensive ongoing monitoring of soil and soil solution chemistry at the Snowdon
320 ECN site should permit assessment of the interannual variability of soil chemistry,
321 and its shorter-term response to climate, deposition and land-use variations in future.

322

323 **5. Conclusion**

324

325 The absence of significant changes in soil composition on Snowdon, over a 40
326 year period in which major changes in key environmental drivers have occurred,
327 provides important insights regarding the sensitivity of soils, and upland ecosystems,
328 to long-term environmental change. Many studies have reported recent changes to
329 soil composition in a UK context, based on large-scale surveys, but our more
330 intensive study of a single location suggests a surprising degree of stability over a
331 longer period. One explanation for this lack of change is that upland soils have been
332 resilient to the (large) environmental pressures to which they have been exposed
333 during the last century. A second interpretation is that soils have been damaged by
334 previous changes, principally acidification, and have not yet recovered. A final
335 possibility is that further damage occurred during the decades following the initial
336 survey in 1968, and that subsequent recovery has been sufficient to reverse this
337 damage, but not to return the system to pre-acidification conditions. This last
338 interpretation appears most consistent with the results of more recent UK-level

339 surveys showing increases in soil pH at broader scales over shorter periods. Site
340 specific monitoring of changes in environmental drivers and their impacts, using
341 historic and existing data, has an important role in providing detailed support to
342 broader-scale survey-based approaches, and in elucidating the interactive effects of
343 multiple pressures on specific ecosystems.

344

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355

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Figure Captions

Fig. 1. **a)** Mean annual temperature residual compared to 1966 -1977 mean, based on Central England Temperature Record (Parker et al., 1992; <http://www.metoffice.gov.uk/hadobs/hadcet/>), ; **b)** total sheep grazing numbers for the County of Gwynedd (adjusted for boundary changes in 1996 based on area change); **c)** UK total sulphur emissions and **d)** UK nitrogen emissions from the UK National Atmospheric Emissions Inventory (<http://naei.defra.gov.uk>); **e)** Mean temperature residual measured at the Snowdon ECN automatic weather station compared to the mean of an earlier 1966 -1977 monitoring period at the same location; **f)** mean weekly sheep numbers at the ECN site; **g)** mean annual wet and dry sulphate concentrations and **h)** mean annual total inorganic nitrogen concentrations in precipitation between 1996/7 and 2011 at the Snowdon ECN site. (Lloyd et al., 2012)

Fig. 2. Measured soil pH in 2008 versus measured pH in 1968. Solid line represents 1:1 line (i.e. no change in pH), dashed line represents best fit based on linear regression ($R^2 = 0.11$, $p = 0.05$, $n=35$).

Fig. 3.

Box plots (median, 25th- and 75th- percentile values, min and max values) of the percentage change relative to 1968, by soil type of a) pH, b) Nitrogen, c) Carbon, d) Carbon/Nitrogen ratio, e) Phosphorus, and f) Total soil exchangeable base cations. Only soils with $n \geq 3$ are shown. AS = All soils $n=31$, BE = brown earth $n= 5$, BP = brown podzolic $n=6$, PP = peaty podzol $n=5$, PR = peat ranker $n= 7$, PG = peaty gley $n= 3$ and PSP = peaty soil and peat $n=5$. Circles represent outliers. Dashed line indicates no change.

Fig. 4.

Box plots (median, 25th- and 75th- percentile values, min and max values) of the percentage change relative to 1968, by parent material of a) pH, b) Nitrogen, c) Carbon, d) Carbon/Nitrogen ratio, e) Phosphorus, and f) Total soil exchangeable base cations. Only parent materials with $n \geq 3$ are shown. APM = All parent materials $n=20$, BPS = bedded pyroclastic series $n= 11$, RBP = rhyolitic & bedded pyroclastic series $n=3$, RBPS = rhyolitic, bedded pyroclastic series & slate $n=3$ and RR = rhyolitic rocks $n=3$. Circles represent outliers. Dashed line indicates no change.

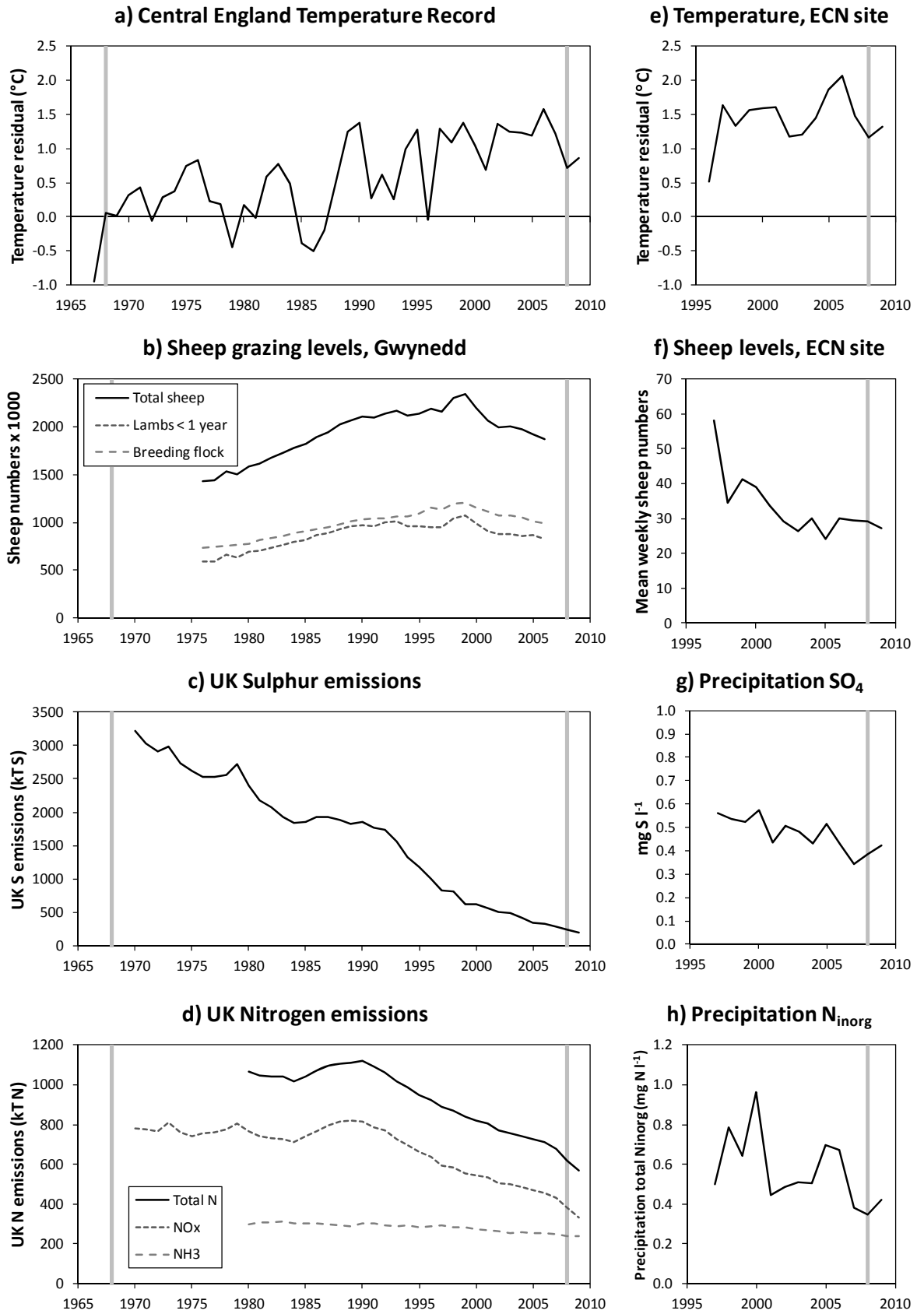


Fig. 1.

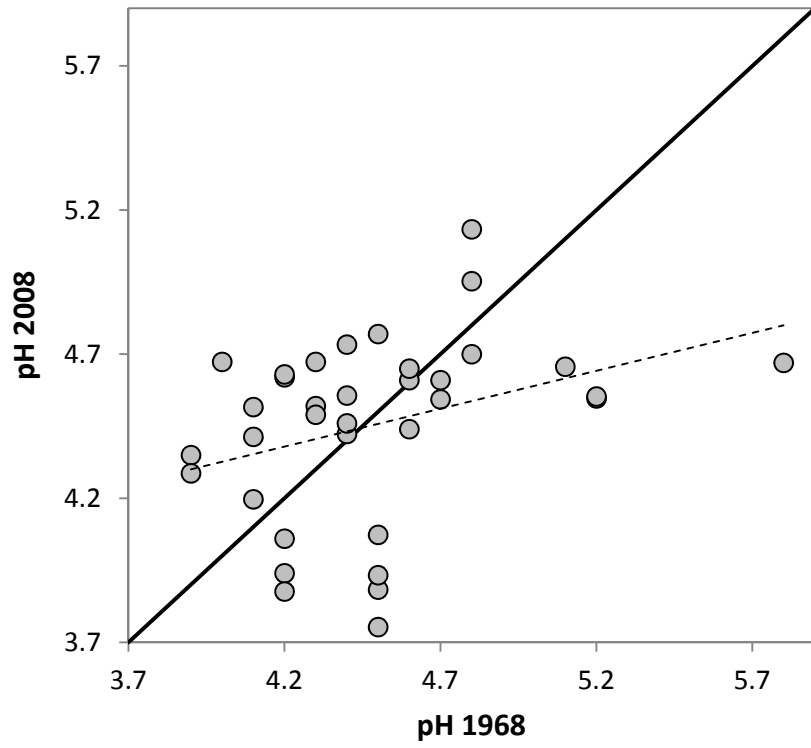


Fig. 2.

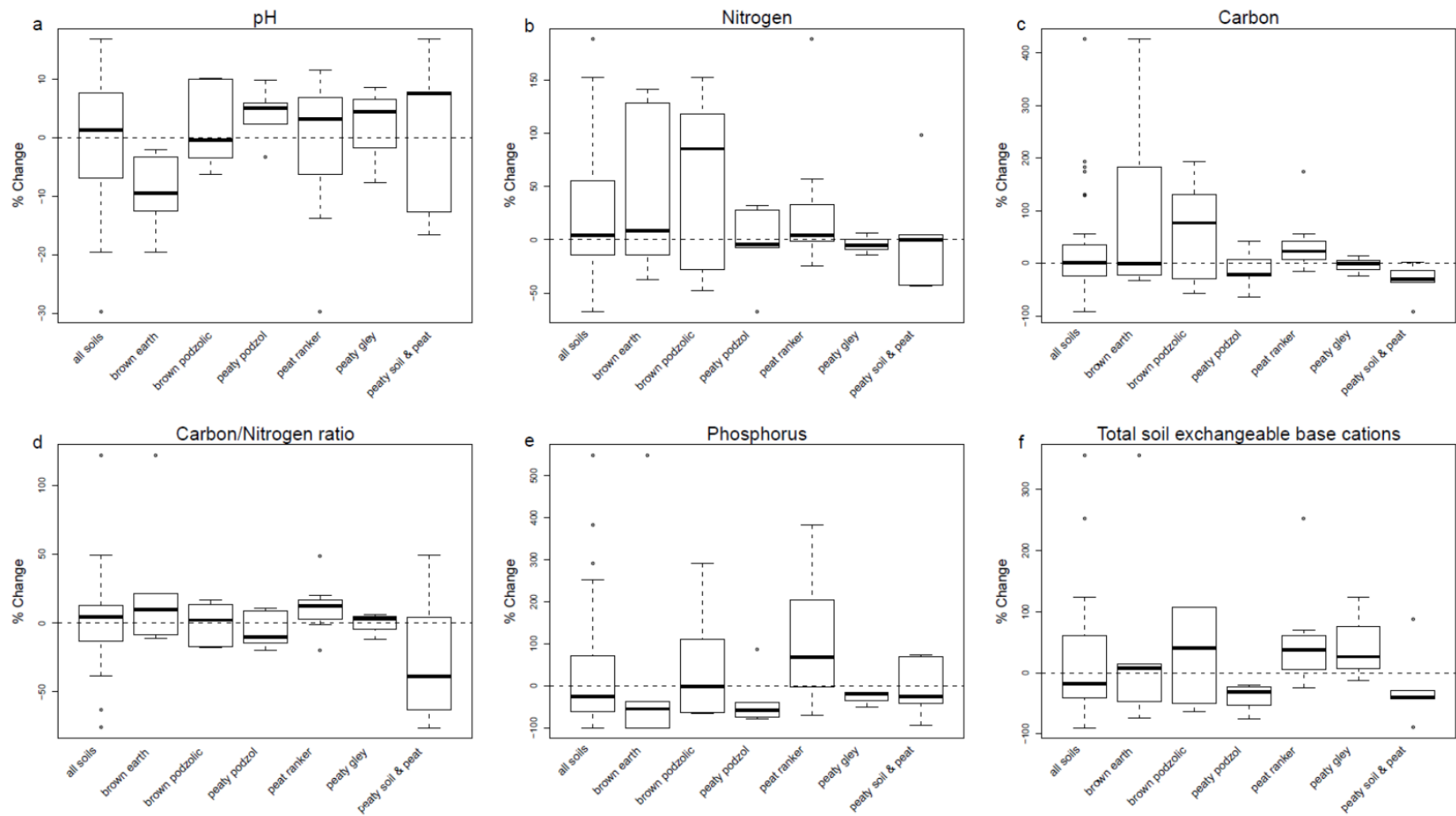


Fig. 3.

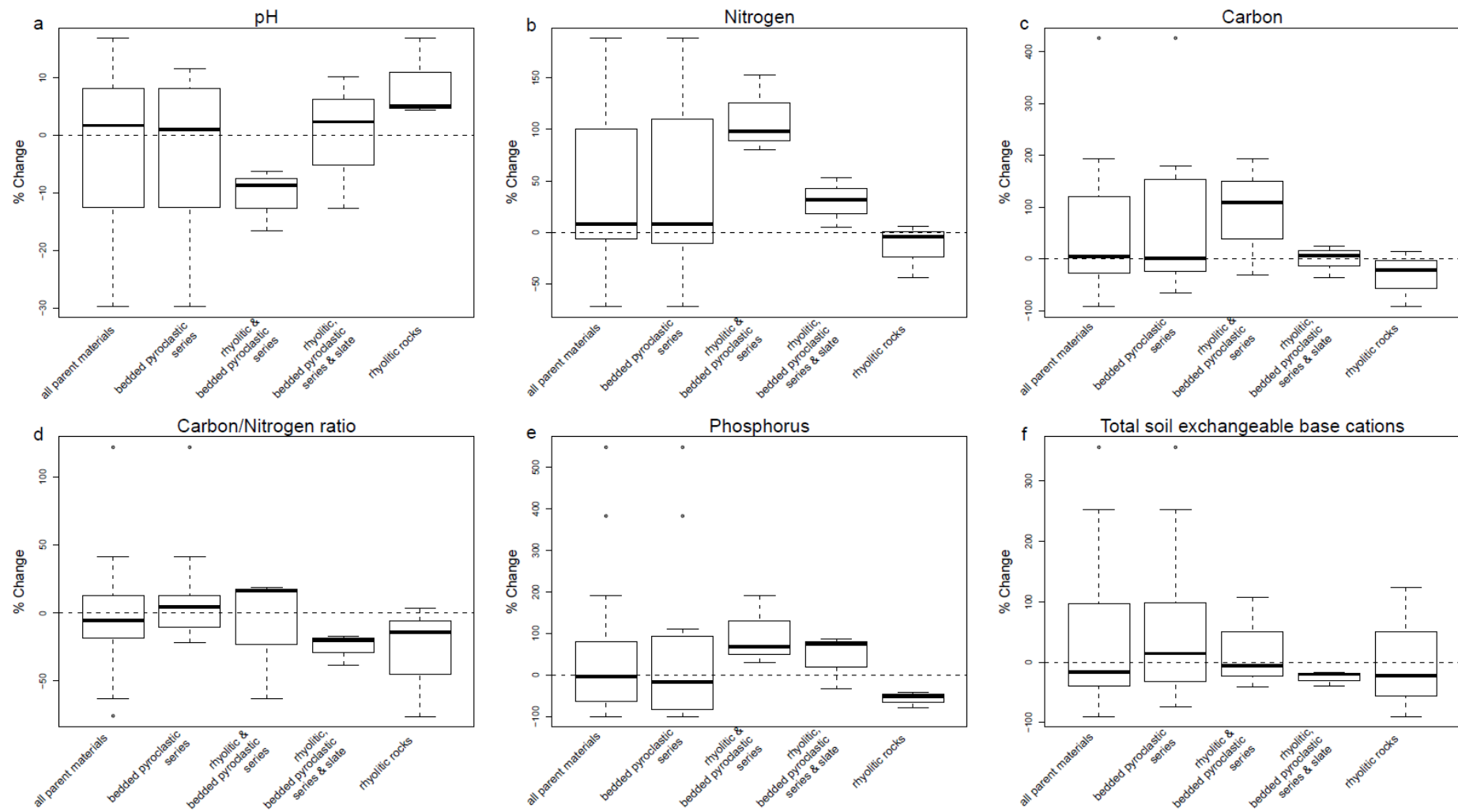


Fig. 4.

Table 1

Results of paired t-tests to determine the effect of sampling year on soil chemical variable. Mean values are displayed and values in parentheses are one standard error of the mean ($n=36$) (\ddagger) $n=35$). Asterisk indicate degree of significance n.s. $P \geq 0.05$, * $P < 0.05$, ** $P < 0.01$, *** $P < 0.001$.

Year	Exchangeable cations (me 100g ⁻¹)							Extractable P ₂ O ₅ (mg 100g ⁻¹)	C (calculated wt %)	Total N (wt %) ^(‡)	C/N Ratio ^(‡)
	pH	Ca ^(‡)	K	Na	Mg	Mn	Total				
1968	4.54 (0.086)	19.44 (2.064)	0.55 (0.072)	0.81 (0.083)	1.37 (0.258)	0.26 (0.062)	3.911 (0.440)	2.99 (0.459)	19.75 (2.029)	1.40 (0.125)	13.09 (0.713)
2008	4.46 (0.052)	20.24 (1.524)	1.08 (0.290)	0.36 (0.030)	1.40 (0.171)	0.03 (0.006)	3.72 (0.441)	3.29 (0.534)	20.72 (1.557)	1.60 (0.119)	13.07 (0.728)
P	n.s.	n.s.	**	***	n.s.	***	n.s.	n.s.	n.s.	n.s.	n.s.