Article (refereed) - postprint

Recovery and non-recovery of freshwater food webs effects of acidification

6   Authors:

7   Clare Gray[1,2] – clare.gray@imperial.ac.uk

8   Alan Hildrew[2,3] – a.hildrew@qmul.ac.uk

9   Xueke Lu[4] - xueke.lu@qmul.ac.uk

10   Athen Ma[4] - athen.ma@qmul.ac.uk

11   David McElroy[5] - david.mcelroy@sydney.edu.au

12   Don Monteith[6] - donm@ceh.ac.uk

13   Eoin O'Gorman[1] - e.ogorman@imperial.ac.uk

14   Ewan Shilland[7] - e.shilland@ucl.ac.uk

15   Guy Woodward[1]* - guy.woodward@imperial.ac.uk

16   *Corresponding author

17   1. Department of Life Sciences, Imperial College London, Silwood Park
18   Campus, Buckhurst Road, Ascot, Berkshire SL5 7PY, UK
19   2. School of Biological and Chemical Sciences, Queen Mary University of
20   London, London E1 4NS, UK
21   3. Freshwater Biological Association, The Ferry Landing, Ambleside, Cumbria,
22   LA22 OLP
23   4. School of Electronic Engineering and Computer Science, Queen Mary
24   University of London, London E1 4NS, UK

25   5. Centre for Research on Ecological Impacts of Coastal Cities, University of
26   Sydney, 2006, Australia
27   6. Centre for Ecology & Hydrology, Lancaster Environment Centre, Lancaster
28   LA1 4AP, UK
29   7. Environmental Change Research Centre, University College London, Gower
30   Street, London WC1E 6BT, UK

31

SUMMARY

33   Many previous attempts to understand how ecological networks respond to
34 and recover from environmental stressors have been hindered by poorly resolved
35 and unreplicated food web data. Few studies have assessed how the topological
36 structure of large, replicated collections of food webs recovers from perturbations.
37 We analysed food web data taken from 23 UK freshwaters, sampled repeatedly over
38 24 years, yielding a collection of 442 stream and lake food webs. Our main goal was
39 to determine the effect of acidity on food web structure and to analyse the way food
40 web structure recovered from the effects of acidity over time.

41 Long-term monotonic reversals of acidification were evident at many of the sites, but
42 the ecological responses were generally far less evident than chemical changes, or
43 absent. Across the acidity gradient, food web linkage density and network efficiency
44 declined with increasing acidity, while node redundancy (i.e. trophic similarity
45 among species within a web) decreased. Within individual sites, connectance,
46 linkage density, trophic height, resource vulnerability and network efficiency tended
47 to increase over time as sites recovered from acidification, while consumer generality
48 and node redundancy tended to decrease. There was evidence for a lag in biological
49 recovery, as those sites showing a recovery in both their biology and their chemistry
50 were a nested subset of those which only showed a chemistry trend.

51   These findings support the notion that food web structure is fundamentally
52 altered by acidity, and that inertia within the food web may be hindering biological
53 recovery. This suggestion of lagged recovery highlights the importance of long-term
54 monitoring when assessing the impacts of anthropogenic stressors on the natural
55 world. This temporal dimension, and recognition that species interactions can shape
56 community dynamics, is missing from most national biomonitoring schemes, which
57 often rely on space-for-time proxies.

I. INTRODUCTION

Natural ecosystems are increasingly exposed to anthropogenic stressors, such as habitat modification, pollution and global climate change (Steffen *et al.* 2011; Smith & Zeder 2013; Sutherland *et al.* 2016). A deeper understanding of how they respond to and recover from such perturbations is important if we are to manage our natural resources effectively in the coming decades (Pimm *et al.* 1995; Woodward *et al.* 2010a).

Biological recovery from the effects of stressors does not necessarily follow from the removal of that stressor, as there may be time lags or ecological hysteresis, even to the extent that alternative equilibria are possible for otherwise identical environmental conditions (O'Neill 1998; Scheffer & Carpenter 2003; Feld *et al.* 2011; Battarbee *et al.* 2014; Murphy *et al.* 2014). These may arise via species interactions, which can alter the rate and/or trajectory of recovery (Scheffer & Carpenter 2003) and confound attempts to scale up predictions made from individuals or species populations to the whole-community or ecosystem, because of the increasing scope for "ecological surprises" to be manifested via complex indirect pathways in the food web (Ings *et al.* 2009; Thompson *et al.* 2012). For instance, artificially high nutrient concentrations can trigger regime shifts in shallow lakes, which may persist even long after nutrient loads have been reduced (Scheffer & Carpenter 2003).

A. Food web recovery research

Due to the difficulties in constructing highly-resolved food webs, however, very few studies have examined how trophic network structure responds to, and recovers from, perturbations, and fewer still have a replicated design. Field experiments have revealed how replicated freshwater food webs respond to drought, through the loss of rare and rare-for-size consumers, as well as the larger taxa high in the food web (e.g. eight stream food webs; Ledger *et al.* 2012; Woodward *et al.* 2012). Other studies have economised on effort and increased their sample sizes, and hence ability to detect responses statistically, by making assumptions about the diet of consumers. Thus, O'Gorman and Emmerson (2010) used 144 marine food webs in a mesocosm study across a range of experimental treatments to investigate how their structure responded to the removal of keystone species. Very few examples exist where the recovery of replicated, natural food webs following a perturbation has been studied, although McLaughlin, Emmerson and O'Gorman (2013) constructed a collection of 96 terrestrial food webs which tracked the recovery of sixteen riparian food webs after a flood over the course of a year. Often, a space-for-time substitution approach is used: for instance, Layer et al (2010b) studied the

94   structure of 20 freshwater food webs sampled once from 20 sites distributed across a
95   wide pH gradient, and this was subsequently set in the context of long-term change
96   in the single model system of Broadstone Stream over four decades of rising pH
97   (Layer *et al.* 2011). However, no studies of which we are aware have analysed the
98   long-term recovery from perturbation of replicated food webs distributed across
99   wide ecological gradients.

B. Freshwater acidification

101   Freshwater acidification is usually caused by atmospheric pollution (though
102   there are naturally rather acidic systems), in which strong mineral acids emitted
103   from industrial sources are deposited on the landscape (e.g. Driscoll *et al.* 2001).
104   Where soils and geology have an insufficient supply of base cations to buffer acidity,
105   runoff to streams and lakes become strongly acidic. At a pH of 5.5, alkalinity falls to
106   zero and inorganic aluminium concentration rises to become toxic to many forms of
107   life, including almost all fish (Sutcliffe & Hildrew 1989). Such anthropogenic
108   acidification has profound ecological impacts, including the loss of many acid-
109   sensitive species from all trophic levels (e.g. Dillon *et al.* 1984; Schindler 1988).
110   Evidence for the causes and consequences of acidification and its effect on species
111   assemblages is long-standing and overwhelming (e.g. Likens & Bormann 1974;
112   Schindler 1988; Hildrew & Ormerod 1995) and comes primarily from north-western
113   Europe and much of north and eastern USA and Canada. This ranges from
114   palaeolimnological reconstructions of lake pH (e.g. Battarbee *et al.* 1988), widespread
115   surveys and descriptions (e.g. Townsend, Hildrew & Francis 1983; Henriksen *et al.*
116   1990), experimental acidification of whole systems (e.g. Hall *et al.* 1980; Webster *et al.*
117   1992; Findlay *et al.* 1999), and biogeochemical modelling (e.g. Jenkins *et al.* 1990). In
118   the UK, intensive research on the long-term and large-scale ecological consequences
119   of acidification on running waters was concentrated in three main study systems:
120   Llyn Brianne in south-west Wales (Durance & Ormerod 2007), the Ashdown Forest
121   of south-eastern England (Hildrew 2009), and via the nationally covering UK Acid
122   Waters Monitoring Network (UKAWMN), which interdigitated to a limited extent
123   by sharing three sites with the other two (Layer et la 2010).

124   In the face of such evidence, reductions of polluting emissions were agreed
125   upon in both Europe and North America, which have resulted in dramatically
126   reduced depositions since the 1970s (Stoddard *et al.* 1999; RoTAP 2012). In 1988, the
127   UK Government set up UKAWMN (incorporated within the latterly expanded
128   Upland Waters Monitoring Network – or UWMN, in 2014) to assess the effectiveness
129   of these measures, which came at considerable economic and social costs. The

Network was designed explicitly to detect any recovery in the quality of surface
waters at its 23 stream and lake sites, as well as any shifts in their biology and
ecology that might be expected to accompany chemical recovery. The network's sites
are distributed across acid sensitive (base poor) regions of the UK (Figure 1), mainly
in the uplands areas of the north and west where precipitation and wet deposition of
acidity tend to be high, although some are in small, acid sensitive areas in the south
and east. A few sites are located in the extreme north and west of Scotland and
Northern Ireland which were thought sufficiently remote from industrial pollutant
sources to have been significantly affected by acid deposition (Patrick *et al.* 1991).

Substantial (though not complete) chemical recovery from acidification has
now occurred at most sites that had been acidified at the outset (Monteith *et al.* 2014).
This has included large reductions in inorganic aluminium concentrations in the
most acidified waters and more widespread but gradual increases in water pH,
while Acid Neutralising Capacity (ANC) has increased in proportion to the rate of
reduction in acid anions. Ubiquitous increases in dissolved organic carbon
concentration also appear to be part of the biogeochemical response, leading to a
partial replacement of mineral acidity by organic acidity that has tempered the pH
response (Evans *et al.* 2008). Evidence for biological recovery, in terms of the
establishment of acid-sensitive assemblages of species, has been much less obvious
(Battarbee *et al.* 2014), but most evident in the diatoms of the epilithon and from the
colonisation of recovering sites by some species of macrophytes. In the last UWMN
data interpretation report (Kernan *et al.* 2010), invertebrate assemblages showed
signs of partial recovery at around half of the chemically recovering sites. Only two
of those sites, both of which were particularly severely acidified at the onset of
monitoring, showed any evidence of recovery of salmonid populations, another
indicator of decreasing acidity (Murphy *et al.* 2014; Malcolm *et al.* 2014). Similar,
"sluggish" biological recovery has also been reported in other acidified systems
elsewhere globally (e.g. Nedbalová *et al.* 2006; Keller *et al.* 2007).

Several hypotheses have been put forward to explain these delays in the
anticipated simple reversal of acidification, including dispersal limitations, pollutant
legacies and attendant recurring acid episodes (e.g. Kowalik *et al.* 2007), interactions
with other stressors (e.g. climate change; Johnson & Angeler 2010), and indirect
food-web effects (Yan *et al.* 2003; Ledger & Hildrew, 2005; Monteith *et al.* 2005).
These mechanisms are not necessarily mutually exclusive explanations and there are
differing levels of support for each. For instance, the role of dispersal is still
uncertain for differing biological components, with evidence for and against its role:

166   Gray & Arnott (2011) suggested it may constrain the recovery of lake zooplankton in
167   Canadian lakes, contrary to previous findings (Keller *et al.* 2002; Yan *et al.* 2004).
168   Evidence for more mobile taxa, such as benthic insects with flying adults, however,
169   suggests that dispersal is usually sufficient to allow rapid recolonisation and thus
170   unlikely to explain delayed biological recovery (Masters *et al.* 2007; Hildrew 2009).
171   The type of waterbody also affects both its rate of chemical recovery and its
172   recolonisation potential: lakes are larger and thus better able to absorb spikes of run-
173   off that can create acid episodes than streams (Evans, Monteith & Harriman 2001)
174   and yet they show similarly limited biological recovery and so acidic episodes alone
175   cannot explain the lag in biological recovery. In addition, these hypotheses may
176   operate additively or even synergistically.

177   To date, no evidence has been found for differing rates of recovery between
178   streams and lakes. Given the evidence that persistent acidic episodes in stream
179   systems can limit biological recovery (Lepori & Ormerod 2005; Kowalik *et al.* 2007)
180   one might expect stream systems to recover more slowly than lakes, although
181   Monteith et al., 2014 found that the magnitude of acid pulses at UWMN sites had
182   declined at a similar rate to mean acidity. Conversely, the dynamic nature of
183   streams, with a natural regime of frequent flow disturbances, and downstream
184   connectivity to pools of less acid-sensitive species in the lower reaches might render
185   them naturally more resilient (e.g. Hildrew & Giller 1994). Thus, we had no clear *a*
186   *priori* hypothesis or expectations of the relative rates of recovery in the communities
187   of lakes and streams.

C. The recovery of acidified food webs

189   The possibility that species interactions within the food web might inhibit
190   ecological recovery requires further testing (Webster *et al.* 1992; Frost, Montz & Kratz
191   1998). Circumstantial evidence from streams suggests that generalist, non-predatory
192   invertebrates (e.g., stoneflies of the families Leuctridae and Nemouridae), that are
193   acid-tolerant and often dominate the benthos of acid streams, may fill the feeding
194   niche of specialist, acid-sensitive grazers and inhibit their return (Ledger & Hildrew
195   2005; Layer, Hildrew & Woodward 2013). Further, dynamic modelling found that
196   the reticulate acidified food webs are more robust, suggesting that they might be
197   more inherently stable and thus less prone to (re)invasion (Layer *et al.* 2010b).
198   Finally, the common reliance on space-for-time proxies may miss the transient
199   dynamics and the possible existence of alternate stable states of a system responding
200   to stress. For instance, if the strong effects of pH on food web properties reported in
201   the space-for-time survey of 20 sites by Layer *et al.* (2010b) are not evident in systems

202 undergoing actual changes in pH over time, then the expected mapping of the biota
203 onto the environmental template might not be evident, and its absence would
204 indeed indicate ecological inertia. This has wider implications for biomonitoring
205 science in general, which is underpinned by space-for-time approaches and rarely
206 has access to truly long-term (i.e. multidecadal) high quality biological time series
207 (Friberg *et al.* 2011). Mismatches between temporal and space-for-time data may
208 provide evidence for time lags or hystereses resulting from the system's own
209 internal dynamics, although the potential importance of biogeographic constraints
210 should also not be discounted. On the other hand, if the two data types match
211 perfectly then indirect food web effects – which would otherwise reshape the simple
212 biota-environment relationship - can effectively be discounted.

213   Repeated assessment of the topology of a large collection of food webs, as
214 they recover from the effects of acidification, is needed to complement previous
215 work, which dealt with single, 'model' systems (e.g. Layer *et al.* 2010a, 2011), or
216 relied on space-for-time substitutions. At present examples of the former case are
217 lacking, but in the latter, Layer *et al.'s* (2010b) suggested the smaller but more
218 interconnected acidified webs had more stable configurations of trophic linkages
219 across a pH gradient of <5 to >8. Linkage density and connectance are both common
220 measures of web complexity, and an abundance of "redundant" interactions can
221 help stabilise the network's structure in the face of perturbations, by preventing the
222 secondary extinctions that arise when consumers are left without resources (Dunne,
223 Williams & Martinez 2002; Thébault & Fontaine 2010). Indeed, linkage density
224 increased with stream pH across the spatial gradient of the 20 sites, which included
225 10 of the UWMN streams used in the current study.

226   Mean food chain length (the number of steps between a basal resource and a
227 particular consumer, see Text Box) gives a measure of the trophic height of the web
228 as a whole (Williams & Martinez 2004), which tends to shorten as environmental
229 stress increases and productivity declines (Woodward *et al.* 2005). Food chains are
230 generally assumed to be shorter than six links, and omnivory, which is
231 commonplace in aquatic systems, tends to truncate them further (Pimm 1980;
232 Hildrew, Townsend & Hasham 1985; Lawton 1989; Yodzis 1989; Williams &
233 Martinez 2000). Although long-term data are still scarce, there is some evidence that
234 suggests that increasing pH leads to higher productivity and an overall lengthening
235 of food chains (Gerson, Driscoll & Roy; Grahn, Hultberg & Landner 1974;
236 Woodward *et al.* 2005; Hildrew 2009). For instance, progressively larger and more
237 acid-tolerant predators have (re)invaded Broadstone Stream as pH has risen since

238   the 1970s, culminating in the recent return of the apex predator, the brown trout

239   (*Salmo trutta*) (Layer *et al.* 2011).

240   In addition to simple food chain metrics, the range of both resources and

241   consumers each species is connected to in the web has important implications for the

242   overall network's dynamical and structural stability, and its ability to respond to or

243   resist environmental change. *Generality* (see Text Box) is a substructural measure of

244   the dietary breadth of a consumer, derived statistically from its number of resources.

245   If a consumer is a specialist (i.e. narrow diet), then it might be more vulnerable to

246   extinction as the loss of only a few species will leave it with insufficient resources.

247   *Vulnerability* (see Text Box) is the converse measure of *Generality*; it is derived from

248   the number of consumers feeding on a particular resource species, and indicates how

249   important that resource is in terms of the consumers it supports. Freshwater

250   predators are commonly gape-limited generalists, so the size and diversity of prey

251   increases with consumer size (Woodward & Hildrew 2002; Woodward *et al.* 2010b).

252   Similarly, the herbivorous consumers in acidified streams are also generalists,

253   feeding on a wide range of detritus and algae (e.g. Ledger & Hildrew 2005; Layer,

254   Hildrew & Woodward 2013). As acidity decreased and acid sensitive, but more

255   specialist, species re-invade, the average generality of consumers (i.e., normalised to

256   the size of the food web) should decrease. The average vulnerability of resources

257   (again normalised to the size of the food web) should increase with decreasing

258   acidity as the consumer guild becomes more speciose (Layer *et al.* 2010b). However,

259   Layer *et al.* (2010b), found that normalised consumer generality and resource

260   vulnerability did not change systematically, and there was no relationship between

261   either the variation (standard deviation) in consumer generality or resource

262   vulnerability and pH, although the sample size was rather small.

263   Under acidified conditions, generalist primary consumers can partially

264   occupy the niche left by the loss of specialist herbivorous species, potentially

265   creating 'ecological inertia' within the food web by slowing the return of the latter as

266   pH rises (Ledger & Hildrew 2005). The effect of acidity on this redundancy of

267   feeding pathways within the whole food web has not been investigated previously

268   using network-based approaches. As acidified systems tend to be species poor, and

269   dominated by generalist consumer species and few specialists (e.g. Hämäläinen &

270   Huttunen 1996; Ledger & Hildrew 2005), there should be greater trophic redundancy

271   (i.e. species of acid streams should have more similar diets and share more predators

272   than more speciose webs of relative specialists), which could make them resistant to

273 perturbations and hence more robust to the loss of food resources than those at
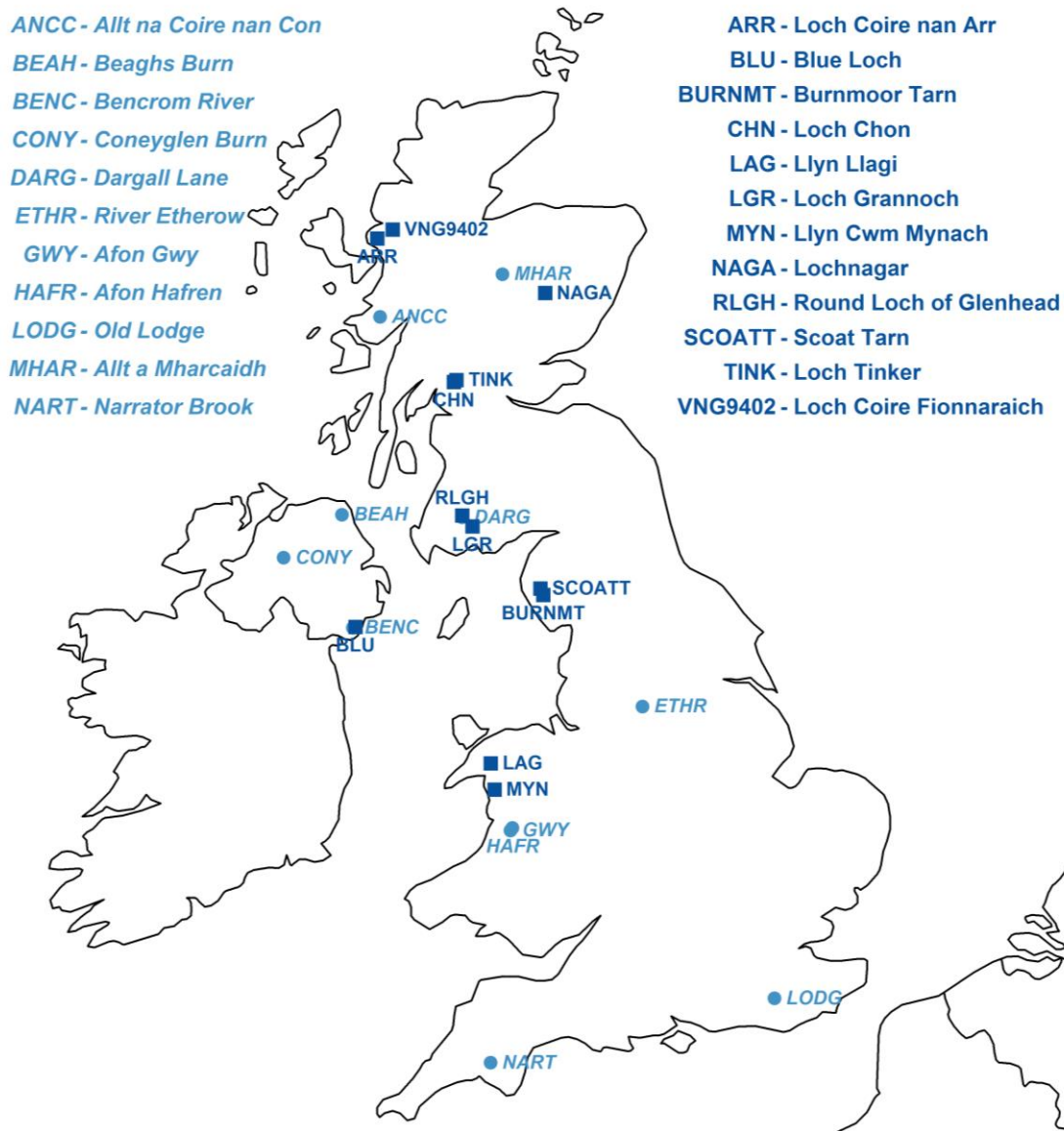274 higher pH (Naeem 1998; Solé *et al.* 2003; Peralta *et al.* 2014).

275   As with other features of the whole network (such as connectance) or parts of
276 the network (e.g. generality) the so-called 'small-world' properties of food webs
277 have also been linked to stability (Watts & Strogatz 1998; Montoya & Solé 2002) and
278 to the rate at which perturbations propagate (Montoya, Pimm & Solé 2006).
279 Essentially, if species are highly connected to the rest of the food web in a 'small-
280 world' network, then perturbations may spread (and dissipate) rapidly, but if there
281 are less well connected (more degrees of separation) this may lead to longer-lived
282 oscillatory dynamics and feedbacks that require a long time to reach equilibrium.
283 Thus, it is not simply the linkage density or strength of connections that are
284 important for determining stability or food-web inertia, but their particular
285 configuration. Even large food webs from circumneutral or higher pH systems can
286 exhibit these properties, with most species being only 1-2 degrees of separation from
287 the rest of the web (Thompson *et al.* 2015). Network efficiency (see Text Box) is a
288 measure of how well connected a network is, as well as the distribution of those
289 connections across a network (Latora & Marchiori 2001), and can enable inferences
290 to be made about the small-world properties of food webs. Although rarely applied
291 to date in food web studies, this metric derived from the wider field of network
292 science could provide new insights into how these small-world aspects of food web
293 topology itself might shape the trajectory of biological recovery (Monteith *et al.* 2005;
294 Layer *et al.* 2010b). The lengthening of food chains associated with the re-invasion of
295 consumers might be expected to increase the overall efficiency of the network, as the
296 wider breadth of diet of new, large, top predators effectively reduces the distance (in
297 terms of number of links) between resources.

298   D. The Upland Waters Monitoring Network

299   The data analysed here (from the UWMN) consist of repeated observations on
300 the same 23 sites (Figure 1) over 24 years (1988 – 2012). Spatially, the sites
301 encompass a wide range of pH (from 3.71 to 7.49), and include some that were
302 strongly acidified at the onset of monitoring (e.g. Old Lodge) and others that were
303 circumneutral and have changed little (e.g. Allt na Coire nan Con). The gradual
304 long-term chemical recovery of many of these sites, particularly among those
305 formerly the most acidic, provides a unique and large-scale picture of the chemical
306 drivers and biological responses in the network over both space and time.
307 Characterizing the 'baseline' variation in food web structure in the near absence of
308 changes in environmental stress is crucial for our understanding of how a

309 community recovers from that stressor and, more broadly, for gauging its potential
310 responses to future environmental changes. The food webs compiled from these data
311 provide the replication and statistical power that has previously been insufficient for
312 rigorous analysis food web responses to acidification and chemical recovery.

313

ANCC - Allt na Coire nan Con
BEAH - Beaghs Burn
BENC - Bencrom River
CONY - Coneyglen Burn
DARG - Dargall Lane
ETHR - River Etherow
GWY - Afon Gwy
HAFR - Afon Hafren
LODG - Old Lodge
MHAR - Allt a Mharcaidh
NART - Narrator Brook

ARR - Loch Coire nan Arr
BLU - Blue Loch
BURNMT - Burnmoor Tarn
CHN - Loch Chon
LAG - Llyn Llagi
LGR - Loch Grannoch
MYN - Llyn Cwm Mynach
NAGA - Lochnagar
RLGH - Round Loch of Glenhead
SCOATT - Scoat Tarn
TINK - Loch Tinker
VNG9402 - Loch Coire Fionnaraich

314
315 *Figure 1. The Upland Waters Monitoring sites, consisting of 11 lakes (dark blue squares) and 12*
316 *streams (light blue circles).*

317

318   We aimed to describe changes in food web structure as water chemistry
319 recovered over three decades of chemical and biological monitoring. Our emphasis
320 was on structural changes in binary networks of the presence/absence of nodes and
321 links, rather than the effects of acidity on the dynamical stability of species

populations *per se*. To determine the effect of environmental variables on food web structure, we tested the following hypotheses, using data gathered across all sites and years:
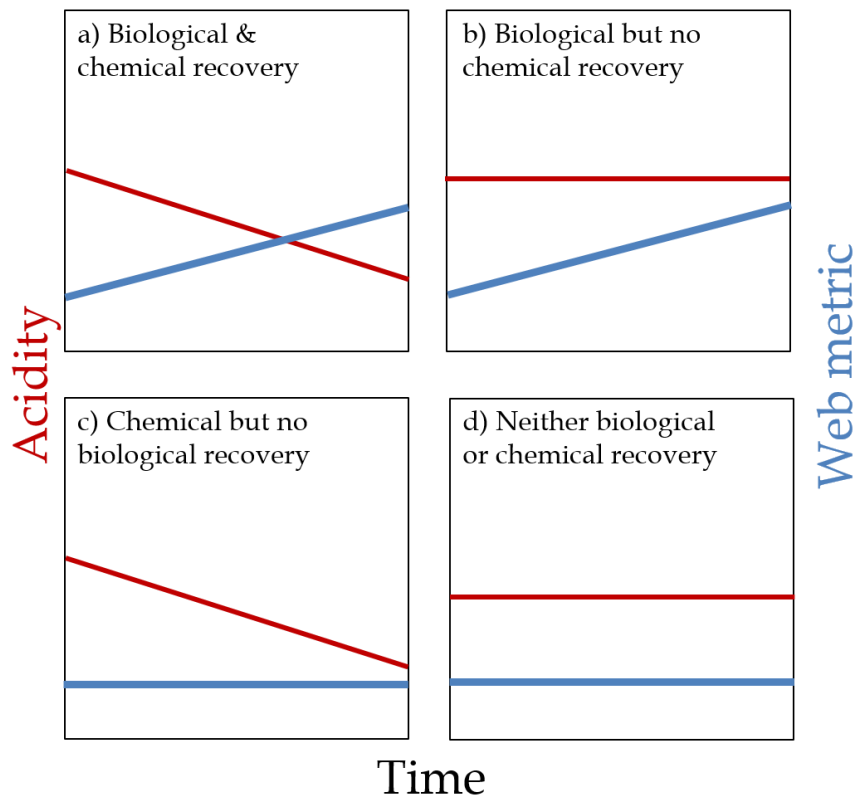
1. When analysed across the entire acidity gradient, food web structure will be directly affected by acidity. Food webs of less-acidified systems should exhibit higher linkage density, food chain length and network efficiency but lower connectance consumer generality and redundancy (Table 1).
2. There will be directional and predictable changes in food web structure at each site through time as acidity decreases (Table 1).
3. If indirect food-web effects arising from biotic interaction are unimportant, changes in network structure through time will match recovery from acidification; i.e. those sites which experience change in their chemistry will also change at a comparable pace in their food web attributes. (Figure 2).

Additionally, in order to investigate if the rates of recovery were different between stream and lake sites, the two ecosystem types were analysed both together and separately.

a) Biological & chemical recovery

b) Biological but no chemical recovery

c) Chemical but no biological recovery

d) Neither biological or chemical recovery

Acidity

Web metric

Time

341

342 *Figure 2. A conceptual figure of the possible outcomes for biological (thick, blue line) and chemical*
343 *(thin, red line) recovery at the UWMN sites over time.*

344

345 *Table 1. Summary of predicted relationships between acidity and food web structure.*

Food web metric	Predicted relationship with decreasing acidity
Connectance	↓
Linkage density	↑
Mean trophic height	↑
Maximum trophic height	↑
Resource vulnerability	↑
Consumer generality	↓
Standard deviation in vulnerability	no change
Standard deviation in generality	no change
Network efficiency	↑
Redundancy	↓

II. METHODS

A. Sites

The UWMN consists of 11 streams and 12 lakes from across the UK (Figure 1): full site descriptions and sampling methodologies are provided in Patrick, Monteith & Jenkins (1995) and Kernan *et al.* (2010). Water chemistry, epilithic diatom, macroinvertebrate and fish sampling began in spring 1988 and has continued annually at most sites up to 2012, except for a few exceptions when access was occasionally restricted (see Kernan *et al.* 2010). The sites are distributed along a latitudinal gradient across the UK, which can be interpreted as a proxy for the degree of acid deposition at the outset: sites at high latitude were exposed to relatively little acid deposition, whilst those sites at lower latitudes were generally more acidified (Patrick *et al.* 1991). One lake site, Loch Coire nan Arr, was affected by damming that increased the water level, so it was replaced in 2001 by the nearby Loch Coire Fionnaraich, which has comparable characteristics (Figure 1).

B. Chemistry

Water samples for chemical analysis were taken in acid-rinsed bottles monthly from streams and quarterly from lakes. A large number of chemical determinands were recorded at each site: for more details see Kernan *et al.* (2010) and Monteith *et al.* (2014). In total 13 variables that are considered key drivers or indicators of acidification (Monteith *et al.* 2014) were used here: pH, alkalinity, H^+, conductivity, nitrate (NO_3), non-labile aluminium, soluble aluminium, labile aluminium, Dissolved Organic Carbon (DOC), sodium (Na+), sulphate (SO_4^{2-}), calcium (Ca^{2+}) and chloride (Cl^-). With the exception of pH, we used the annual arithmetic mean of all chemical data as summary statistics for each site. Annual average pH was calculated by first converting pH to H^+ concentration, calculating the annual arithmetic mean, and then converting back to pH.

C. Biota

Benthic diatoms, macroinvertebrates and fish were sampled annually from 1988-2012. Benthic diatoms were sampled according to standard UWMN protocols (Patrick *et al.* 1991), by selecting five cobble-sized stones from the streambed, or from the permanently submerged littoral zone in lakes. Three samples were taken from each site, in streams at the top, middle and bottom of fixed 50m reaches, and in the lakes from three discrete locations on the shoreline (away from inflow or outflow streams). The biofilm on the surface of the stone was scrubbed into a funnel, washed into a plastic vial with stream or lake water, and immediately preserved in Lugol's iodine solution. In the laboratory, samples were prepared by digestion in hydrogen

382   peroxide (H_2O_2) and diluted with distilled water. To enable subsequent examination

383   by light microscopy at 1000x magnification, a sub-sample was pipetted onto a

384   coverslip, dried and mounted onto a slide using Naphrax (Battarbee *et al.* 2001).

385   Three hundred diatom valves from each sample were identified to species to give

386   the diatom species assemblage per site per year.

387   Macroinvertebrates were sampled according to standard UWMN protocols

388   (Patrick *et al.* 1991), by taking five separate one-minute kick samples with a standard

389   hand net (330μm mesh) from riffle sections of streams and from the dominant littoral

390   habitat of lakes. Macroinvertebrates were subsequently sorted and preserved with

391   70% Industrial Methylated Spirit. Oligochaeta, Diptera and Bivalvia were identified

392   to class, family and genus, respectively, while all others were identified to species.

393   All taxa were counted and the counts from the five samples summed to represent the

394   macroinvertebrate assemblage per site per year.

395   Annual electrofishing surveys were conducted according to standard UWMN

396   protocols (Patrick *et al.* 1991). Surveys were undertaken between mid-September and

397   mid-October at each stream site or, for lakes, in the outflow stream immediately

398   downstream. It was assumed that composition of the fish assemblage in lake

399   outflows could serve as a proxy for that of fish in the lake itself. Three 50m reaches,

400   distributed across 500m of the stream or lake outflow, were isolated using stop nets

401   and electrofished. Depletion electrofishing was employed and all salmonids were

402   counted, while the presence of any other fishes was also recorded.

D. Food web construction

404   Species lists were compiled for each site in each year for which there was

405   complete biological and chemical data, yielding 442 food webs. Binary food webs

406   were constructed using the WebBuilder function in R (Gray *et al.* 2015) and the

407   database of freshwater aquatic trophic interactions contained therein, based on the

408   presence/absence of species (nodes) at each site in each year and the occurrence of a

409   trophic linkage in the feeding link database. This method is based on the assumption

410   that all feeding links between specific pairs of species that have been reported

411   previously would be realized, wherever and whenever both species co-exist at a

412   study site (Hall & Raffaelli 1991; Martinez 1991; Layer *et al.* 2010b; Pocock, Evans &

413   Memmott 2012), although many of the feeding links within that database were, in

414   fact, derived from direct observation from previous UWMN surveys (Ledger &

415   Hildrew 2005; Layer *et al.* 2010b; a, 2011). When species-specific trophic interactions

416   had not previously been described for some rare or understudied taxa (nodes),

417   feeding links were assigned on the basis of taxonomic similarity; for instance, by

418   assuming that different species within the same genus consumed and were
419   consumed by the same species. This method is often used when constructing
420   freshwater food webs as in these systems consumer diets tend to be highly
421   generalist, and determined primarily by the size of their prey (e.g. Layer *et al.* 2010b;
422   a; Layer, Hildrew & Woodward 2013). Food webs built in this manner are
423   structurally comparable to those built solely through analysis of consumers gut
424   contents; for instance, the method has predicted the links of four well documented
425   freshwater food webs with 40-60% accuracy (on a scale from -100% to 100%, Gray *et*
426   *al.* 2015).

E. Network metrics

428   Several commonly used metrics were calculated for each food web, including:
429   connectance ($C = L/S^2$; where L= the number of trophic links, S = the number of
430   species) and linkage density (LD = L/S), mean trophic height (after Levine 1980:
431   defined as 1 plus the mean trophic level of a consumer's resources, averaged across
432   all consumers) and maximum trophic height (defined in the same way, except that
433   the maximum value across all consumers was taken). Mean generality (G; number of
434   resources per consumer, see Text Box) and vulnerability (V; number of consumers
435   per resource, see Text Box) was calculated, as was normalised G and V for each taxon
436   k, as:

$$G_k = \frac{1}{L/S} \sum_{i=1}^{S} a_{ik} \qquad (1)$$

$$V_k = \frac{1}{L/S} \sum_{j=1}^{S} a_{jk} \qquad (2)$$

437

438   Where S is the number of nodes and L the number of links in a food web. $a_{ik} = 1$ if
439   taxon k consumes taxon i (otherwise $a_{ik} = 0$), and $a_{jk} = 1$ if taxon k is being
440   consumed by taxon j (otherwise $a_{jk} = 0$). Mean G_k and V_k in any given food web
441   equal 1, making their standard deviations, which give an indication of the variability
442   in G and V respectively across a network, comparable across networks of different
443   size. These metrics were all calculated using the R package cheddar (Hudson *et al.*
444   2013).

445   Network efficiency (Latora & Marchiori 2001, see Text Box) describes the
446   'reachability' of each node by any other node, and is a measure of overall
447   connectivity, and was calculated using the sna R package (Butts 2013) as:

$$E = \frac{1}{S(S-1)} \sum_{i \neq j \in G} \frac{1}{d_{ij}} \qquad (3)$$

448

449   Where d_{ij} is the shortest path length between node i and j.

450   The proportional node redundancy (see Text Box) of each network was
451   calculated by grouping nodes into trophic species (i.e. nodes with common resources
452   and consumers) and then calculated as:

$$Redundancy = 1 - \frac{T}{S} \qquad (4)$$

453

454   Where T is the number of trophic species within the network. Redundancy was
455   calculated using functions from the cheddar package (Hudson *et al.* 2013) in R.

456

F. Statistical analyses
457
458   All statistical analyses were done in R version 3.1.1 (R Core Team 2013),
459   PRIMER-E with PERMANOVA + (2006). To simplify the chemical data, Principal
460   Component Analysis (PCA) was performed on all 13 water chemistry variables
461   across all sites and years, using a resemblance matrix constructed from Euclidean
462   distances. As some of the variables were measured on different scales (i.e. NO_3 vs
463   pH), and to reduce the influence of extremely large or small values, each variable
464   were centred to zero and scaled by their standard deviations (van den Berg *et al.*
465   2006). Sample scores on the first PC axis (PC1) were extracted for use as a proxy for a
466   general gradient in overall acidity in further analysis.

Effects of acidity on food web structure
467
468   For data-visualisation purposes only, as we were unable to fully account for
469   both temporal and spatial pseudoreplication of our data simultaneously using
470   multivariate analysis, principal coordinates analysis was used on all data across all
471   sites and time points. The resemblance matrix of food web metrics was constructed
472   from square root transformed variables, using Bray-Curtis distances. More rigorous
473   statistical inferences were drawn from univariate approaches, in which
474   pseudoreplication was addressed within the variance structure of the relevant
475   model(s), as explained below.

476   To assess the effect of acidity on food web structure (our first hypothesis),
477 each network metric was regressed against the derived acidity gradient (PC1), and
478 any trend assessed with Generalised Linear Mixed Effects models. Alongside acidity
479 (PC1), site type (lake or stream) was fitted as a fixed effect, and any potential
480 interactions with acidity (PC1) were assessed on the basis of stepwise model
481 simplification and model AIC (Crawley 2013). For each model, site was fitted as
482 having a random effect on the intercept of the model, and year was fitted to have a
483 random effect on the slope and intercept of the model.

### *Directional change in food web structure*

485   Mann–Kendall trend tests were used to determine if there were significant
486 monotonic trends in the acidity and food web structure over time at each site (our
487 second hypothesis). The acidity gradient (PC1) extracted from the PCA above, and
488 all the network metrics described above, were calculated for each site in each year.
489 These variables were then assessed for monotonic trends over time at each site.

Food web recovery from acidification

491   To test our third hypothesis we used χ^2 contingency tests to assess the extent
492 to which sites that exhibited clear decreases in acidity also showed evidence of
493 directional change in their food web structure (as in Murphy *et al.* 2014). For acidity
494 (PC1), and for each network metric, we counted the number of sites (out of 23) that
495 exhibited (a) a biological and a chemical trend, (b) a biological but not a chemical
496 trend, (c) a chemical but not a biological trend (i.e., evidence for ecological inertia),
497 and (d) neither a biological nor a chemical trend (Figure 2). The χ^2 test assessed
498 whether the distribution of sites across these four categories was due to chance.

III.   RESULTS

500   The PCA of the chemical variables revealed that the first axis was strongly
501 correlated with pH, H^+ ions and $SO_4.$ It was therefore was used a proxy for the
502 acidity-related stress to which each food web was exposed (Figure *3*), as it
503 encompassed the variation in these pH related chemistry variables. From here on
504 PC1 is called 'acidity' and refers not only to pH, but to the chemical stress associated
505 with low pH.
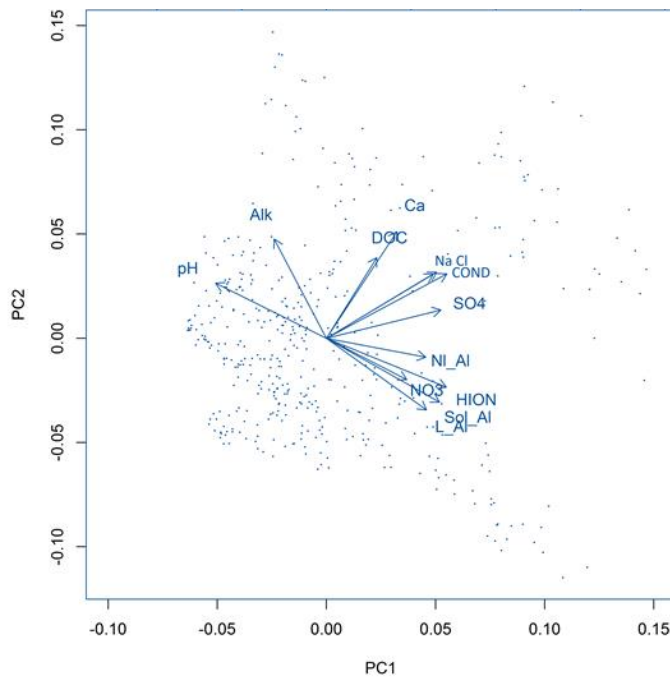
Variable	Correlation with PC1
pH	0.311
Alk	0.147
HION	-0.336
Cond	-0.337
NO3	-0.225
Nl_Al	-0.278
Sol_Al	-0.317
DOC	-0.141
L_Al	-0.281
Na	-0.309
Ca	-0.198
Cl	-0.299
SO4	-0.321

506

507 *Figure 3. PCA ordination of chemical variables. The correlation between each variable and acidity*
508 *(PC1) is given in the accompanying table. The first axis, PC1, is strongly related to pH, SO_4 and*
509 *aluminium such that PC1 can be interpreted as an 'acidity gradient'.*

510 ## A. Effects of acidity on food web structure
511 When the food web data were analysed at the regional (UK) scale, and modelled
512 against the acidity gradient extracted from Figure 3, some clear trends in food web
513 structure emerged, although several did not match or even ran counter to our initial
514 hypotheses. Contrary to our expectations (Table 1) connectance, and trophic height
515 were unrelated to the acidity gradient (

516   Table 2; more acid sites are to the right in Figure 5). As predicted, linkage density
517   increased with decreasing acidity (

518   Table 2; Figure 5), lake food webs had lower linkage density than stream food webs
519   (Figure 5). Normalised consumer generality and normalised vulnerability did not
520   change (

521   Table 2; Figure 6), nor did their standard deviations, across the acidity gradient. As

522   predicted, however, network efficiency was lower in more acidified conditions (

523   Table 2; Figure 7), suggesting that more acidified food webs were connected such
524   that the average path length between nodes was greater than for circumneutral food
525   webs. Node redundancy was highest in more acidified food webs (

526 Table 2; Figure 7), suggesting that circumneutral food webs had more unique
527 feeding pathways, confirming our prediction. Overall, lake food webs had lower
528 network efficiency and higher node redundancy than streams (

529   Table 2; Figure 7). Figure 4 indicates more variation in lake food web structure
530   along PCO1, contrasting with our expectation that stream food webs might be more
531   dynamic and variable than lake food webs.
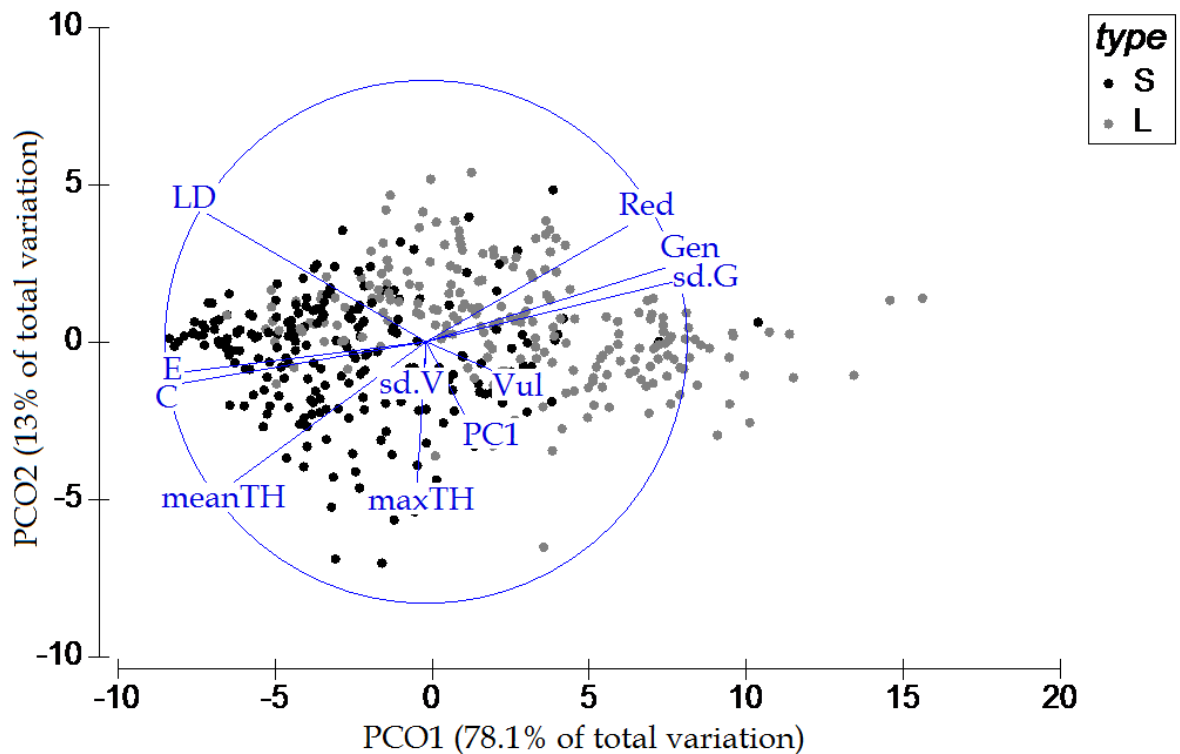
532

533   *Figure 4. Ordination of food webs (points) based on the resemblance matrix created from food web*
534   *metrics using Bray-Curtis distances, which was analysed with PERMANOVA. Site type is shown as*
535   *either black points (streams) or grey points (lakes). This plot allows comparison of food web metrics*
536   *(blue vectors and names) with the predictor variable PC1 extracted from Figure 3, in multivariate*
537   *space. Longer vectors indicate a stronger correlation. The blue circle indicates the boundary for a*
538   *correlation of 1. C = connectance, LD = linkage density, meanTH = mean trophic height, maxTH =*
539   *maximum trophic height, Vul = resource vulnerability, Gen = consumer generality, E = network*
540   *efficiency, Red = node redundancy*

541
542

Table 2. Statistics of fit for the multiple mixed effects models. All models include a random effect of site on the intercept of the linear relationship, and year on the slope of the linear relationship. Bold p-values indicate significance at α = 0.05.

| Response variable	Predictor variable	d.f.	F-value	P-value
Connectance	PC1	99	-1.537	0.1270
	type	1	7.991	**<0.0001**
Linkage density	PC1	72	-3.902	**<0.0001**
	type	1	2.686	**0.0130**
Mean trophic height	PC1	76	-0.017	0.9864
	type	1	4.294	**0.0003**
Maximum trophic height	PC1	74	1.407	0.1640
	type	1	-0.068	0.9460
Network efficiency	PC1	101	-2.306	**0.0231**
	type	1	8.288	**<0.0001**
Normalised vulnerability	PC1	54	-0.264	0.7929
	type	1	-2.478	**0.0208**
Normalised generality	PC1	67	1.122	0.2666
	type	1	-6.531	**<0.0001**
sd(Vulnerability)	PC1	115	-0.395	0.6940
	type	1	-0.450	0.6570
sd(Generality)	PC1	112	1.952	0.0534
	type	1	-7.228	<0.0001
Redundancy	PC1	91	3.577	0.0005
	type	1	-5.269	<0.0001

546

547

548
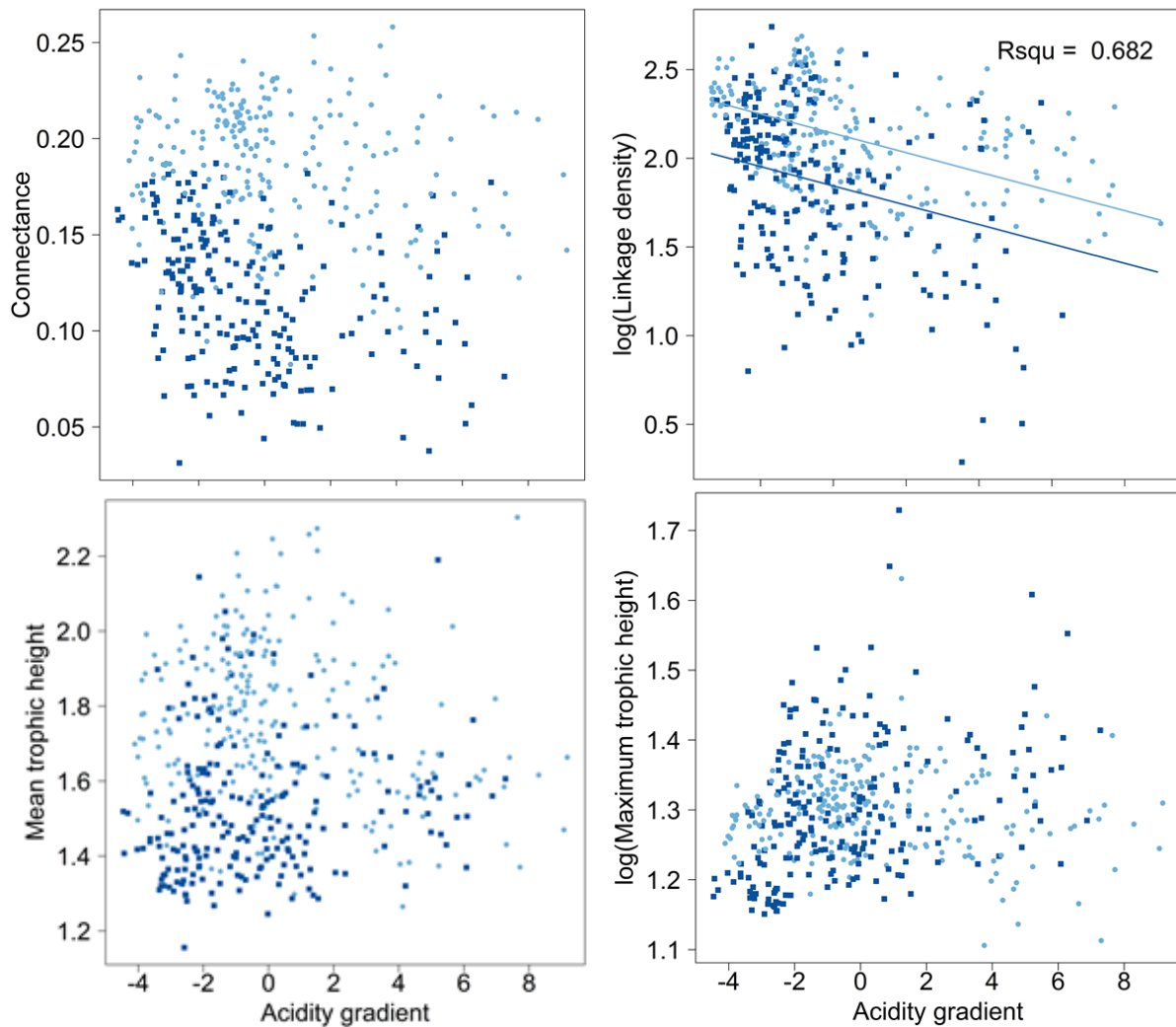
Figure 5. The relationship between connectance, linkage density, mean and maximum trophic height, and environmental stress. The acidity gradient is PC1 extracted from Figure 3, and is strongly related to pH, SO_4 & labile aluminium, with increasing environmental stress (acidity) from left to right, such that the most acidified food webs are to the right of each plot. Lines indicate fitted values from GLMM; where $p < 0.05$, the conditional R^2 as an indication of overall model explanatory power is shown (Johnson 2014). Where site type (lake or stream) was found to be a significant predictor variable, separate lines are given for each site type. Lake food webs are indicated by dark blue squares, while streams are light blue circles.

557

558

559
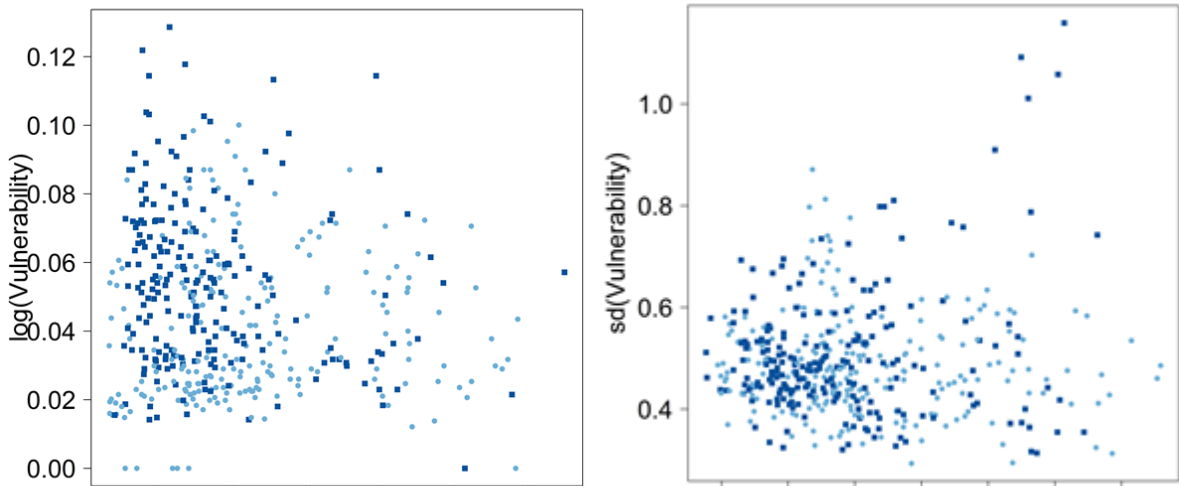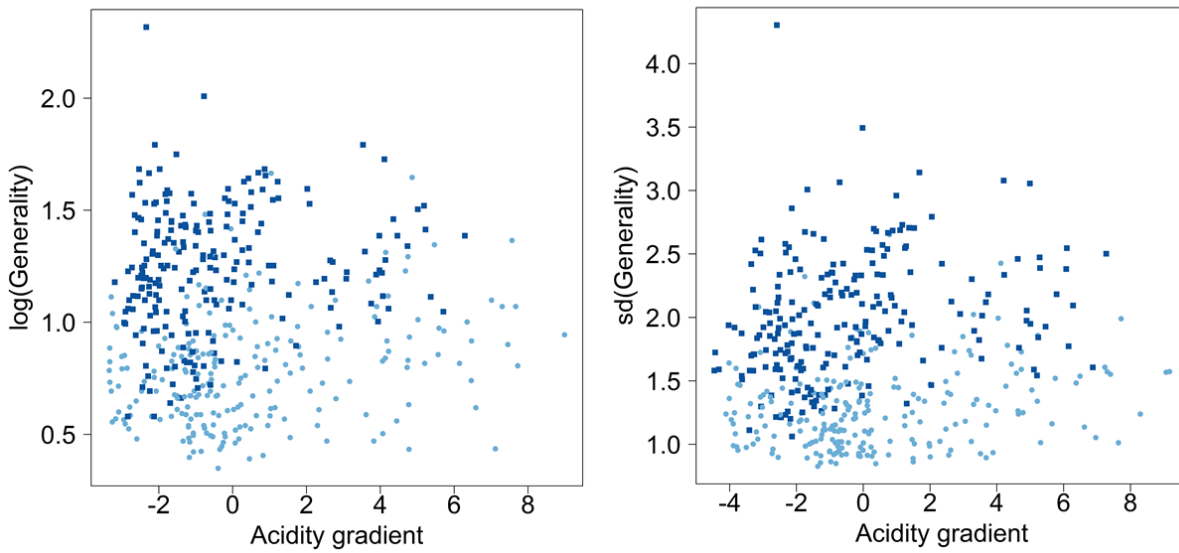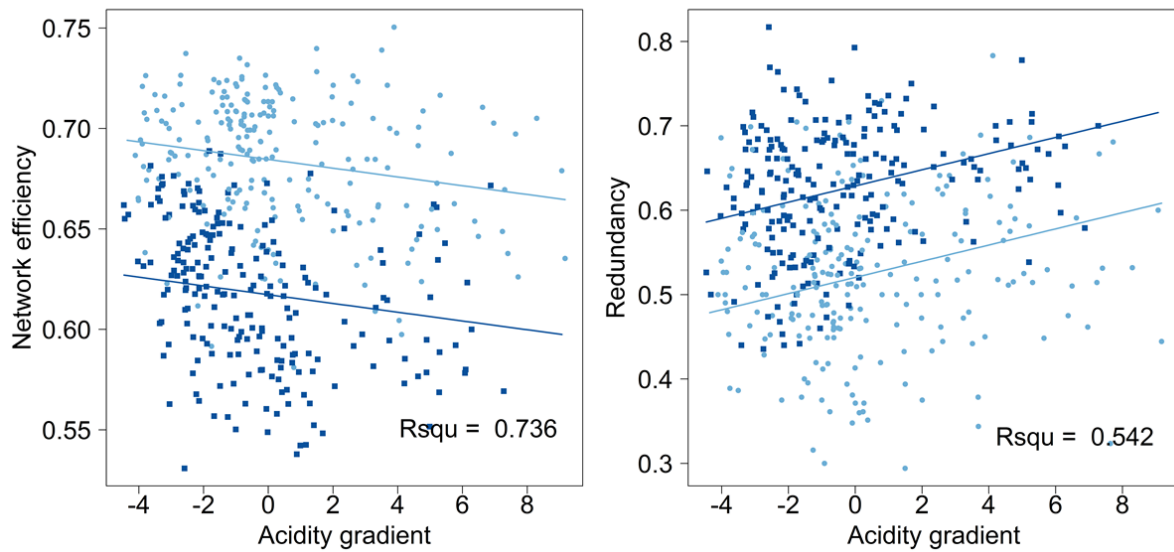
Figure 6. Variation in resource vulnerability and consumer generality (both normalised to species richness) across the stress (acidity) gradient (greater acidity to the right, see Figure 5). Lake food webs are indicated by dark blue squares, while streams are light blue circles.
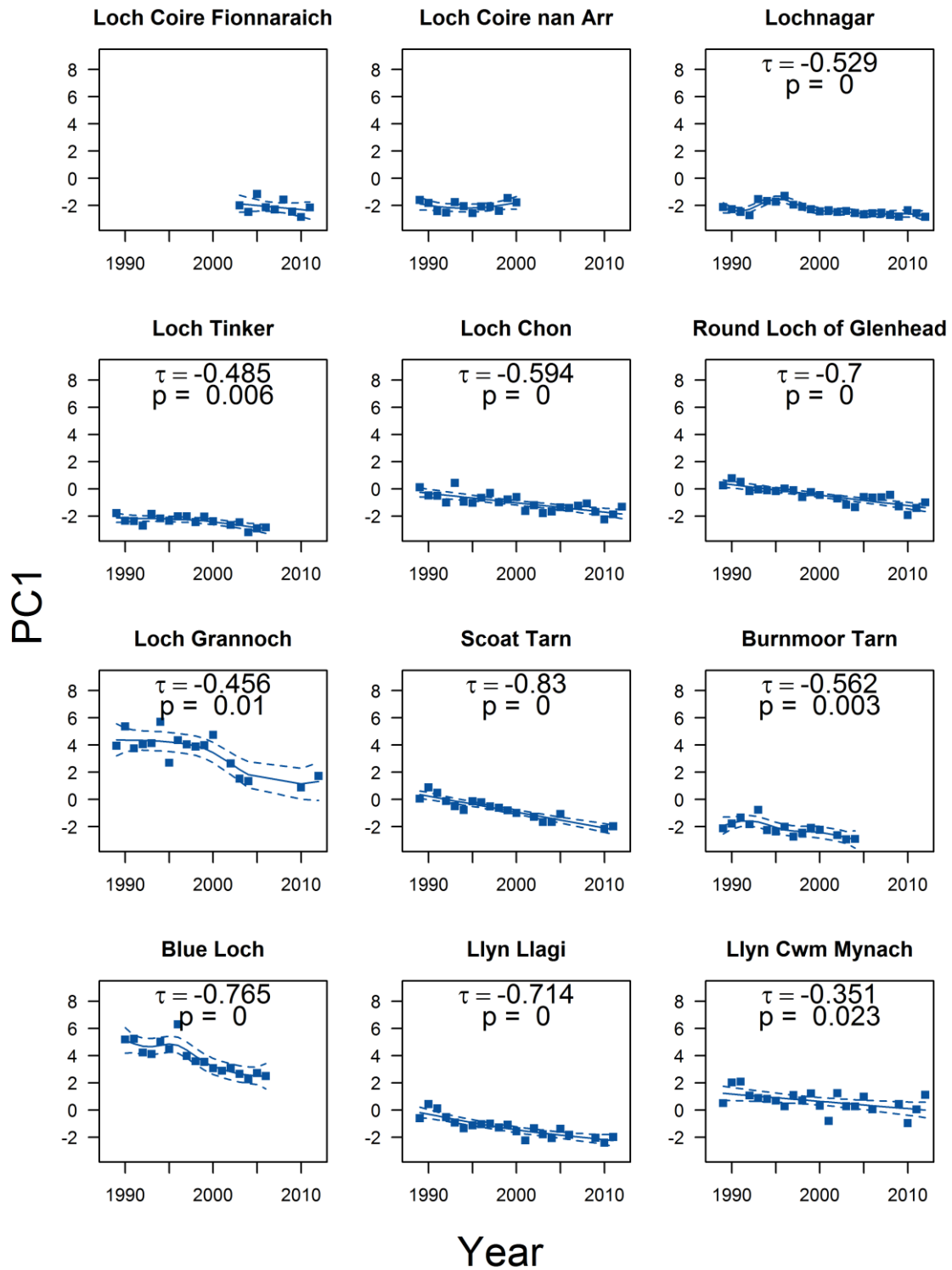
560

561

562

563

564

Figure 7. Network efficiency increases with decreasing environmental stress (acidity, greater acidity to the right, see Figure 5), node redundancy decreases with decreasing environmental stress. Lines indicate fitted values from GLMM; where p < 0.05 the conditional R^2 as an indication of overall model explanatory power is shown (Johnson 2014). Where site type (lake or stream) was found to be a significant predictor variable, separate lines (light blues streams; dark blue lakes) are given for each site type. Lake food webs are indicated by dark blue squares, while streams are light blue circles.

B. Directional change in food web structure

There was overall a clear directional trend in chemical recovery: eighteen of the 23 sites exhibited a monotonic declining trend in their PC1 axis scores (i.e. decreasing acidity) over time (Figure 8;Figure 9). Three of the five sites which showed no trend in their PC1 scores (i.e. no directional change in acidity over time) were located in the north of Scotland, which always experienced less acid deposition and so were not highly acidified at the outset of monitoring (Figure 1)(Patrick, Monteith & Jenkins 1995). This suggests that at least partial chemical recovery from acidification has occurred at most sites at which it was expected (Monteith *et al.* 2014).

Figure 8. Trends in overall acidity (PC1 extracted from Figure 3) at each of the UWMN lake sites. For those sites showing significant monotonic temporal trends (as determined through Mann–Kendall trend tests, see Methods) the test statistic and associated p-value are shown. Sites are arranged in order of their decreasing latitude, which can be used as a proxy for their initial acidified state, more acidified sites were generally in the south (bottom panels), while the least acidified sites were more northern (top panels).
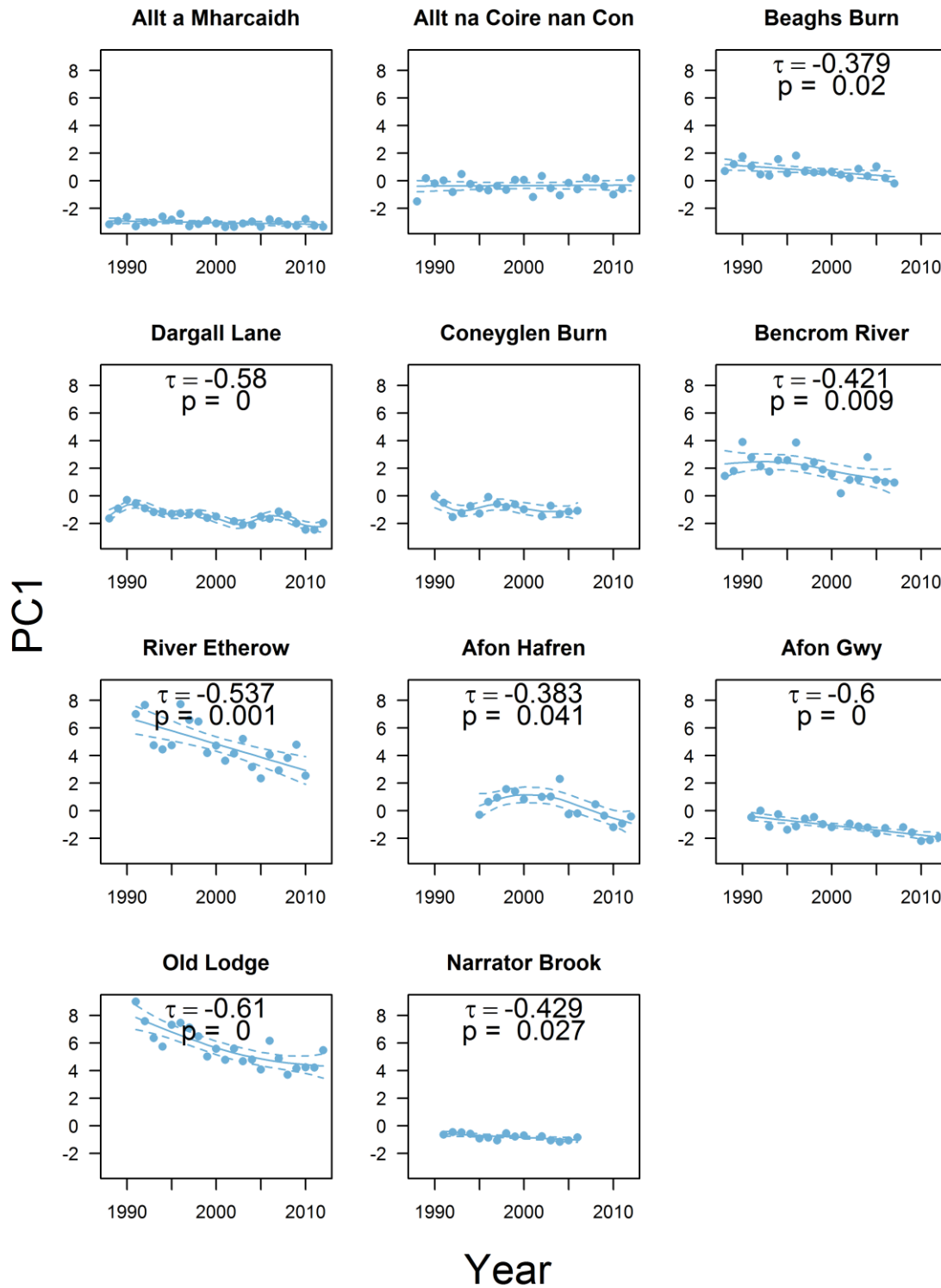
Figure 9. Trends in overall acidity (PC1 extracted from Figure 3) at each of the UWMN stream sites.
For those sites showing significant monotonic temporal trends (as determined through Mann–Kendall
trend tests, see Methods) the test statistic and associated p-value are shown. Sites are arranged in
order of their decreasing latitude, which can be used as a proxy for their initial acidified state, more
acidified sites were generally in the south (bottom panels), while the least acidified sites were more
northern (top panels).

597   Directional change in food web structure was also evident across many of the
598   UWMN sites, in line with the chemical trends and with our second main hypothesis,
599   although it was far from ubiquitous. Of the eighteen sites showing chemical
600   recovery, around half also showed significant increasing trends in connectance (9
601   sites; Table 3; Table 4), linkage density (7 sites; Table 3; Table 4), mean trophic height
602   (8 sites; Table 3; Table 4), resource *Vulnerability* (6 sites; ; Table 3; Table 4), standard
603   deviation in resource *Vulnerability* (5 sites, Figure 10; Figure 11) and network
604   efficiency (7 sites; Table 3; Table 4). Of the eighteen showing chemical recovery,
605   significant declines were evident in consumer *Generality* (10 sites; Table 3; Table 4),
606   redundancy (10 sites; Table 3) and the standard deviation of *Generality* (10 sites;
607   Figure 10; Figure 11). Maximum trophic height increased in one site, decreased in
608   two, and showed no trend in the other 20 (Figure 10; Figure 11). See Appendices for
609   more detailed plots of each trend over time.

610   There was evidence for a delay in food web recovery after chemical recovery
611   at the UWMN sites; most sites occupied the 'both biological and chemical recovery'
612   or 'chemical but no biological recovery' portions of the conceptual recovery figure
613   (Figure 2) for each of their food web metrics (Table 3; Table 4). Very few sites
614   exhibited change in their food web structure in the absence of directional change in
615   their acidity (PC1); the food webs of Loch Coire Fionnaraich and Allt na Coire nan
616   Con both showed increasing linkage density and resource vulnerability over time, in
617   the absence of a significant temporal trend in acidity (Figure 10, Figure 11). Similarly,
618   the food web of Coneyglen Burn decreased in redundancy over time, despite no
619   significant temporal trend in acidity (Figure 10, Figure 11).

620

621

622

623

624

625

626

627

628

630 *Table 3. The number of the twelve lake sites showing a significant temporal trend in their food web*
631 *metrics over time, as determined by Mann–Kendall trend tests (see Methods). Chemical recovery here*
632 *is indicated by a significant temporal trend in acidity (PC1). C = connectance, LD = linkage density,*
633 *meanTH = mean trophic height, maxTH = maximum trophic height, Vul = resource vulnerability,*
634 *Gen = consumer generality, E = network efficiency, Red = node redundancy.*

| Biological & chemical recovery		Biological but no chemical recovery	
C = 6	LD = 2	C = 0	LD = 1
meanTH = 4	maxTH = 0	meanTH = 0	maxTH = 0
Vul = 3	Gen = 6	Vul = 0	Gen = 1
E = 4	Red = 4	E = 0	Red = 0
Chemical but no biological recovery		No biological or chemical recovery	
C = 4	LD = 8	C = 2	LD = 1
meanTH = 6	maxTH = 8	meanTH = 2	maxTH = 2
Vul = 6	Gen = 4	Vul = 2	Gen = 1
E = 6	Red = 6	E = 2	Red = 2

635

636

637 *Table 4. The number of the eleven stream sites showing a significant temporal trend (in the direction*
638 *predicted in hypotheses; recovery) in their food web metrics over time, as determined by Mann–*
639 *Kendall trend tests (see Methods). Chemical recovery here is indicated by a significant temporal trend*
640 *in acidity (PC1). C = connectance, LD = linkage density, mean.TH = mean trophic height, max.TH =*
641 *maximum trophic height, Vul = resource vulnerability, Gen = consumer generality, E = network*
642 *efficiency, Red = node redundancy.*

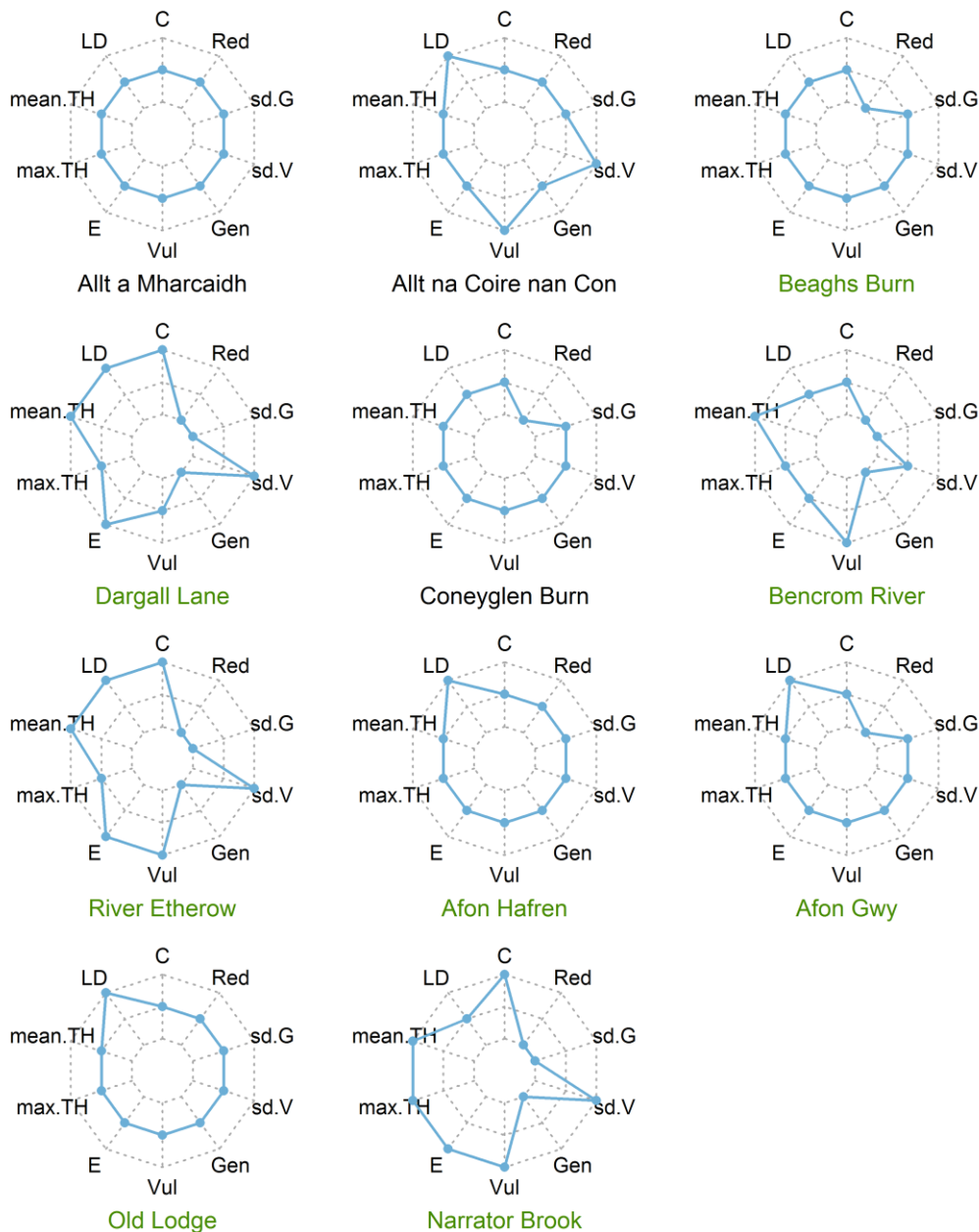| Biological & chemical recovery		Biological but no chemical recovery	
C = 3	LD = 5	C = 0	LD = 1
meanTH = 4	maxTH = 1	meanTH = 0	maxTH = 0
Vul = 3	Gen = 4	Vul = 1	Gen = 0
E = 3	Red = 6	E = 0	Red = 1
Chemical but no biological recovery		No biological or chemical recovery	
C = 5	LD = 3	C = 3	LD = 2
meanTH = 4	maxTH = 7	meanTH = 3	maxTH = 3
Vul = 5	Gen = 4	Vul = 2	Gen = 3
E = 5	Red = 2	E = 3	Red = 2

643

644
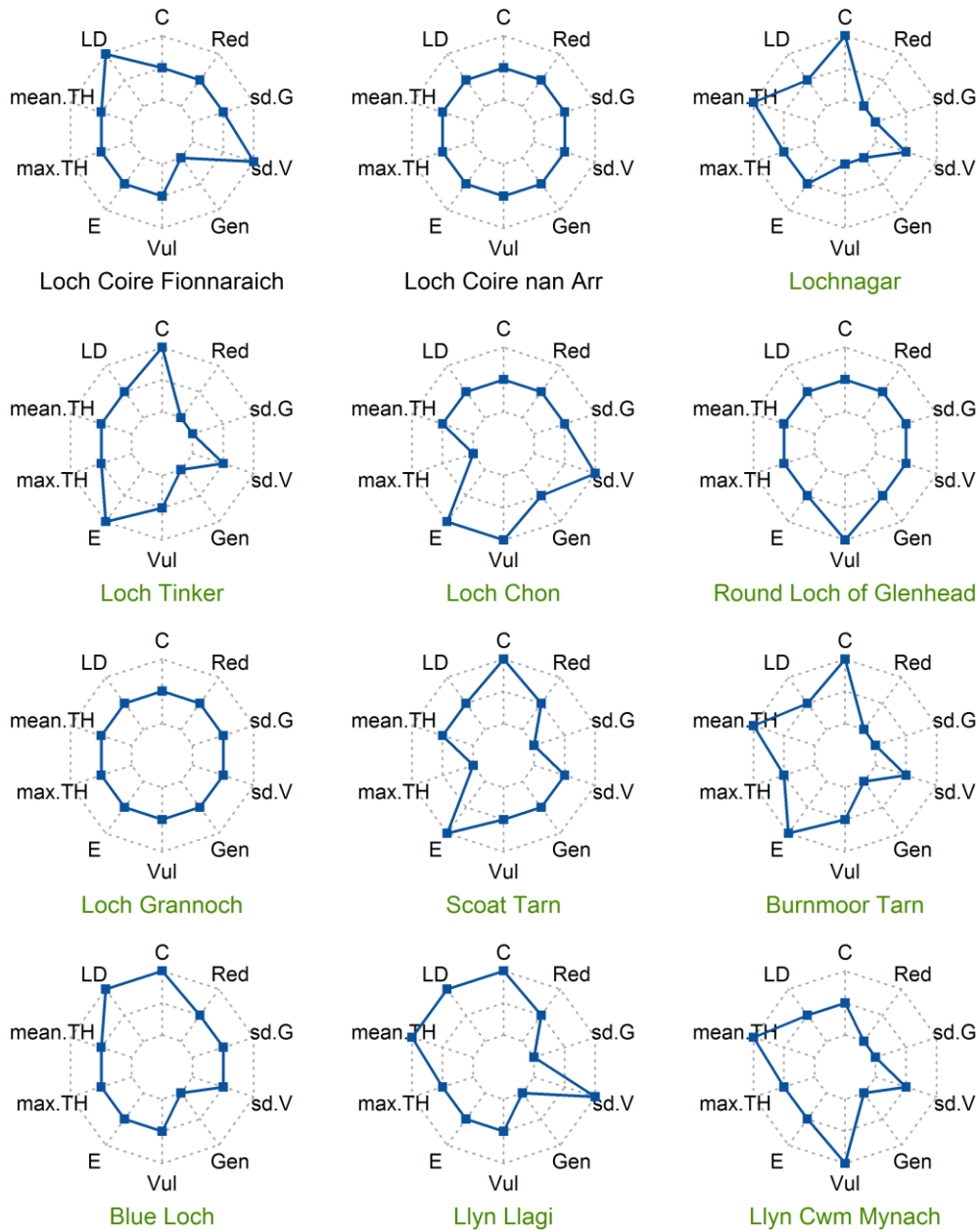
Figure 10. Trends in food web metrics at each of the UWMN lake sites. Sites are arranged in order of their decreasing latitude (top left to bottom right; see Figure 1), which can be used as a proxy for their initial acidified state (generally least acid to the top left, more acidified sites at the bottom). Site names in green indicate a monotonic decreasing trend in acidity over time at that site, site names in black indicate no trend in acidity. Points on the inner ring of each radial plot indicated a negative trend in that variable at that site over time, points on the middle ring indicate no significant trend while points on the outer ring indicate a positive trend. C = connectance, LD = linkage density, mean.TH = mean trophic height, max.TH = maximum trophic height, E = network efficiency, Vul = resource vulnerability, Gen = consumer generality, sd.V = standard deviation in resource vulnerability, sd.G = standard deviation in consumer generality, Red = node redundancy. See Appendices for detailed plots of each trend over time.

Figure 11. Trends in food web metrics at each of the UWMN stream sites. Sites are arranged in order of their decreasing latitude (top left to bottom right; see Figure 1), which can be used as a proxy for their initial acidified state (generally least acid to the top left, more acidified sites at the bottom). Site names in green indicate a monotonic decreasing trend in acidity over time at that site, site names in black indicate no trend in acidity. Points on the inner ring of each radial plot indicated a negative trend in that variable at that site over time, points on the middle ring indicate no significant trend while points on the outer ring indicate a positive trend. C = connectance, LD = linkage density, mean.TH = mean trophic height, max.TH = maximum trophic height, E = network efficiency, Vul = resource vulnerability, Gen = consumer generality, sd.V = standard deviation in resource vulnerability, sd.G = standard deviation in consumer generality, Red = node redundancy. See Appendices for detailed plots of each trend over time.

C. Food web recovery from acidification

Trends in chemistry over time were not linearly related to shifts in food web structure, or at least have not yet related to the latter, strengthening the evidence for inertia in food web recovery. The χ^2 tests revealed that there was little congruence between those sites exhibiting chemical and biological recovery (Table 5). However, the χ^2 tests did reveal that those sites showing a trend in standard deviation in consumer *Generality* also tended to show a trend in acidity (Table 5). This generally refutes our third hypothesis, and provides more evidence for a lag or inertia in food web recovery; those sites recovering from acidification over time showed little systematic change in their food web structure, suggesting that biological recovery (in terms of food web structure) does not directly track chemical recovery at these sites.

Table 5. Results from the χ^2 contingency test (see main text). Bold p-values indicate significance at α = 0.05. See the legend of Table 3 for abbreviations.

	PC1
C	0.113
LD	1
mean.TH	0.123
max.TH	0.574
E	0.146
Vulnerability	1
Generality	1
sd.V	1
sd.G	**0.046**
Redundancy	0.325

IV. Discussion

Our analyses reveals fundamental structural changes occurring in food web structure in response to decreasing acidity over the three decades of the study. These structural changes could have profound implications for the stability of the systems' food webs, and could be hindering biological recovery. Confirming our hypotheses, when analysed at the regional (UK) scale, acidified food webs had lower linkage density, and network efficiency but had more redundancy within their feeding pathways. Contrary to our other hypotheses, we found no effect on connectance, trophic height, nor on resource *Vulnerability* and consumer *Generality* or the standard deviations of both. When analysed at the site scale some further trends in network metrics over time became clearer, but overall these were mixed and often harder to associate with decreasing acidity *per se*. There was strong evidence for a lag in biological recovery, as those sites showing a recovery in both their biology and their chemistry tended to be a nested subset of those that only showed a chemical trend.

A. Food web recovery across the UWMN sites and acidity gradient

The general increasing mean trophic height of food webs over time at each site (see also Figure D Appendices) reflected the reverse of the typical responses to acidification, where species are lost throughout the food web, but top predators such as fish (Henriksen, Fjeld & Hesthagen 1999) and many predatory macroinvertebrates are especially vulnerable (Layer *et al.* 2011). The return of these acid-sensitive species over time causes food chain lengths and the trophic height of the web as a whole to increase (Woodward & Hildrew 2001; Layer et al 2011) – in our dataset all of the sites which experienced this lengthening of food chains were also decreasing in their acidity. However, not all sites with falling acidity also exhibited increases in trophic height, which is again suggestive of food-web inertia. Often site-specific trends were not evident, but were when the data were analysed across the full acidity gradient, also suggesting that other environmental drivers might be modulating the relationship with the food web. For instance DOC, which was closely related to PC2, is known to limit secondary production in lakes (Finstad *et al.* 2014).

Along the derived acidity gradient, normalised *Vulnerability* and *Generality* were unchanged. Over time at individual sites, however, the latter tended to decrease and the former to increase, but across the 23 sites as a whole only seven showed increasing *Vulnerability* and most showed complex and non-linear patterns over time (see Appendices). Decreasing *Generality* and increasing *Vulnerability* with decreasing acidity is consistent with the proposition that specialist consumers and

724 also larger top predators re-colonise communities following chemical recovery
725 (Woodward & Hildrew 2001; Layer, Hildrew & Woodward 2013). This should result
726 in increased *Vulnerability* (more consumers per resource due to greater consumer
727 species richness) and reduced *Generality* (fewer resources per consumer due to
728 increased specialism). The reappearance of acid-sensitive consumers including both
729 invertebrates (such as species of the mayflies *Baetis spp.* and *Caenis spp.*, or the snail
730 *Radix balthica*) and salmonid fish at high pH should lead to both a general elongation
731 and greater compartmentalisation in the web and specialism becomes more
732 prevalent both within and across trophic levels.

733   The connectivity of the food webs as a whole changed across the derived
734 acidity gradient: network efficiency, which describes how 'reachable' each node is
735 from every other, increased with decreasing acidity. If pockets of species are poorly
736 connected to other species, the average shortest path length between all pairs of
737 nodes will increase. Thus, species within more acidified food webs were less well-
738 connected on average across the whole network. The increased species richness and
739 addition of top predators such as salmonid fish to the system (Woodward & Hildrew
740 2001) may explain the increased efficiency of these less-acidified food webs. The top
741 predator of these freshwater systems, the brown trout (*Salmo trutta*) is a highly
742 generalist engulfing predator which will consume anything within a given size
743 range of prey. The addition of these (acid-sensitive) generalist interactions between
744 top predators and those macroinvertebrates within its prey-size range may well
745 increase the reachability between those resource nodes, as well as ultimately linking
746 together different feeding pathways (e.g., the allochthonous vs autochthonous
747 resource base of the food web), even though these may be becoming more
748 compartmentalised horizontally among their increasingly specialist primary
749 consumers.

750   Acidified food webs contained proportionally more redundant feeding
751 pathways than their circumneutral counterparts, the proportion of 'trophic species',
752 nodes feeding on and being fed on by the same species, is larger in the smaller, more
753 acidified food webs. This is consistent with the increase is specialist consumers as
754 acidity decreases. Additionally, acidified food webs tend to have few species and
755 few links (Layer *et al.* 2010b), making the scope for unique feeding pathways small.

756   Contrasting trends emerged when our data were analysed at the site or
757 regional scale. When our data were analysed at the individual site scale, trends were
758 mixed and were not necessarily always related to decreasing acidity, while clearer
759 trends often emerged from the regional scale analysis. This could arise if

760 communities are highly variable when released from a stressor, and other drivers
761 (e.g. nutrients) that were previously uninfluential start to shape local habitat filtering
762 (e.g. Micheli *et al.* 1999). Additionally, site scale sources of variation, such as
763 potentially powerful contingent site characteristics, might have swamped potential
764 underlying trends in food web structure over time. Indeed, site identity was a
765 necessary variable in our models that encompassed a range of site specific variables,
766 such as latitude. Additionally, weather conditions were uncontrolled and extreme
767 events close to the small sampling widow for each site, has caused some sites to lose
768 and regain their significant trends in biotic recovery over time (Monteith & Evans
769 1998, 2005; Kernan *et al.* 2010). Additionally, the portion of the acidity gradient that
770 each site is exposed to is small relative to that of the whole dataset.

771 ## B. The recovery of freshwater food webs from acidification
772   Although some clear responses were evident, the food web metrics used here
773 might not be the most appropriate for detecting recovery from acidification. There
774 were considerable inter-site differences in food web structure, but not all were
775 sensitive to changes in acidity and there was still considerable unexplained variation
776 in the models. It seems likely that, as our understanding grows, more sensitive
777 measures of food web structure will emerge, perhaps through analysis of
778 substructure rather than 'whole network' properties, and that these might be better
779 at capturing ecological responses to environmental change.

780   That acidified ecosystems might exhibit 'ecological inertia' has increasingly been
781 suggested as mechanism to explain the delay in biological recovery (Lundberg,
782 Ranta & Kaitala 2000; Ledger & Hildrew 2005; Kernan *et al.* 2010; Layer *et al.* 2010b).
783 Various lines of evidence are increasingly suggesting that acidified food webs are
784 dynamically stable and resistant to re-colonisation by acid-sensitive species, even as
785 chemical conditions start to improve. Townsend *et al.* (1987) measured the
786 persistence of 27 stream invertebrate communities across a pH gradient, and found
787 that those communities from the most acidified sites were indeed the most
788 persistent, although data on species interactions and network structure were not
789 available at the time. Later, Layer *et al.* (2010b) used dynamic modelling to determine
790 the robustness of stream food webs to species extinctions, and found that food webs
791 from more acidified conditions were more robust, but the long-term temporal data
792 were not available to test this prediction empirically. Here we provide the largest
793 scale evidence to test these ideas, which broadly support the general notion that
794 redundancy is an important component of stability that could confer robustness on
795 the system. In ecosystems redundancy can increase the reliability of process rates

796 and buffer the effects of species loss (Naeem 1998; Peralta *et al.* 2014): we found that
797 food webs from acidified waters had higher redundancy, suggesting that they might
798 be more robust, and might therefore provide more stable (albeit often slower)
799 process rates (Naeem 1998; Peralta *et al.* 2014). As acidity decreases in fresh waters,
800 decomposition of leaf-litter, which fuels much of the food web, does indeed
801 accelerate (Jenkins, Woodward & Hildrew 2013), although the extent to which
802 species richness modulates this relationship is still largely unknown (but see Jonsson
803 *et al.* 2002). Additionally, we found that more acidified food webs had lower global
804 efficiency, which is associated with reduced small-world properties. Ecological
805 networks that are small worlds are often relatively stable (Solé & Montoya 2001;
806 Dunne, Williams & Martinez 2002), as they offer many alternative pathways of
807 interaction. These apparently contrasting responses to different dimensions of
808 stability warrant further investigation to reveal if acidified food webs are indeed
809 more (or less) stable in some regards and not others (e.g. Donohue *et al.* 2013).

C. Caveats and future directions

811   The use of inferred feeding links in food web studies has been criticised on
812 the basis that they might over estimate diet breadth, and fail to detect behavioural
813 differences between sites (Hall & Raffaelli 1997; Raffaelli 2007), yet to build complete
814 food webs *de novo* from replicated natural systems is simply logistically unfeasible,
815 so a trade-off between replication and realism is inevitable. The use of 'summary'
816 food webs, which include the full complement of known possible tropic interactions
817 can still be a useful tool for understanding community structure, especially as in
818 freshwaters most species are highly generalist and their diets are largely size-driven
819 and consistent among systems when presented with the same potential prey species
820 (Woodward *et al.* 2010b; Layer, Hildrew & Woodward 2013; Gray *et al.* 2015). Indeed,
821 given the nature of building summary food webs, that they tend to overestimate
822 interactions between species, they are more likely to be insensitive to environmental
823 change rather than reveal erroneous trends (i.e. it is more likely that the structure of
824 summary food webs is conserved given that any changes will be entirely driven by
825 changes in species composition rather than feeding behaviour). Hence, we contend
826 that the trends revealed here are broadly realistic, and warrant further examination,
827 especially as the feeding links described in many of our webs had been observed in
828 the same system, albeit only for a snapshot of the full set of sampling occasions.
829 Future work could involve a more formal validation of randomly selected portions
830 of the network via direct analysis of gut contents (as in Woodward, Speirs &
831 Hildrew 2005; Layer *et al.* 2010b) and also the application of new molecular

832 approaches that could potentially capture a more complete picture of the entire food
833 web with a fraction of the current effort required (Gray *et al.* 2014).

834   Another potential limitation to the food webs produced here is that they do
835 not include the full freshwater community, in particular the meiofauna and
836 microfauna (e.g. Schmid-Araya *et al.* 2002) and true apex predators such as the
837 European Dipper (*Cinclus cinclus)* or Otter (*Lutra lutra).* Top predators can have
838 varying effects on food web structure in these systems (Woodward & Hildrew 2001;
839 Layer *et al.* 2011), and so their exclusion may be omitting an important source of
840 variation in this data. However, this was unavoidable in this study, as in almost all
841 other food webs described to date, because the presence of these cryptic or very rare
842 species has not been systematically recorded. Additionally, although the fish
843 assemblage of the lakes were sampled from the lake outflows, all these low-
844 productivity upland sites are typically dominated by brown trout (*Salmo trutta*) and
845 the occasional European eel (*Anguilla anguilla*) in both the running and standing
846 waters across the acidity gradient: of the 434 sampling occasions on which fish were
847 present at a site, brown trout were always present, reflecting its dominance in these
848 systems. The next most common species was the European eel (*Anguilla anguilla*),
849 was found on 136 occasions and all other species (*Esox lucius, Gasterosteus aculeatus,*
850 *Lampetra spp., Phoxinus phoxinus* and *Salmo salar*) were found on <60 sampling
851 occasions.

852

D. Conclusion

854   It is clear from this study that both spatial and temporal scales are important
855 considerations when assessing food web responses to environmental change in real
856 time (Chave 2013). When our data were analysed at the individual site scale, trends
857 were mixed and were not necessarily always related to decreasing acidity. When the
858 data were analysed at the regional (UK) scale, some clear and significant trends
859 emerged, highlighting the need for large, replicated collections of food webs as well
860 as the need for caution when extrapolating from small collections or individual food
861 webs. Identifying the effects of individual chemical drivers was often challenging
862 given the range of potential drivers in a nationwide dataset that also spans several
863 decades.

864   To the best of our knowledge this is the largest collection of food webs that
865 span both large temporal and spatial gradients: the next largest set of empirical food
866 webs from natural systems of which we are aware is less than half the size (the 170

867 soil webs described by Mulder *et al.* 2011) and the remainder are far smaller still,
868 with most studies being conducted on unreplicated singletons (Ings et al 2009). Our
869 study is thus one of the first to address macroecological questions relating to the
870 structure of food webs across time and a broad environmental gradient in a
871 (relatively) standardised manner. Our analysis reveals fundamental structural
872 changes occurring in the food webs as they respond to changes in acidity, these
873 structural changes could have profound implications for the stability of the system,
874 and may be limiting biological recovery. It would be instructive to investigate
875 further the stability of these food webs, in order to explore more fully whether
876 intrinsic inertia is indeed limiting their recovery, and how that might possibly be
877 manipulated to accelerate the rate of recovery.

878

V.   Acknowledgements

891

892

893

VI.   References

Battarbee, R.W., Anderson, N.J., Appleby, P.G., Flower, R.J., Fritz, S.C., Haworth, E.Y., Higgitt, S., Jones, V.J., Kreiser, A., Munro, M.A.R., Natkanski, J., Oldfield, F., Patrick, S.T., Richardson, N.G., Rippey, B. & Stevenson, A.C.L.E. (1988) *Lake Acidification in the United Kingdom 1800–1986: Evidence from Analysis of Lake Sediments.* ENSIS Ltd, London.

Battarbee, R.W., Jones, V.J., Flower, R.J., Cameron, N.G., Bennion, H., Carvalho, L. & Juggins, S. (2001) Diatoms. *Tracking Environmental Change Using Lake Sediments. Volume 3: Terrestrial, Algal and Siliceous Indicators* pp. 151–202. Kluwer Academic Publishers, Dordrecht, The Netherlands.

Battarbee, R.W., Simpson, G.L., Shilland, E.M., Flower, R.J., Kreiser, A., Yang, H. & Clarke, G. (2014) Recovery of UK lakes from acidification: An assessment using combined palaeoecological and contemporary diatom assemblage data. *Ecological Indicators*, **37, Part B**, 365–380.

van den Berg, R. a, Hoefsloot, H.C.J., Westerhuis, J. a, Smilde, A.K. & van der Werf, M.J. (2006) Centering, scaling, and transformations: improving the biological information content of metabolomics data. *BMC genomics*, **7**, 142.

Briand, F. & Cohen, J.E. (1984) Community food webs have scale-invariant structure. *Nature*, **307**, 264–267.

Butts, C. (2013) sna: Tools for Social Network Analysis. R package version 2.3-2. http://CRAN.R-project.org/package=sna.

Chave, J. (2013) The problem of pattern and scale in ecology: What have we learned in 20 years? *Ecology Letters*, **16**, 4–16.

Cohen, J.E. & Briand, F. (1984) Trophic links of community food webs. *Proceedings of the National Academy of Sciences of the United States of America*, **81**, 4105–4109.

Crawley, M.J. (2013) *The R Book*, second.

Dillon, P.J., Yan, N.D., Harvey, H.H. & Schindler, D.W. (1984) Acidic deposition: effects on aquatic ecosystems. *CRC Critical reviews in Environmental Control*, **13**, 167–194.

Donohue, I., Petchey, O.L., Montoya, J.M., Jackson, A.L., Mcnally, L., Viana, M., Healy, K., Lurgi, M., O'Connor, N.E. & Emmerson, M.C. (2013) On the dimensionality of ecological stability. *Ecology Letters*, **16**, 421–9.

Driscoll, C.T., Lawrence, G.B., Bulger, A.J., Butler, T.J., Cronan, C.S., Eagar, C., Lambert, K.F., Likens, G.E., Stoddard, J.L. & Weathers, K.C. (2001) Acidic Deposition in the Northeastern United States: Sources and Inputs, Ecosystem Effects, and Management Strategies. *BioScience*, **51**, 180.

Dunne, J.A., Williams, R.J. & Martinez, N.D. (2002) Food-web structure and network theory: The role of connectance and size. *Proceedings of the National Academy of Sciences of the United States of America*, **99**, 12917–12922.

933 Durance, I. & Ormerod, S.J. (2007) Climate change effects on upland stream
934   macroinvertebrates over a 25-year period. *Global Change Biology*, **13**, 942–957.

935 Evans, C.D., Monteith, D.T. & Harriman, R. (2001) Long-term variability in the
936   deposition of marine ions at west coast sites in the UK Acid Waters Monitoring
937   Network: Impacts on surface water chemistry and significance for trend
938   determination. *Science of the Total Environment*, **265**, 115–129.

939 Evans, C.D., Monteith, D.T., Reynolds, B. & Clark, J.M. (2008) Buffering of recovery
940   from acidification by organic acids. *Science of the Total Environment*, **404**, 316–325.

941 Feld, C.K., Birk, S., Bradley, D.C., Hering, D., Kail, J., Marzin, A., Melcher, A.,
942   Nemitz, D., Pedersen, M.L., Pletterbauer, F., Pont, D., Verdonschot, P.F.M. &
943   Friberg, N. (2011) From Natural to Degraded Rivers and Back Again: A Test of
944   Restoration Ecology Theory and Practice. *Advances in Ecological Research*, **44**,
945   119–209.

946 Findlay, D., Kasian, S., Turner, M. & Stainton, M. (1999) Responses of phytoplankton
947   and epilithon during acidification and early recovery of a lake. *Freshwater
948   Biology*, **42**, 159–175.

949 Finstad, A.G., Helland, I.P., Ugedal, O., Hesthagen, T. & Hessen, D.O. (2014)
950   Unimodal response of fish yield to dissolved organic carbon. *Ecology Letters*, **17**,
951   36–43.

952 Friberg, N., Bonada, N., Bradley, D.C., Dunbar, M.J., Edwards, F.K., Grey, J., Hayes,
953   R.B., Hildrew, A.G., Lamouroux, N., Trimmer, M. & Woodward, G. (2011)
954   Biomonitoring of Human Impacts in Freshwater Ecosystems: The Good, the Bad
955   and the Ugly. *Advances in Ecological Research*, **44**, 1–68.

956 Frost, T.M., Montz, P.K. & Kratz, T.K. (1998) Zooplankton community responses
957   during recovery from acidification in Little Rock Lake, Wisconsin. *Restoration
958   Ecology*, **6**, 336–342.

959 Gerson, J.R., Driscoll, C.T. & Roy, K.M. Patterns of nutrient dynamics in Adirondack
960   lakes recovering from acid deposition. *Ecological Applications*.

961 Grahn, O., Hultberg, H. & Landner, L. (1974) Oligotrophication: a self-accelerating
962   process in lakes subjected to excessive supply of acid substances. *Ambio*, **3**, 93–
963   94.

964 Gray, D.K. & Arnott, S.E. (2011) Does dispersal limitation impact the recovery of
965   zooplankton communities damaged by a regional stressor? *Ecological
966   Applications*, **21**, 1241–1256.

967 Gray, C., Baird, D.J., Baumgartner, S., Jacob, U., Jenkins, G.B., O'Gorman, E.J., Lu, X.,
968   Ma, A., Pocock, M.J.O., Schuwirth, N., Thompson, M. & Woodward, G. (2014)
969   Ecological networks: the missing links in biomonitoring science. *Journal of
970   Applied Ecology*, **51**, 1444–1449.

971 Gray, C., Figueroa, D.H., Hudson, L.N., Ma, A., Perkins, D. & Woodward, G. (2015)
972   Joining the dots: an automated method for constructing food webs from

973   compendia of published interactions. *Food Webs*, **5**, 11–20.

974   Hall, R.J., Likens, G.E., Fiance, S.B. & Hendrey, G.R. (1980) Experimental
975   Acidification of a Stream in the Hubbard Brook Experimental Forest, New
976   Hampshire. *Ecology*, **61**, 976–989.

977   Hall, S.J. & Raffaelli, D. (1991) Food-Web Patterns: Lessons from a Species-Rich Web.
978   *Journal of Animal Ecology*, **60**, 823–841.

979   Hall, S.J. & Raffaelli, D.G. (1997) Food web patterns: What do we really know?
980   *Multitrophic Interactions* (eds A.C. Gange), & V.K. Brown), pp. 395–417.
981   Blackwells Scientific Publications, Oxford, UK.

982   Hämäläinen, H. & Huttunen, P. (1996) Inferring the minimum pH of streams from
983   macroinvertebrates using weighted averaging regression and calibration.
984   *Freshwater Biology*, **36**, 697–709.

985   Henriksen, A., Fjeld, E. & Hesthagen, T. (1999) Critical load exceedance and damage
986   to fish populations. *Ambio*, **28**, 583–586.

987   Henriksen, A., Lien, L., Traan, T.S., Rosseland, B.O. & Sevaldrud, I.S. (1990) The
988   1000-lake survey in Norway 1986. *The Surface Waters Acidification Programme* (ed
989   B.J. Mason), pp. 199–212. Cambridge University Press, Cambridge, UK.

990   Hildrew, A.G. (2009) Sustained Research on Stream Communities: A Model System
991   and The Comparative Approach. *Advances in Ecological Research*, **41**, 175–312.

992   Hildrew, A. & Ormerod, S. (1995) Acidification: causes, consequences and solutions.
993   *The ecological basis for river management* (eds D.M. Harper), & A.J.D. Ferguson),
994   pp. 147–160. J. Wiley.

995   Hildrew, A.G., Townsend, C.R. & Hasham, A. (1985) The predatory chironomidae of
996   an iron-rich stream - feeding ecology and food web structure. *Ecological
997   Entomology*, **10**, 403–413.

998   Hudson, L.N., Emerson, R., Jenkins, G.B., Layer, K., Ledger, M.E., Pichler, D.E.,
999   Thompson, M.S.A., O'Gorman, E.J., Woodward, G. & Reuman, D.C. (2013)
1000   Cheddar: analysis and visualisation of ecological communities in R. *Methods in
1001   Ecology and Evolution*, **4**, 99–104.

1002   Ings, T.C., Montoya, J.M., Bascompte, J., Bluthgen, N., Brown, L., Dormann, C.F.,
1003   Edwards, F., Figueroa, D., Jacob, U., Jones, J.I., Lauridsen, R.B., Ledger, M.E.,
1004   Lewis, H.M., Olesen, J.M., van Veen, F.J.F., Warren, P.H. & Woodward, G.
1005   (2009) Ecological networks - beyond food webs. *Journal of Animal Ecology*, **78**,
1006   253–269.

1007   Jenkins, A., Whitehead, P.G., Cosby, B.J. & Birks, H.J.B. (1990) Modelling long-term
1008   acidification: A comparison with diatom reconstructions and the implications
1009   for reversibility. *Philosophical Transactions of the Royal Society B: Biological Sciences*,
1010   **327**, 435–440.

1011   Jenkins, G.B., Woodward, G. & Hildrew, A.G. (2013) Long-term amelioration of

1012   acidity accelerates decomposition in headwater streams. *Global Change Biology*,
1013   **19**, 1100–1106.

1014   Johnson, P.C.D. (2014) Extension of Nakagawa & Schielzeth's R 2 GLMM to random
1015   slopes models. *Methods in Ecology and Evolution*, **5**, 944–946.

1016   Johnson, R.K. & Angeler, D.G. (2010) Tracing recovery under changing climate:
1017   response of phytoplankton and invertebrate assemblages to decreased
1018   acidification. *Journal of the North American Benthological Society*, **29**, 1472–1490.

1019   Jonsson, M., Dangles, O., Malmqvist, B. & Guérold, F. (2002) Simulating species loss
1020   following perturbation: assessing the effects on process rates. *Proceedings.*
1021   *Biological sciences / The Royal Society*, **269**, 1047–52.

1022   Keller, W., Yan, N.D., Gunn, J.M. & Heneberry, J. (2007) Recovery of Acidified Lakes:
1023   Lessons From Sudbury, Ontario, Canada. *Acid Rain - Deposition and Recovery* p.
1024   pp 317–322.

1025   Keller, W., Yan, N.D., Somers, K.M. & Heneberry, J.H. (2002) Crustacean
1026   zooplankton communities in lakes recovering from acidification. *Canadian*
1027   *Journal of Fisheries and Aquatic Sciences*, **59**, 726–735.

1028   Kernan, M., Battarbee, R.W., Curtis, C., Monteith, D.T. & Shillands, E.M. (2010)
1029   *Recovery of Lakes and Streams in the UK from Acid Rain. The United Kingdom Acid*
1030   *Waters Monitoring Network 20 Year Interpretative Report*. Report to the
1031   Department for Environment, Food and Rural Affairs.

1032   Kowalik, R.A., Cooper, D.M., Evans, C.D. & Ormerod, S.J. (2007) Acidic episodes
1033   retard the biological recovery of upland British streams from chronic
1034   acidification. *Global Change Biology*, **13**, 2439–2452.

1035   Latora, V. & Marchiori, M. (2001) Efficient Behavior of Small-World Networks.
1036   *Physical Review Letters*, **87**, 198701.

1037   Lawton, J.H. (1989) Food webs. *Ecological concepts* (ed J.M. Cherrett), pp. 43–78.
1038   Blackwell Scientific, Oxford, UK.

1039   Layer, K., Hildrew, A.G., Jenkins, G.B., Riede, J.O., Rossiter, S.J., Townsend, C.R. &
1040   Woodward, G. (2011) Long-Term Dynamics of a Well-Characterised Food Web:
1041   Four Decades of Acidification and Recovery in the Broadstone Stream Model
1042   System (ed G Woodward). *Advances in Ecological Research*, **44**, 69–117.

1043   Layer, K., Hildrew, A., Monteith, D. & Woodward, G. (2010a) Long-term variation in
1044   the littoral food web of an acidified mountain lake. *Global Change Biology*, **16**,
1045   3133–3143.

1046   Layer, K., Hildrew, A.G. & Woodward, G. (2013) Grazing and detritivory in 20
1047   stream food webs across a broad pH gradient. *Oecologia*, **171**, 459–471.

1048   Layer, K., Riede, J.O., Hildrew, A.G. & Woodward, G. (2010b) Food Web Structure
1049   and Stability in 20 Streams Across a Wide pH Gradient. *Advances in Ecological*
1050   *Research*, **42**, 265–299.

1051   Ledger, M.E., Brown, L.E., Edwards, F.K., Milner, A.M. & Woodward, G. (2012)
1052   Drought alters the structure and functioning of complex food webs. *Nature*
1053   *Climate Change*, **3**, 223–227.

1054   Ledger, M.E. & Hildrew, A.G. (2005) The ecology of acidification and recovery:
1055   changes in herbivore-algal food web linkages across a stream pH gradient.
1056   *Environmental Pollution*, **137**, 103–118.

1057   Lepori, F. & Ormerod, S.J. (2005) Effects of spring acid episodes on
1058   macroinvertebrates revealed by population data and in situ toxicity tests.
1059   *Freshwater Biology*, **50**, 1568–1577.

1060   Levine, S. (1980) Several measures of trophic structure applicable to complex food
1061   webs. *Journal of Theoretical Biology*, **83**, 195–207.

1062   Likens, G.E. & Bormann, F.H. (1974) Acid rain: A serious regional environmental
1063   problem. *Science*, **184**, 1176–1179.

1064   Lundberg, P., Ranta, E. & Kaitala, V. (2000) Species loss leads to community closure.
1065   *Ecology Letters*, **3**, 465–468.

1066   Martinez, N.D. (1991) Artifacts or attributes? Effects of resolution on the Little Rock
1067   Lake food web. *Ecological Monographs*, **61**, 367–392.

1068   Masters, Z.O.E., Peteresen, I., Hildrew, A.G. & Ormerod, S.J. (2007) Insect dispersal
1069   does not limit the biological recovery of streams from acidification. *Aquatic*
1070   *Conservation: Marine and Freshwater Ecosystems*, **17**, 375–383.

1071   McLaughlin, Ó.B., Emmerson, M.C. & O'Gorman, E.J. (2013) *Habitat Isolation Reduces*
1072   *the Temporal Stability of Island Ecosystems in the Face of Flood Disturbance*, 1st ed.
1073   Elsevier Ltd.

1074   Micheli, F., Cottingham, Kathryn L., Bascompte, J., Bjørnstad, O.N., Eckert, G.L.,
1075   Fischer, J.M., Keitt, T.H., Kendall, B.E., Klug, J.L. & Rusak, J.A. (1999) The Dual
1076   Nature of Community Variability. *Oikos*, **85**, 161–169.

1077   Monteith, D.T. & Evans, C.D. (1998) *United Kingdom Acid Waters Monitoring Network*
1078   *10 Year Report. Analysis and Interpretation of Results, April 1988 - March 1998.*
1079   *Report to the Department for Environment, Food and Rural Affairs (Contract EPG*
1080   *1/3/160).*

1081   Monteith, D.T. & Evans, C.D. (2005) The United Kingdom Acid Waters Monitoring
1082   Network: a review of the first 15 years and introduction to the special issue.
1083   *Environmental Pollution*, **137**, 3–13.

1084   Monteith, D.T., Evans, C.D., Henrys, P.A., Simpson, G.L. & Malcolm, I.A. (2014)
1085   Trends in the hydrochemistry of acid-sensitive surface waters in the UK 1988–
1086   2008. *Ecological Indicators*, **37**, 287–303.

1087   Monteith, D.T., Hildrew, A.G., Flower, R.J., Raven, P.J., Beaumont, W.R.B., Collen,
1088   P., Kreiser, A.M., Shilland, E.M. & Winterbottom, J.H. (2005) Biological
1089   responses to the chemical recovery of acidified fresh waters in the UK.

1090   *Environmental Pollution*, **137**, 83–101.

1091   Montoya, J.M., Pimm, S.L. & Solé, R. V. (2006) Ecological networks and their
1092   fragility. *Nature*, **442**, 259–264.

1093   Montoya, J.M. & Solé, R. V. (2002) Small world patterns in food webs. *Journal of*
1094   *Theoretical Biology*, **214**, 405–412.

1095   Mulder, C., Boit, A., Bonkowski, M., De Ruiter, P.C., Mancinelli, G., Van der Heijden,
1096   M.G.A., Van Wijnen, H.J., Vonk, J.A. & Rutgers, M. (2011) A Belowground
1097   Perspective on Dutch Agroecosystems: How Soil Organisms Interact to Support
1098   Ecosystem Services. *Advances in Ecological Research*, **44**, 277–357.

1099   Murphy, J.F., Winterbottom, J.H., Orton, S., Simpson, G.L., Shilland, E.M. &
1100   Hildrew, A.G. (2014) Evidence of recovery from acidification in the
1101   macroinvertebrate assemblages of UK fresh waters: A 20-year time series.
1102   *Ecological Indicators*, **37**, 330–340.

1103   Naeem, S. (1998) Species redundancy and ecosystem reliability. *Conservation Biology*,
1104   **12**, 39–45.

1105   Nedbalová, L., Vrba, J., Fott, J., Kohout, L., Kopáček, J., Macek, M. & Soldán, T.
1106   (2006) Biological recovery of the Bohemian Forest lakes from acidification.
1107   *Biologia*, **61**, 453–465.

1108   O'Gorman, E.J. & Emmerson, M.C. (2010) *Manipulating Interaction Strengths and the*
1109   *Consequences for Trivariate Patterns in a Marine Food Web*, 1st ed. Elsevier Ltd.

1110   O'Neill, R. V. (1998) Recovery in complex ecosystems. *Journal of Aquatic Ecosystem*
1111   *Stress and Recovery*, **6**, 181–187.

1112   Patrick, S., Monteith, D.T. & Jenkins, A. (Eds.). (1995) *UK Acid Waters Monitoring*
1113   *Network: The First Five Years. Analysis and Interpretation of Results, April 1988 -*
1114   *March 1993*. London.

1115   Patrick, S., Waters, D., Juggins, S. & Jenkins, A. (1991) *The United Kingdom Acid*
1116   *Waters Monitoring Network: Site Descriptions and Methodology Report. Report to the*
1117   *Department of the Environment and Department of the Environment Northern Ireland*.
1118   London.

1119   Peralta, G., Frost, C.M., Rand, T.A., Didham, R.K. & Tylianakis, J.M. (2014)
1120   Complementarity and redundancy of interactions enhance attack rates and
1121   spatial stability in host-parasitoid food webs. *Ecology*, **95**, 1888–1896.

1122   Pimm, S.L. (1980) Properties of Food Webs. *Ecology*, **61**, 219–255.

1123   Pimm, S.L., Russell, G.J., Gittleman, J.L. & Brooks, T.M. (1995) The Future of
1124   Biodiversity. *Science*, **269**, 347–350.

1125   Pocock, M.J.O., Evans, D.M. & Memmott, J. (2012) The Robustness and Restoration
1126   of a Network of Ecological Networks. *Science*, **335**, 973–977.

1127   R Core Team. (2013) R: A language and environment for statistical computing.

1128 Raffaelli, D. (2007) Food Webs, Body Size and the Curse of the Latin Binomial. *From*
1129   *Energetics to Ecosystems: The Dynamics and Structure of Ecological Systems* (eds N.
1130   Rooney),, K.S. McCann), & D.L.G. Noakes), pp. 53–64. Springer Netherlands.

1131 RoTAP. (2012) *Review of Transboundary Air Pollution. Acidification, Eutrophication,*
1132   *Ground Level Ozone and Heavy Metals in the UK. Contract Report to the Dept of the*
1133   *Environment, Food and Rural Affairs*.

1134 Scheffer, M. & Carpenter, S.R. (2003) Catastrophic regime shifts in ecosystems:
1135   linking theory to observation. *Trends in Ecology and Evolution*, **18**, 648–656.

1136 Schindler, D.W. (1988) Effects of Acid rain on freshwater ecosystems. *Science*, **239**,
1137   149–157.

1138 Schmid-Araya, J.M., Hildrew, a. G., Robertson, a., Schmid, P.E. & Winterbottom, J.
1139   (2002) The importance of meiofauna in food webs: Evidence from an acid
1140   stream. *Ecology*, **83**, 1271–1285.

1141 Schoener, W. & Schoenerz, T.W. (1989) Food Webs From the Small To the Large.
1142   *Ecology*, **70**, 1559–1589.

1143 Smith, B.D. & Zeder, M.A. (2013) The onset of the Anthropocene. *Anthropocene*, **4**, 8–
1144   13.

1145 Solé, R. V., Ferrer-Cancho, R., Montoya, J.M. & Valverde, S. (2003) Selection,
1146   tinkering, and emergence in complex networks. *Complexity*, **8**, 20–33.

1147 Solé, R. V. & Montoya, J.M. (2001) Complexity and fragility in ecological networks.
1148   *Proceedings of the Royal Society B: Biological Sciences*, **268**, 2039–2045.

1149 Steffen, W., Grinevald, J., Crutzen, P. & McNeill, J. (2011) The Anthropocene:
1150   conceptual and historical perspectives. *Philosophical Transactions of the Royal*
1151   *Society A*, **369**, 842–867.

1152 Stoddard, J.L., Jeffries, D.S., Lukewille, A., Clair, T.A., Dillon, P.J., Driscoll, C.T.,
1153   Forsius, M., Johannessen, M., Kahl, J.S., Kellogg, J.H., Kemp, A., Mannio, J.,
1154   Monteith, D.T., Murdoch, P.S., Patrick, S., Rebsdorf, A., Skjelkvale, B.L.,
1155   Stainton, M.P., Traaen, T., van Dam, H., Webster, K.E., Wieting, J. & Wilander,
1156   A. (1999) Regional trends in aquatic recovery from acidification in North
1157   America and Europe. *Nature*, **401**, 575–578.

1158 Sutcliffe, D.W. & Hildrew, A.G. (1989) Invertebrate communities in acid streams.
1159   *Acid Toxicity and Aquatic Animals* (eds R. Morris),, E.W. Taylor),, D.J.A. Brown),
1160   & J.A. Brown), pp. 13–29. Seminar Series of the Society for Experimental
1161   Biology, Cambridge University Press.

1162 Sutherland, W.J., Broad, S., Caine, J., Clout, M., Dicks, L. V., Doran, H., Entwistle,
1163   A.C., Fleishman, E., Gibbons, D.W., Keim, B., LeAnstey, B., Lickorish, F.A.,
1164   Markillie, P., Monk, K.A., Mortimer, D., Ockendon, N., Pearce-Higgins, J.W.,
1165   Peck, L.S., Pretty, J., Rockström, J., Spalding, M.D., Tonneijck, F.H., Wintle, B.C.,
1166   Wright, K.E., William J. Sutherland, Steven Broad, Jacqueline Caine, Mick Clout,
1167   Lynn V. Dicks, Helen Doran, Abigail C. Entwistle, Erica Fleishman, David W.

Gibbons, Brandon Keim, Becky LeAnstey, Fiona A. Lickorish, Paul Markillie, Kathryn A. Monk, Diana Mortimer, Nancy Ockendon, James W. Pearce-Higgins, Lloyd S. Peck, Jules Pretty, Johan Rockström, Mark D. Spalding, Femke H. Tonneijck, Bonnie C. Wintle & Katherine E. Wright. (2016) A Horizon Scan of Global Conservation Issues for 2016. *Trends in Ecology and Evolution*, **31**, 44–53.

Thébault, E. & Fontaine, C. (2010) Stability of Ecological Communities and the Architecture of Mutualistic and Trophic Networks. *Science*, **329**, 853–856.

Thompson, M.S.A., Bankier, C., Bell, T., Dumbrell, A.J., Gray, C., Ledger, M.E., Lehman, K., McKew, B.A., Sayer, C.D., Shelley, F., Trimmer, M., Warren, S.L. & Woodward, G. (2015) Gene-to-ecosystem impacts of a catastrophic pesticide spill: testing a multilevel bioassessment approach in a river ecosystem. *Freshwater Biology*.

Thompson, R.M., Brose, U., Dunne, J.A., Hall, R.O., Hladyz, S., Kitching, R.L., Martinez, N.D., Rantala, H., Romanuk, T.N., Stouffer, D.B. & Tylianakis, J.M. (2012) Food webs: reconciling the structure and function of biodiversity. *Trends in Ecology and Evolution*, **27**, 689–697.

Townsend, C.R., Hildrew, A.G. & Francis, J. (1983) Community structure in some southern English streams: the influence of physicochemical factors. *Freshwater Biology*, **13**, 521–544.

Townsend, C.R., Hildrew, A.G. & Schofield, K. (1987) Persistence of Stream Invertebrate Communities in Relation to Environmental Variability. *Journal of Animal Ecology*, **56**, 597–613.

Tylianakis, J.M., Tscharntke, T. & Lewis, O.T. (2007) Habitat modification alters the structure of tropical host-parasitoid food webs. *Nature*, **445**, 202–205.

Warren, P.H. (1994) Making connections in food webs. *Trends in Ecology & Evolution*, **9**, 136–141.

Watts, D.J. & Strogatz, S.H. (1998) Collective dynamics of 'small-world' networks. *Nature*, **393**, 440–2.

Webster, K.E., Frost, T.M., Watras, C.J., Swenson, W.A., Gonzalez, M. & Garrison, P.J. (1992) Complex biological responses to the experimental acidification of Little Rock Lake, Wisconsin, USA. *Environmental Pollution*, **78**, 73–78.

Williams, R.J. & Martinez, N.D. (2000) Simple rules yield complex food webs. *Nature*, **404**, 180–183.

Williams, R.J. & Martinez, N.D. (2004) Limits to trophic levels and omnivory in complex food webs: theory and data. *The American Naturalist*, **163**, 458–68.

Woodward, G., Benstead, J.P., Beveridge, O.S., Blanchard, J., Brey, T., Brown, L.E., Cross, W.F., Friberg, N., Ings, T.C., Jacob, U., Jennings, S., Ledger, M.E., Milner, A.M., Montoya, J.M., O'Gorman, E.J., Olesen, J.M., Petchey, O.L., Pichler, D.E., Reuman, D.C., Thompson, M.S.A., Van Veen, F.J.F. & Yvon-Durocher, G. (2010a) Ecological Networks in a Changing Climate. *Advances in Ecological Research*, **42**,

1208   71–138.

1209   Woodward, G., Blanchard, J., Lauridsen, R.B., Edwards, F.K., Jones, J.I., Figueroa, D.,
1210   Warren, P.H. & Petchey, O.L. (2010b) Individual-Based Food Webs: Species
1211   Identity, Body Size and Sampling Effects. *Advances in Ecological Research*, **43**,
1212   211–266.

1213   Woodward, G., Brown, L.E., Edwards, F.K., Hudson, L.N., Milner, A.M., Reuman,
1214   D.C. & Ledger, M.E. (2012) Climate change impacts in multispecies systems:
1215   drought alters food web size structure in a field experiment. *Philosophical*
1216   *Transactions of the Royal Society B: Biological Sciences*, **367**, 2990–2997.

1217   Woodward, G. & Hildrew, A.G. (2001) Invasion of a stream food web by a new top
1218   predator. *Journal of Animal Ecology*, **70**, 273–288.

1219   Woodward, G. & Hildrew, A.G. (2002) Body-size determinants of niche overlap and
1220   intraguild predation within a complex food web. *Journal of Animal Ecology*, **71**,
1221   1063–1074.

1222   Woodward, G., Speirs, D.C. & Hildrew, A.G. (2005) Quantification and resolution of
1223   a complex, size-structured food web. *Advances in Ecological Research*, **36**, 85–135.

1224   Woodward, G., Thompson, R., Townsend, C.R. & Hildrew, A.G. (2005) Pattern and
1225   process in food webs: evidence from running waters. *Aquatic Food Webs: An*
1226   *Ecosystem Approach* (eds A. Belgrano),, U.M. Scharler),, J. Dunne), & R.E.
1227   Ulanowicz), pp. 51–66. Oxford University Press, Oxford, UK.

1228   Yan, N.D., Girard, R., Heneberry, J.H., Keller, W.B., Gunn, J.M. & Dillon, P.J. (2004)
1229   Recovery of copepod, but not cladoceran, zooplankton from severe and chronic
1230   effects of multiple stressors. *Ecology Letters*, **7**, 452–460.

1231   Yan, N.D., Leung, B., Keller, W., Arnott, S.E., Gunn, J.M. & Raddum, G.G. (2003)
1232   Developing conceptual frameworks for the recovery of aquatic biota from
1233   acidification. *Ambio*, **32**, 165–169.

1234   Yodzis, P. (1989) *Introduction to Theoretical Ecology*. Harper & Row, Cambridge, UK.

1235

1236

VII.   Text Box

1239   Definitions of food web metrics used in this study

| Food Web metric	Definition
Connectance (C)	Number of links (L) / Number of species $(S)^2$. The proportion of potential trophic links that do occur (Warren 1994).
Linkage density	L/S. Number of links per taxon. A measure of average diet specialisation across the food web (Tylianakis, Tscharntke & Lewis 2007).
Generality (G)	The mean number of prey per consumer (Schoener & Schoenerz 1989).
Vulnerability (V)	The mean number of consumers per prey (Schoener & Schoenerz 1989).
Mean food chain length	Average number of links found in a food chain across a food web (Levine 1980; Williams & Martinez 2000).
Maximum food chain length	The maximum number of links found in any food chain in a food web (Levine 1980; Williams & Martinez 2000).
Efficiency	How well connected a network is, as well as the distribution of those connections across a network. High efficiency indicates that the species of a food web are all closely connected to one another (Latora & Marchiori 2001)
Redundancy	The trophic similarity among species within a web, high redundancy indicates that many of the species in a food webs are the same resources and consumers; many of the feeding pathways are the same (Briand & Cohen 1984; Cohen & Briand 1984).

1240

II.   Appendices

1242
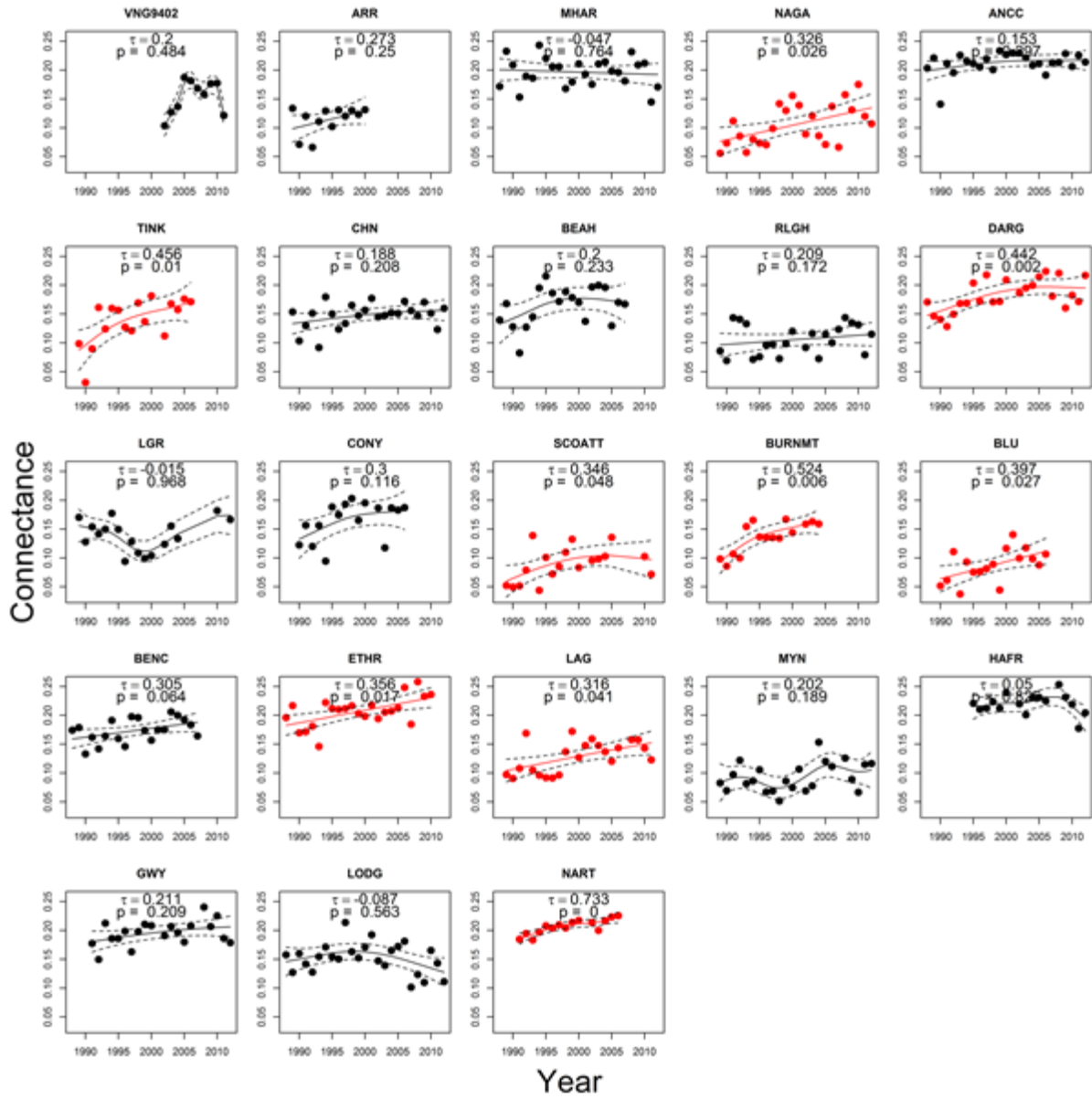
1243

Figure A. Trends in connectance at each of the UWMN sites. Sites are arranged in order of their
decreasing latitude, which can be used as a proxy for their initial acidified state, more acidified sites
were generally in the south (bottom of plot), while the least acidified sites were more northern (top of
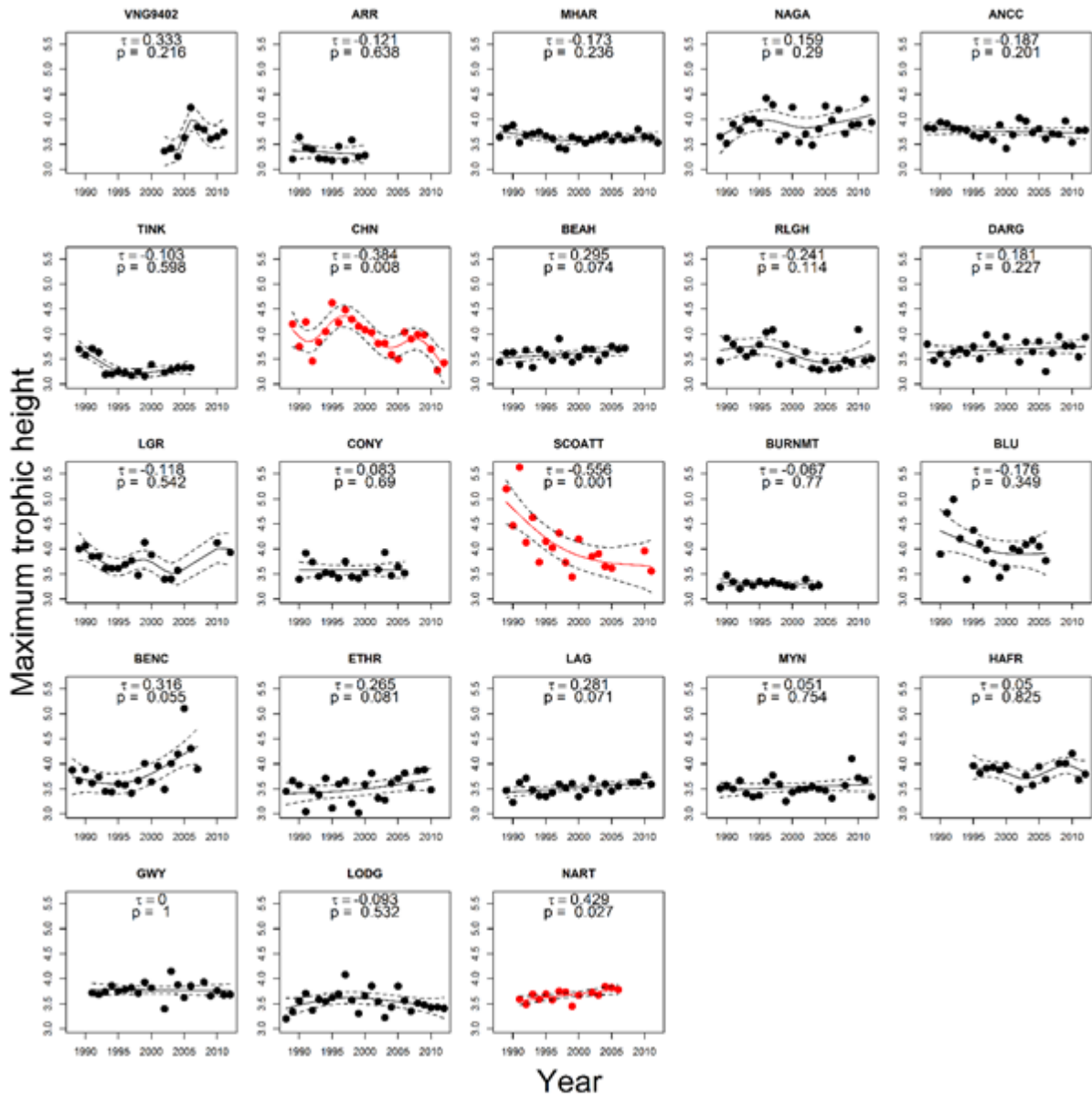*plot). See Figure 1 for site name abbreviations.*

1248
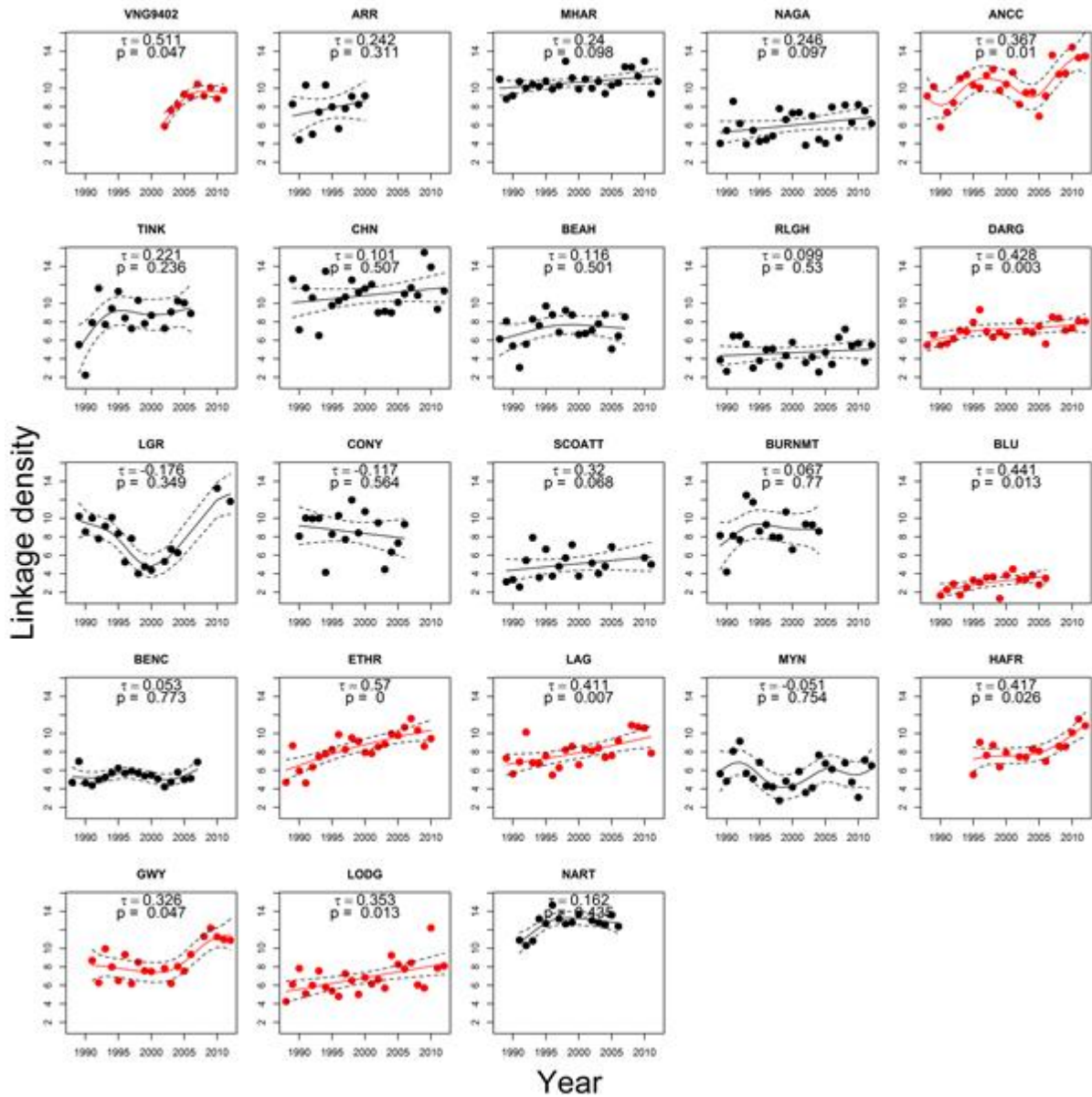
1249

Figure B. Trends in linkage density at each of the UWMN sites. Site ordering is explained in the
legend of Figure A. See Figure 1 for site name abbreviations.
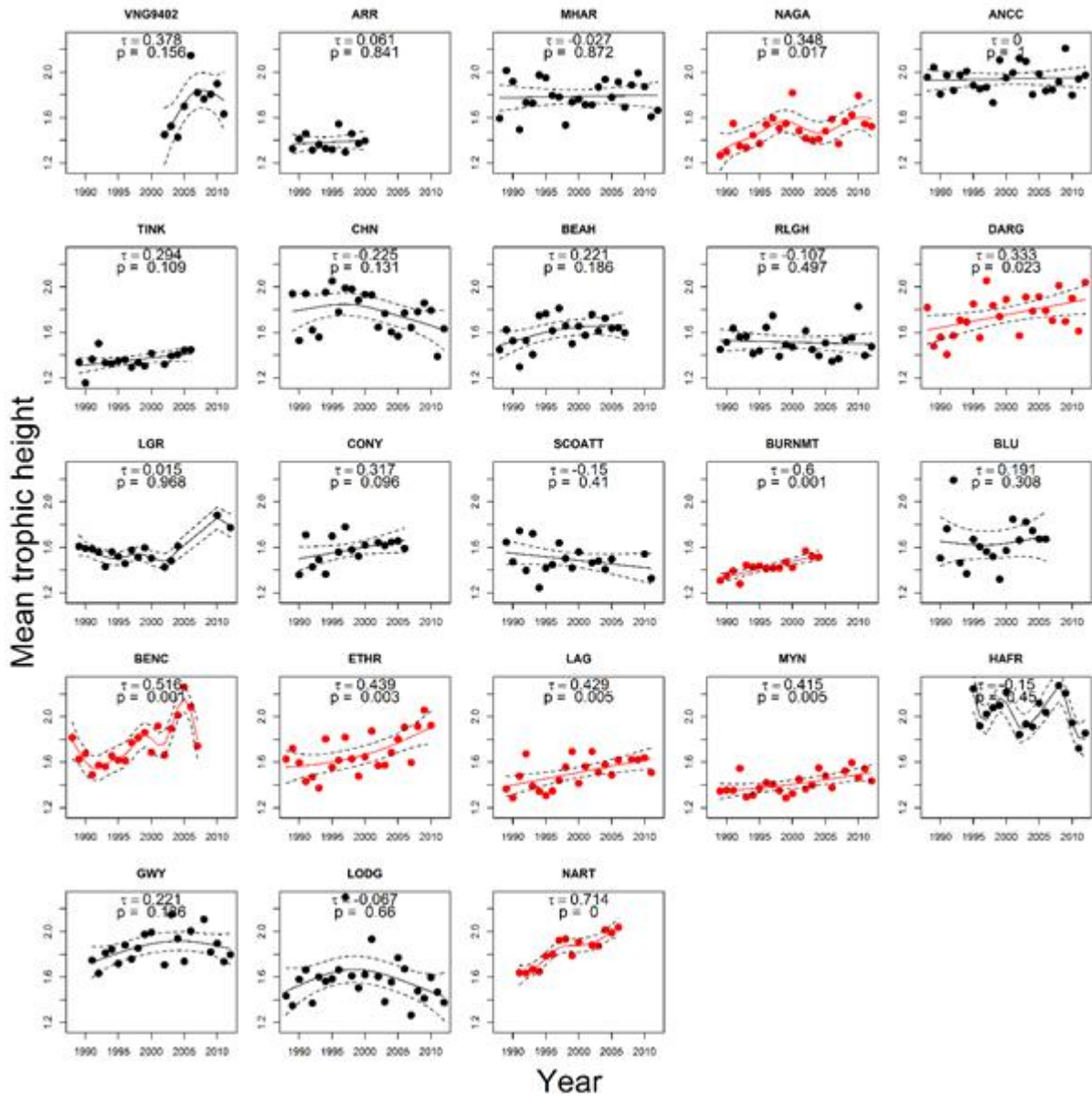
1252

Figure C. Trends in mean trophic height at each of the UWMN sites. Site ordering is explained in the
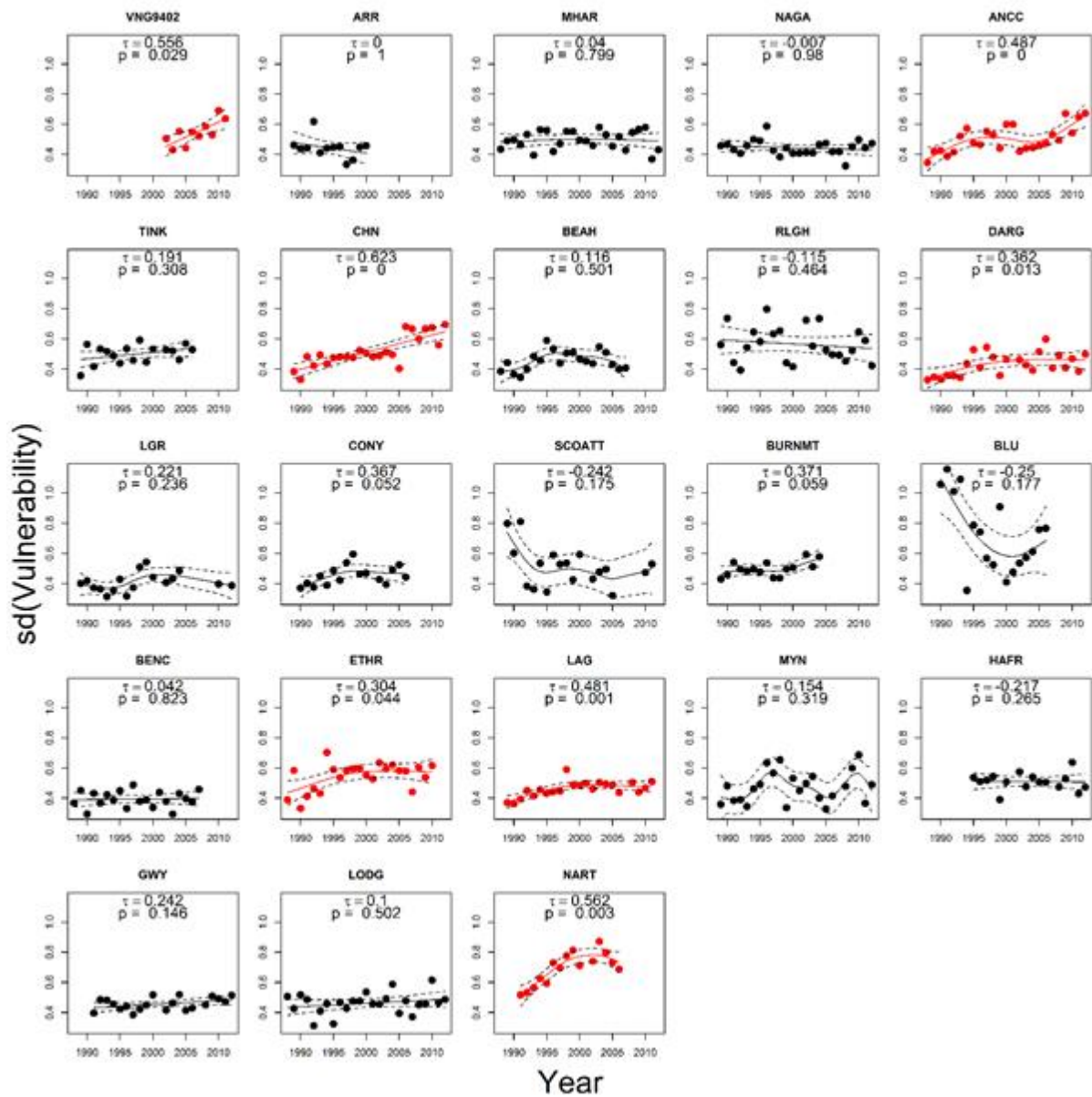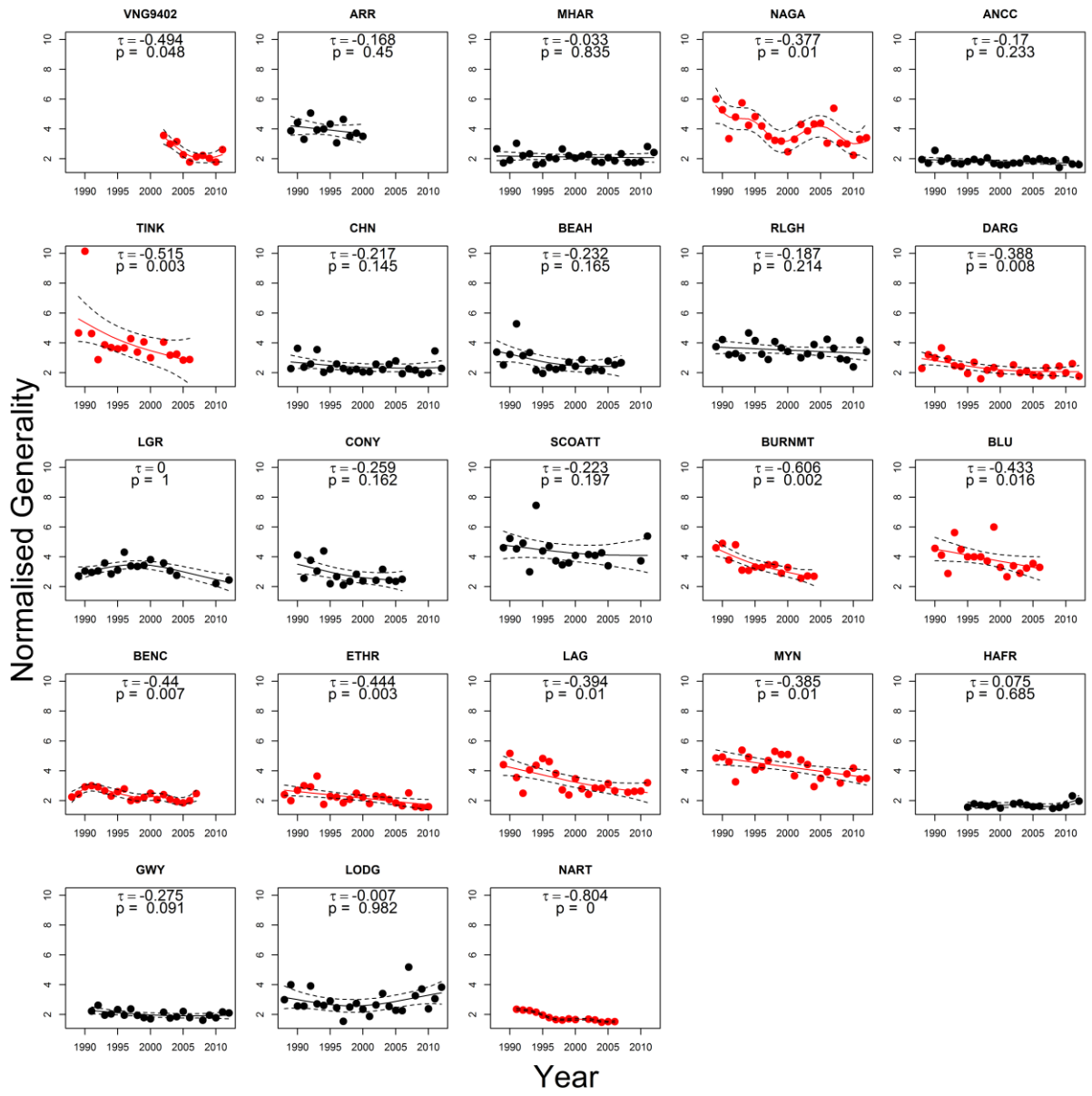*legend of Figure A. See Figure 1 for site name abbreviations.*

1255

Figure D. Trends in maximum trophic height at each of the UWMN sites. Site ordering is explained
in the legend of Figure A. See Figure 1 for site name abbreviations.
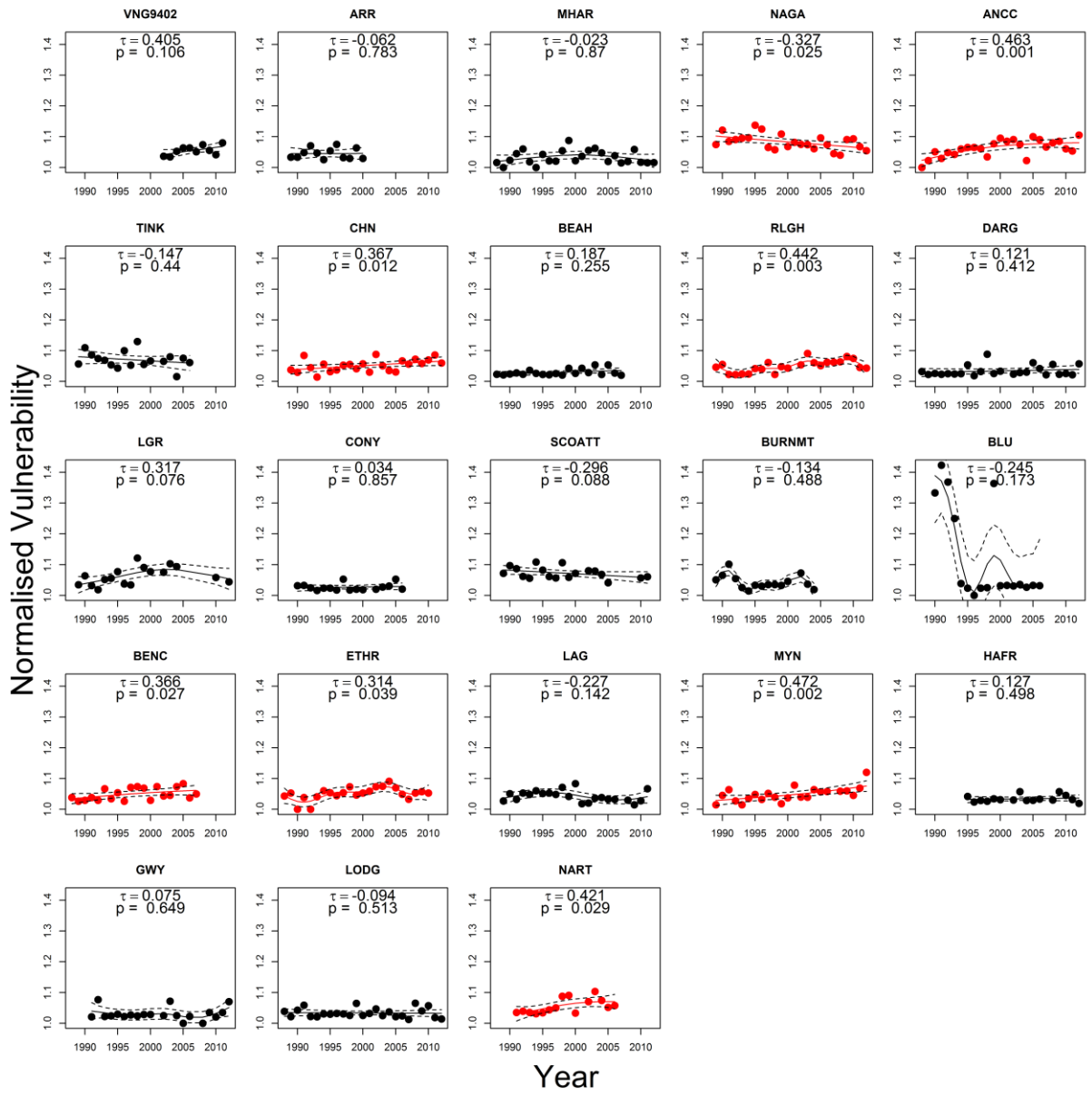
1258

Figure E. Trends in average food web vulnerability at each of the UWMN sites. Site ordering is
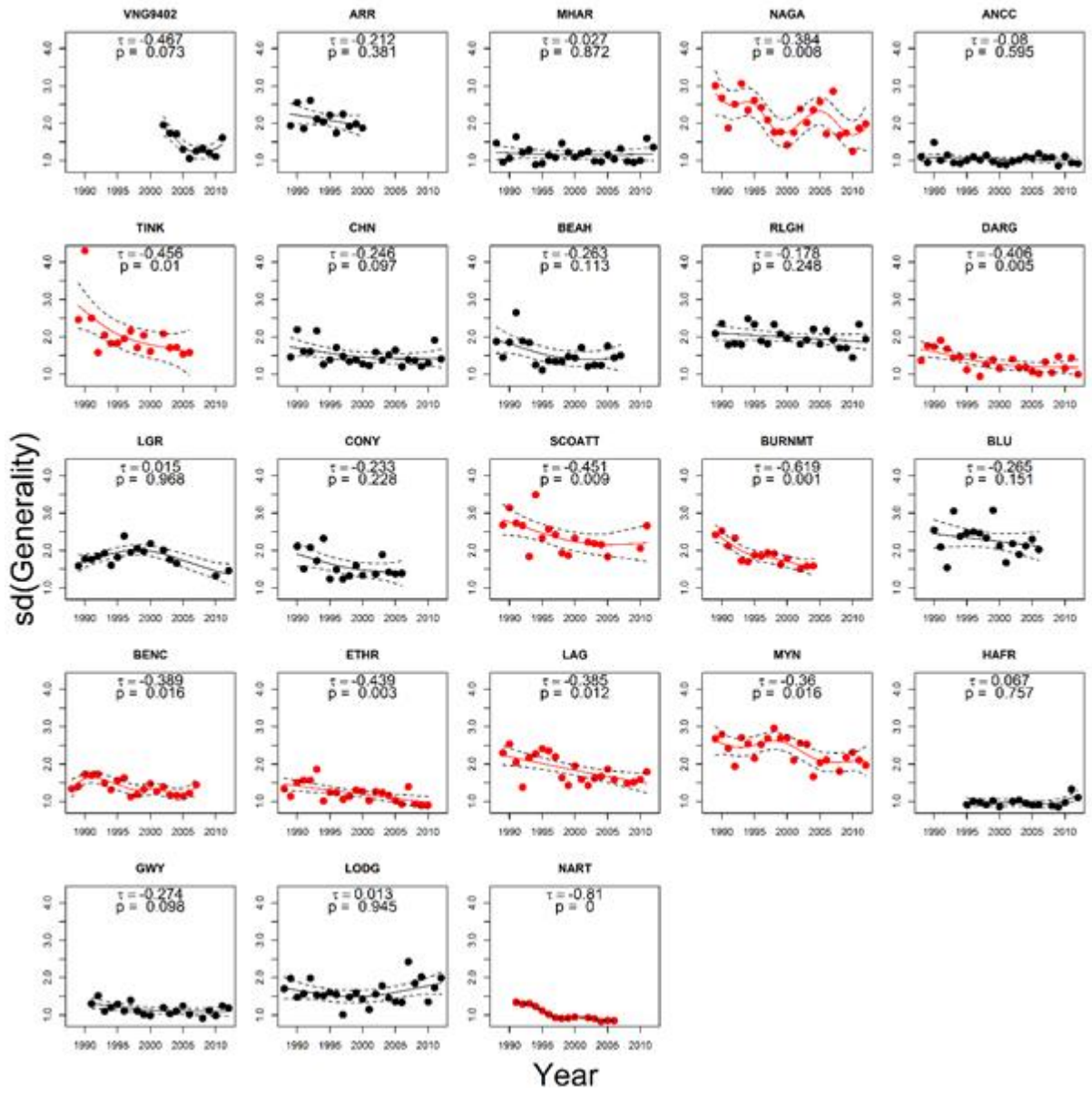*explained in the legend of Figure A. See Figure 1 for site name abbreviations.*

1259

1260

1261

Figure F. Trends in the standard deviation of food web vulnerability at each of the UWMN sites. Site ordering is explained in the legend of Figure A. See Figure 1 for site name abbreviations.

1264

Figure G. Trends in food web generality at each of the UWMN sites. Site ordering is explained in the

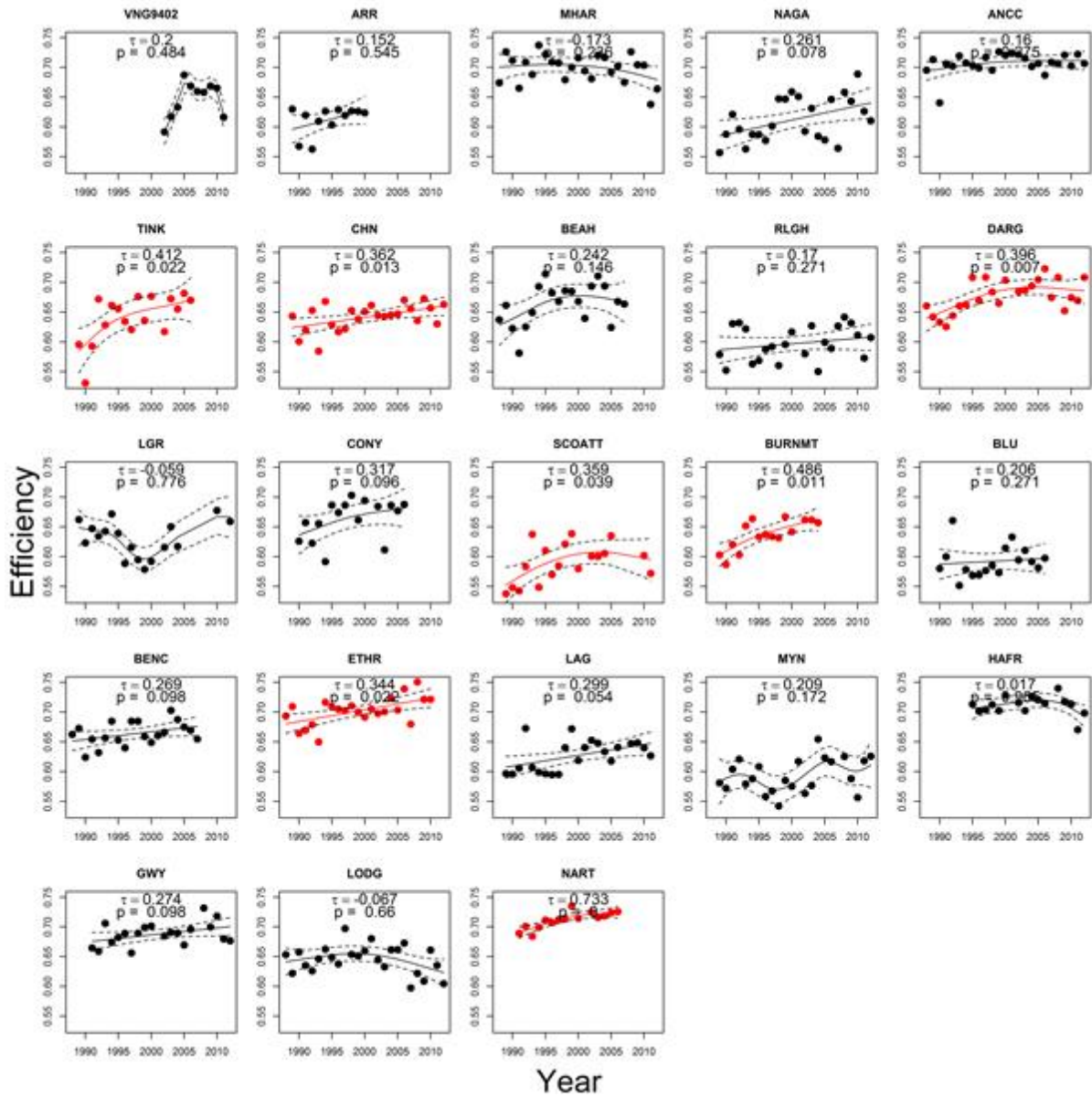*legend of Figure A. See Figure 1 for site name abbreviations.*

1267

Figure H. Trends in the standard deviation of food web generality at each of the UWMN sites. Site ordering is explained in the legend of Figure A. See Figure 1 for site name abbreviations.
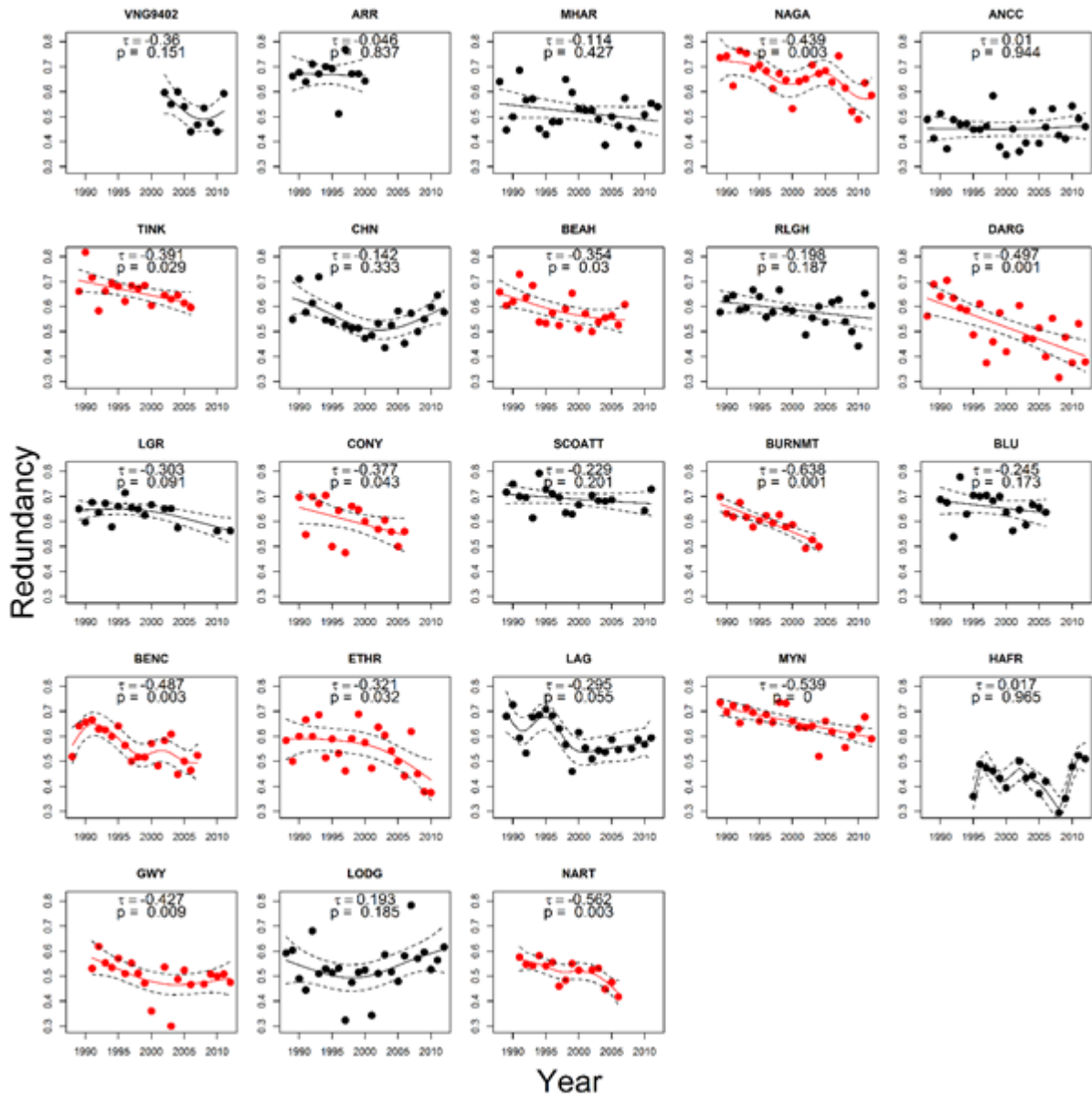
1270

1271

1272 *Figure I. Trends in food web efficiency at each of the UWMN sites. Site ordering is explained in the*
1273 *legend of Figure A. See Figure 1 for site name abbreviations.*

1274

1275

1276

1277

Figure J. Trends in food web redundancy at each of the UWMN sites. Site ordering is explained in the
legend of Figure A. See Figure 1 for site name abbreviations.

1278
1279

1280