R packages used: vegan, dismo

Hierarchical Clustering

For each of the macroinvertebrate taxa matrices, Bray-Curtis distances were computed using the vegdist function in the vegan package. Hierarchical clustering was performed using the respective distance matrices (Figures 1 and 2), and the resulting dendrograms were "pruned" by eye to provide grouping identifiers. Those identifiers were passed to the respective environmental matrices, and used to color-code subsequent plots for a frame of reference.

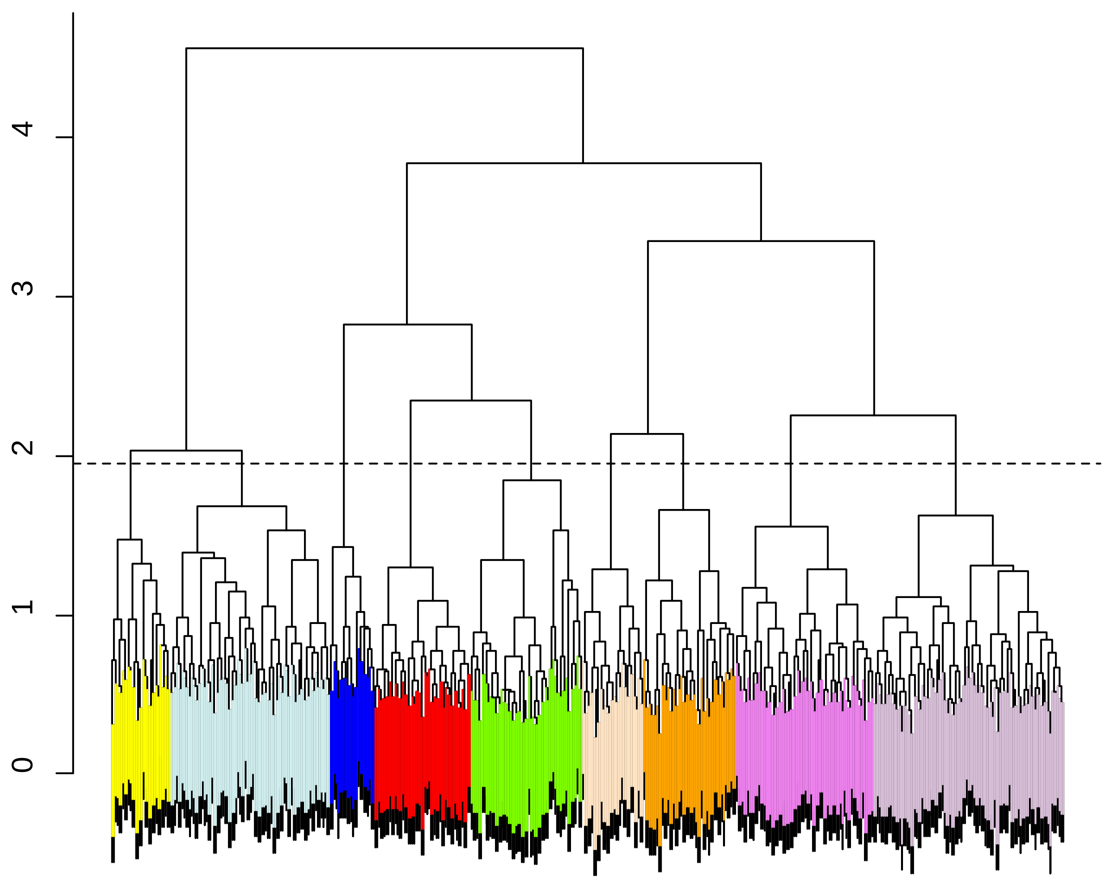

Figure 1. A cluster dendrogram of sampling sites based on distance measures of macroinvertebrate assemblages sampled from the glaciated portion of Ohio. The dendrogram was cut at a height of 1.95 based on inspection. Except for the blue-colored group (see ensuing results), the colors are arbitrary, but applied consistently in ensuing plots.

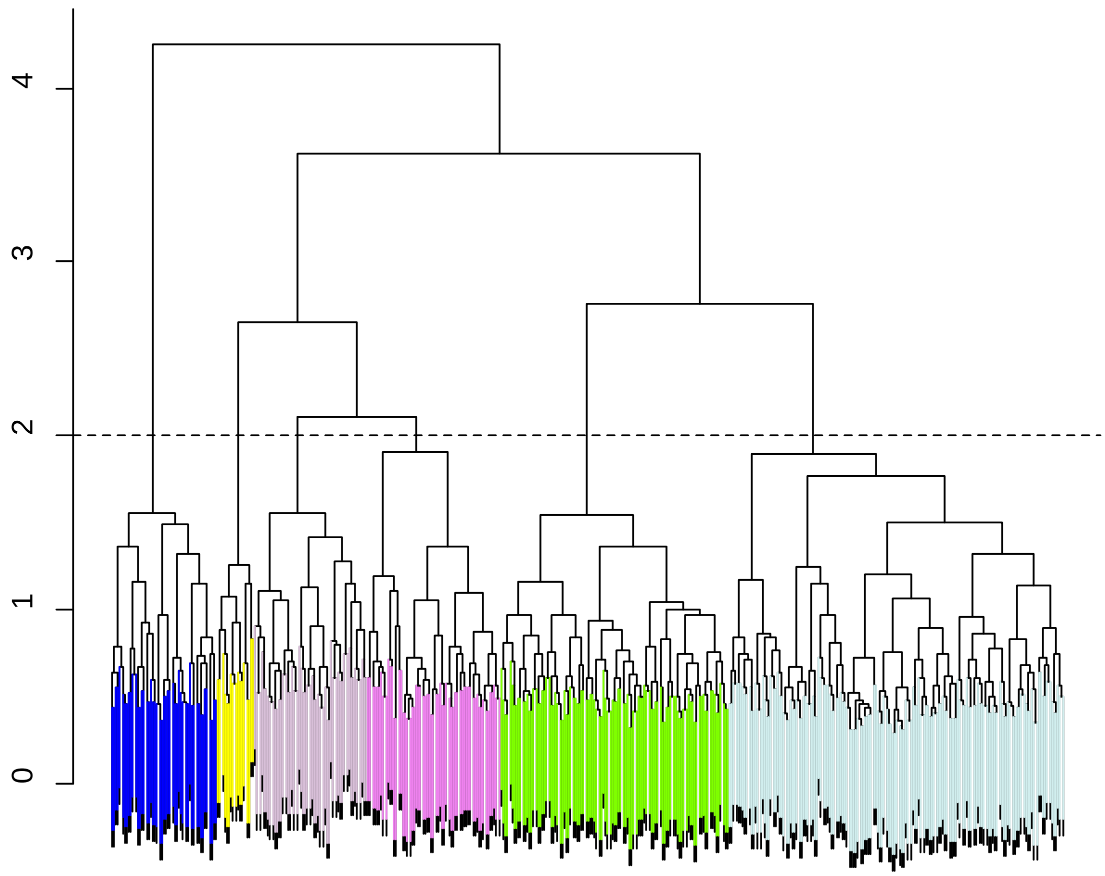

Figure 2. A cluster dendrogram of sampling sites based on distance measures of macroinvertebrate assemblages sampled from the Western Allegheny Plateau ecoregion of Ohio. The dendrogram was cut at a height of 2 based on inspection. Except for the blue-colored group (see ensuing results), the colors are arbitrary, but applied consistently in ensuing plots.

Nonmetric Multidimensional Scaling (NMDS)

NMDS is analogous to hierarchical clustering in that it allows for a graphical representation of how sites are related to each other in ordination space, but also allows for a representation of how the sites are arrayed along environmental gradients. NMDS was applied to the respective (i.e., glaciated and WAP ecoregions) distance matrices using the metaMDS function in vegan, and environmental variables were fit to the resulting axis scores using the envfit function in the vegan package. For sites sampled from the glaciated ecoregions, the distance matrix was represented by four axes:

```
Call:
metaMDS(comm = glac.dist, k = 4, trymax = 999)

global Multidimensional Scaling using monoMDS

Data: glac.dist
Distance: bray

Dimensions: 4
Stress: 0.1660693
Stress type 1, weak ties
Two convergent solutions found after 429 tries
Scaling: centring, PC rotation
Species: scores missing
```

And for sites sampled from the WAP ecoregion, a solution with 3 axes was found:

```
Call:
metaMDS(comm = wap.dist, k = 3, trymax = 9999, wascores = TRUE)

global Multidimensional Scaling using monoMDS

Data: wap.dist
Distance: bray

Dimensions: 3
Stress: 0.1884462
Stress type 1, weak ties
Two convergent solutions found after 162 tries
Scaling: centring, PC rotation
Species: scores missing
```

Note that the number of dimensions for each NMDS was selected by examining the decrease in stress from successive runs starting with a dimension of 1; if an additional axis resulted in a marginal decrease in stress, and the final stress from the previous solution was less than 0.2, then the previous solution was used. A general rule of thumb is that stress needs to be at least less than 0.2 to have a useful result (from Clarke's rule of thumb described in McCune and Grace (2002; Analysis of Ecological Communities).

Because of the high level of missingness of chemistry variables in the environmental matrices, the environmental matrices were fit to the respective NMDS plots twice, once for all variables, and once excluding chemistry variables. This allows one to gauge the influence missingness has on vectors drawn from complete variables – e.g., if the direction and degree of association an environmental variable has with one (or more) of the NMDS axes doesn't change appreciably whether or not the chemistry variables are included, then it's reasonable to conclude that that vector is unbiased with respect to the subset of sites where chemistry was included.

Figures 3 and 4 show the NMDS plots for the glaciated ecoregions and the WAP, respectively. NMDS were added to the respective environmental matrices.

Redundancy Analysis (RDA)

RDA is an ordination technique that, like hierarchical clustering and NMDS, shows how sampling locations relate to each other based on shared similarities (or dissimilarities) in assemblages, but differs from NMDS in that it is considered a constrained technique. That is, the projection of sites in ordination space is based on a linear function of combinations of environmental variables related to the distance matrix. Because it is a constrained technique, the projection of both sites and environmental vectors is sensitive to missingness. This differs from NMDS, where the projection of sites is independent of the environmental variables. One advantage of RDA appears to be the ability to project both sites and taxa in the same plot. The ensuing output here is based on RDA (or more formally Constrained Analysis of Principal Coordinates) preformed using environmental variables selected based on inspection of the NMDS plots and respective output of the environmental fit (appended at the end of the document) that were chosen to minimize the influence of missingness. Notice that for the first test based on data from the WAP ecoregion, habitat variables were included, and in the second test habitat variables were excluded. The biplots resulting from each test are shown in Figure 5. For the glaciated ecoregions, only one test was performed using variables without high degree of missingness. The resulting biplot is shown in Figure 7. Site scores from testfit2 (WAP ecoregion) and testfit3 (glaciated ecoregions) were added to the respective environmental matrices. Species scores were extracted and saved.

Western Allegheny Plateau

```
> testfit
Call: capscale(formula = wapbug.sub ~ bawx + balt5 + mwdrained + poordrain +
minecnt + soilslope + pctclay + cationx + SUBSTRATE + GRADE + PRECIP + totgrass
+ lowurb + rowcrop + PROB_1DAY + DA + QHEI + meancelt, data = emwap, distance =
"bray", na.action = na.exclude)
```

	Inertia	Proportion	Eigenvals	Rank
Total	85.2674	1.0000	112.9863	
Constrained	17.9637	0.2107	19.1965	18
Unconstrained	67.3037	0.7893	93.7898	144
Imaginary			-27.7189	186

Inertia is squared Bray distance
107 observations deleted due to missingness

```
Eigenvalues for constrained axes:
 CAP1  CAP2  CAP3  CAP4  CAP5  CAP6  CAP7  CAP8  CAP9 CAP10 CAP11 CAP12 CAP13 CAP14
5.836 2.934 1.942 1.681 1.272 0.905 0.814 0.574 0.506 0.463 0.411 0.368 0.334 0.290
CAP15 CAP16 CAP17 CAP18
0.246 0.233 0.220 0.167

Eigenvalues for unconstrained axes:
 MDS1  MDS2  MDS3  MDS4  MDS5  MDS6  MDS7  MDS8
4.793 4.283 3.167 3.011 2.526 2.430 2.311 2.014
(Showed only 8 of all 144 unconstrained eigenvalues)

> testfit2
Call: capscale(formula = wapbug.sub ~ bawx + balt5 + mwdrained + poordrain +
minecnt + soilslope + pctclay + cationx + PRECIP + totgrass + lowurb + rowcrop
+ PROB_1DAY + DA + meancelt, data = emwap, distance = "bray", na.action =
na.exclude)
```

	Inertia	Proportion	Eigenvals	Rank
Total	111.6770	1.0000	150.1389	
Constrained	22.3174	0.1998	23.4623	15
Unconstrained	89.3596	0.8002	126.6766	163
Imaginary			-38.4619	234

```
Inertia is squared Bray distance
40 observations deleted due to missingness

Eigenvalues for constrained axes:
 CAP1  CAP2  CAP3  CAP4  CAP5  CAP6  CAP7  CAP8  CAP9 CAP10 CAP11 CAP12 CAP13 CAP14
8.340 5.343 1.929 1.645 1.251 0.994 0.941 0.589 0.501 0.426 0.422 0.331 0.287 0.268
CAP15
0.197

Eigenvalues for unconstrained axes:
 MDS1  MDS2  MDS3  MDS4  MDS5  MDS6  MDS7  MDS8
6.664 5.254 3.938 3.632 3.207 2.986 2.896 2.735
(Showed only 8 of all 163 unconstrained eigenvalues)
```

Glaciated Ecoregions

```
> testfit3
Call: capscale(formula = glacbugs[c(3:643)] ~ GRADE + FOREST + gaqyld + bawx +
Lsand + mwdrained + poordrain + soilslope + pctclay + cationx + totgrass +
lowurb + rowcrop + PROB_1DAY + DA + meancelt, data = emglac, distance = "bray",
na.action = na.exclude)

              Inertia  Proportion  Eigenvals  Rank
Total         142.877       1.000    199.513
Constrained    26.581       0.186     27.975    16
Unconstrained 116.296       0.814    171.537   185
Imaginary                            -56.635   320
Inertia is squared Bray distance
72 observations deleted due to missingness

Eigenvalues for constrained axes:
 CAP1  CAP2  CAP3  CAP4  CAP5  CAP6  CAP7  CAP8  CAP9 CAP10 CAP11 CAP12 CAP13 CAP14
7.891 5.839 4.054 2.840 1.453 1.113 0.988 0.796 0.620 0.477 0.427 0.366 0.318 0.284
CAP15 CAP16
0.256 0.255

Eigenvalues for unconstrained axes:
  MDS1   MDS2   MDS3   MDS4   MDS5   MDS6   MDS7   MDS8
10.278  6.057  5.701  4.973  4.113  3.671  3.576  3.394
(Showed only 8 of all 185 unconstrained eigenvalues)
```

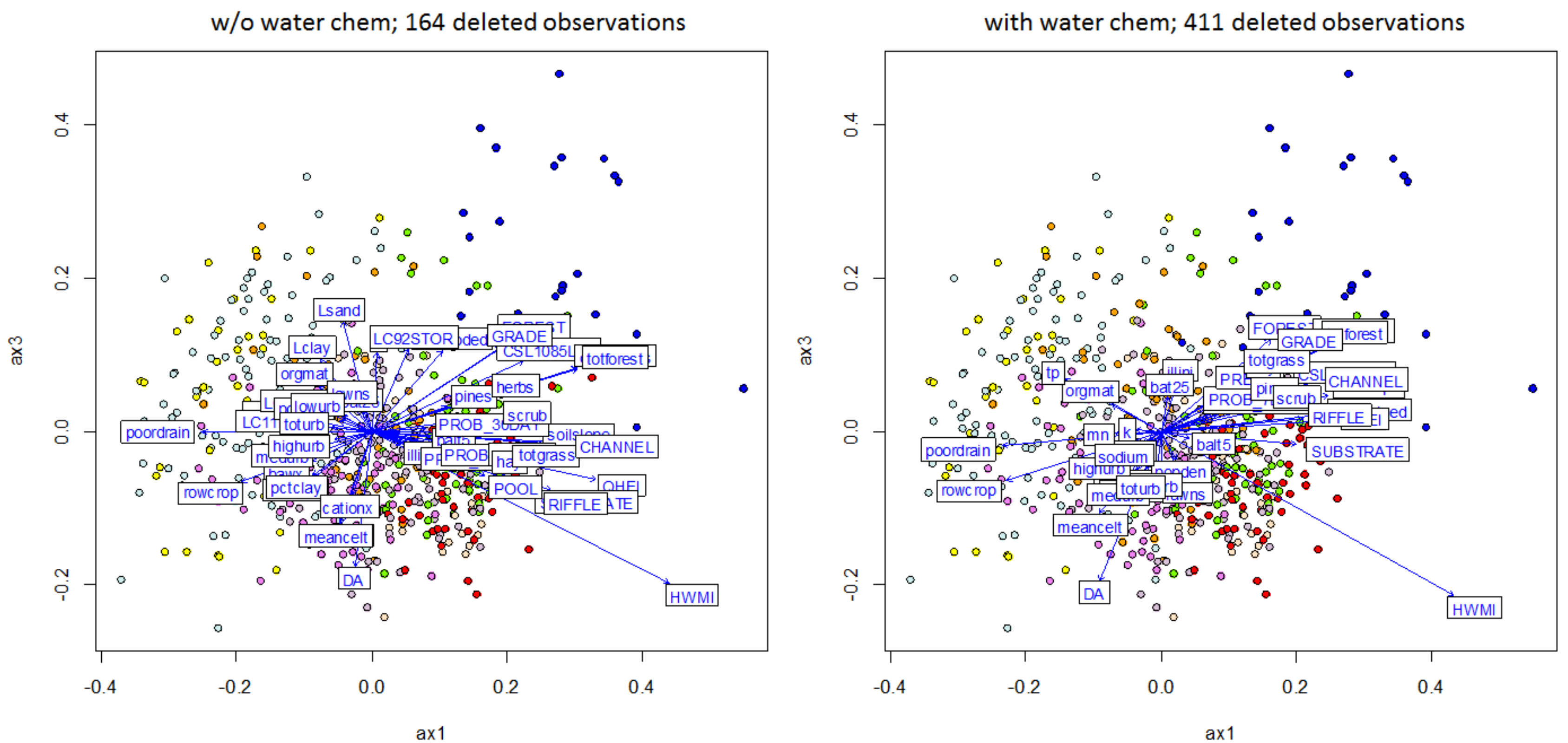

Figure 3. Very cluttered ordination plots resulting from a NMDS of the macroinvertebrate distance matrix for the glaciated ecoregions. The plot on the left includes a fit of environmental variables sans chemistry variables. The plot on the right includes chemistry variables. The x-axis (ax1) broadly represents a counter gradient of habitat quality and catchment row crop agriculture, and the y-axis (ax3) represents a gradient of decreasing temperature and stream size and increasing forest cover. Only variables with highly significant associations ($p<0.0001$, based on permutation tests) with an NMDS axis are plotted. The points are colored-coded corresponding to groups identified in Figure 1.

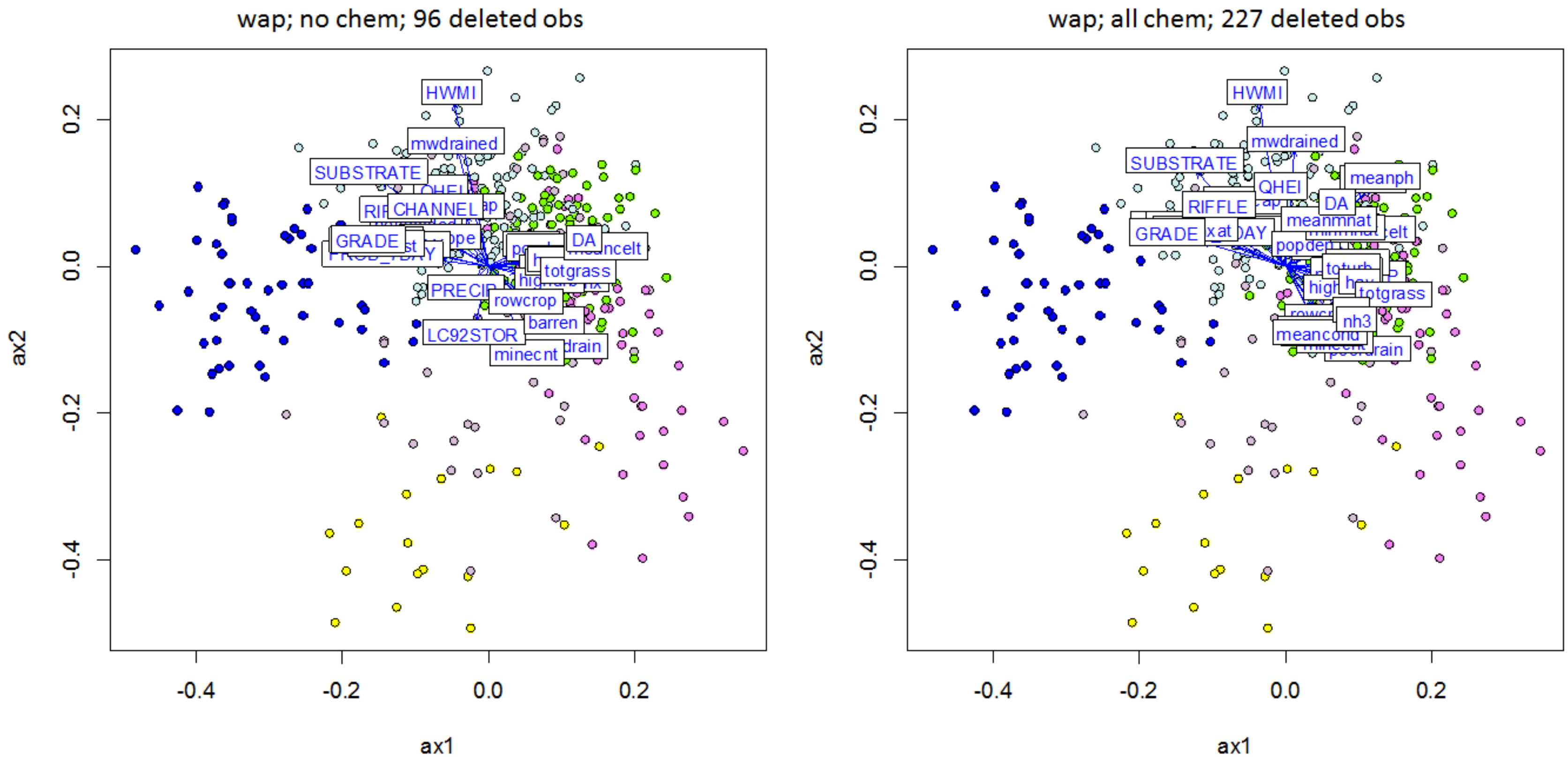

Figure 4. Less cluttered plots resulting from a NMDS of the macroinvertebrate distance matrix for the WAP ecoregion. The plot on the left includes a fit of environmental variables sans chemistry variables. The plot on the right includes chemistry variables (and shows the level of missingness in the chemistry data). The x-axis (ax1) represents a gradient of stream size and temperature (hint, look for celt behind DA). The y-axis (ax2) represents a counter gradient of mining and overall macroinvertebrate assemblage quality (as given by the HWMI biotic index). Only variables with highly significant associations (p<0.0001, based on permutation tests) with an NMDS axis are plotted. Note that habitat quality aligns with both axes, increasing in the “northwest” direction. The points are colored-coded corresponding to groups identified in Figure 2.

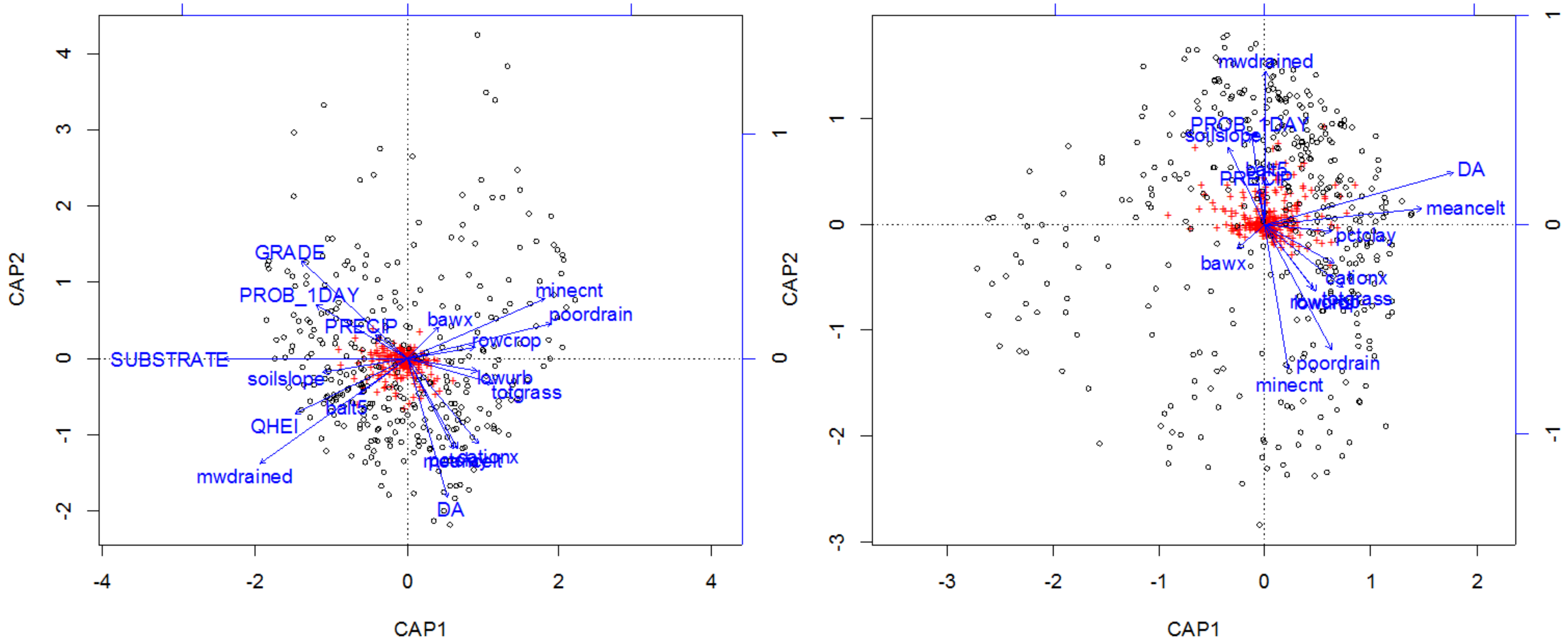

Figure 5. Biplots from RDA performed on data from the WAP ecoregion. The plot on the left was fit based on the inclusion of habitat variables, the plot on the right was fit without the habitat variables. The plot on the right is reproduced in Figure 6 with sample sites color-coded base on the hierarchical clustering from Figure 1.

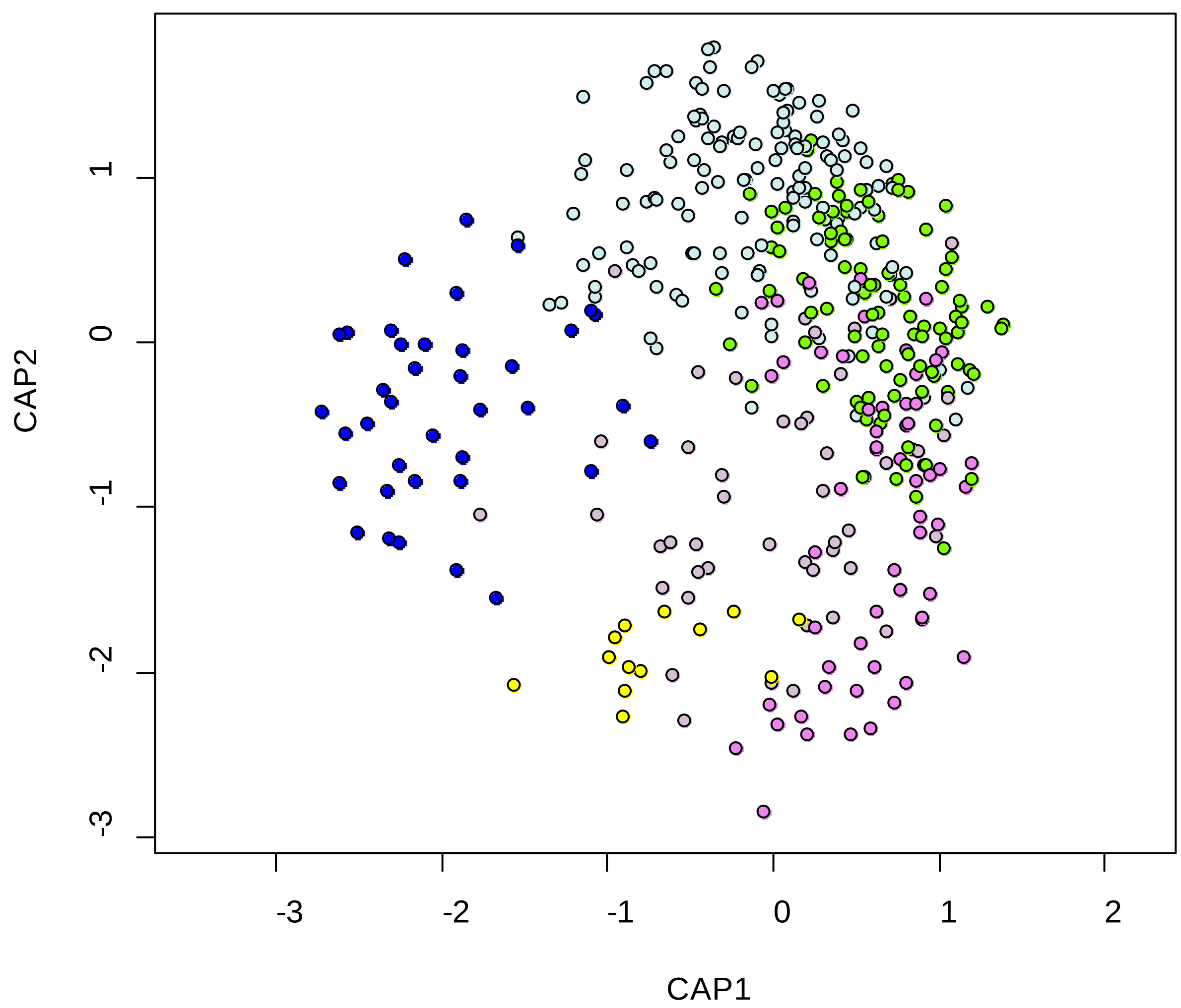

Figure 6. The same plot as the left panel from Figure 5, but with sites color-coded from hierarchical clustering as in Figure 1.

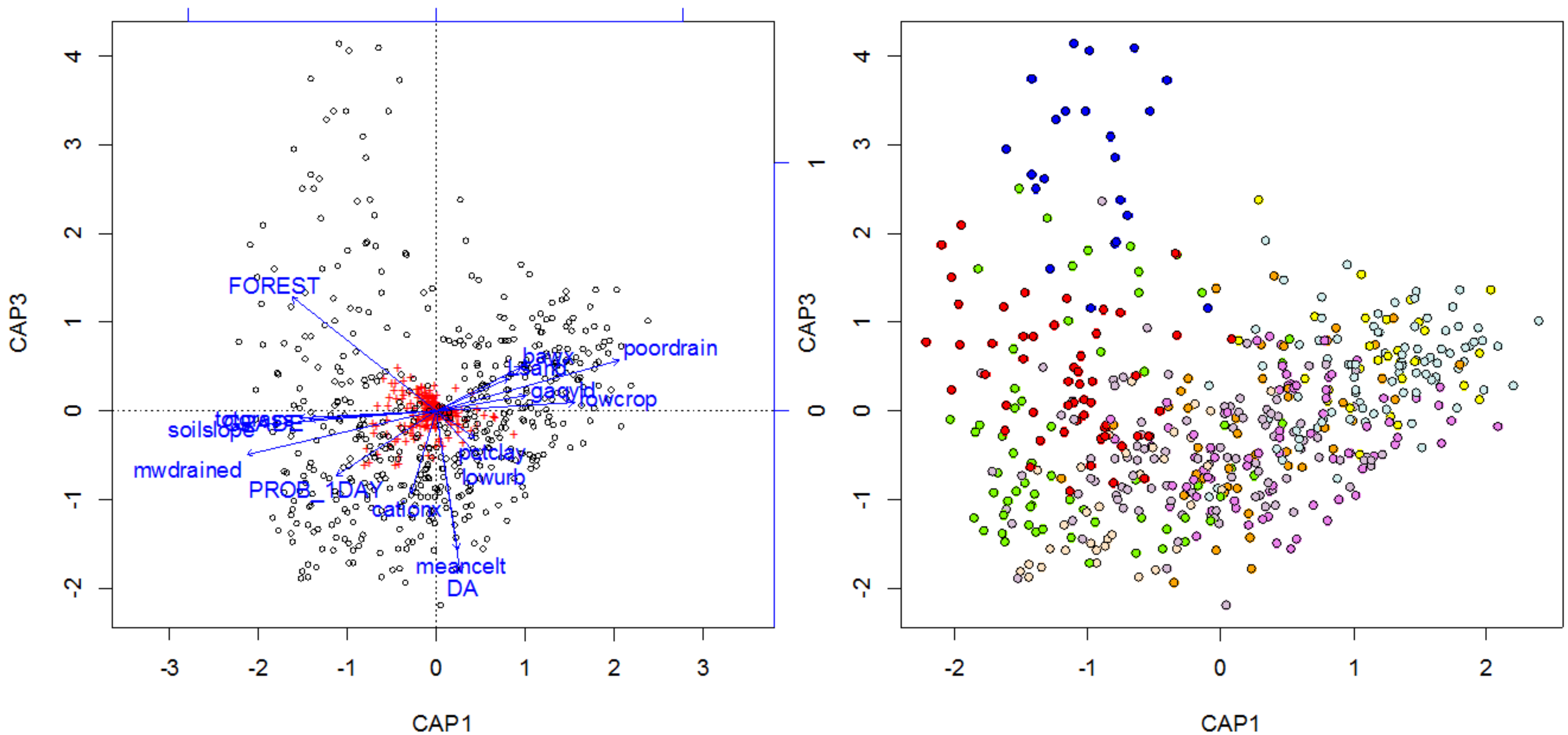

Figure 7. Left panel, a biplot resulting from an RDA performed on data from the glaciated ecoregions. The right panel plots the site scores (same as the left panel) color-coded to the groups identified from hierarchical clustering in Figure 2. The y-axis (CAP3) represents a gradient of stream size and temperature.

Graphical Representation

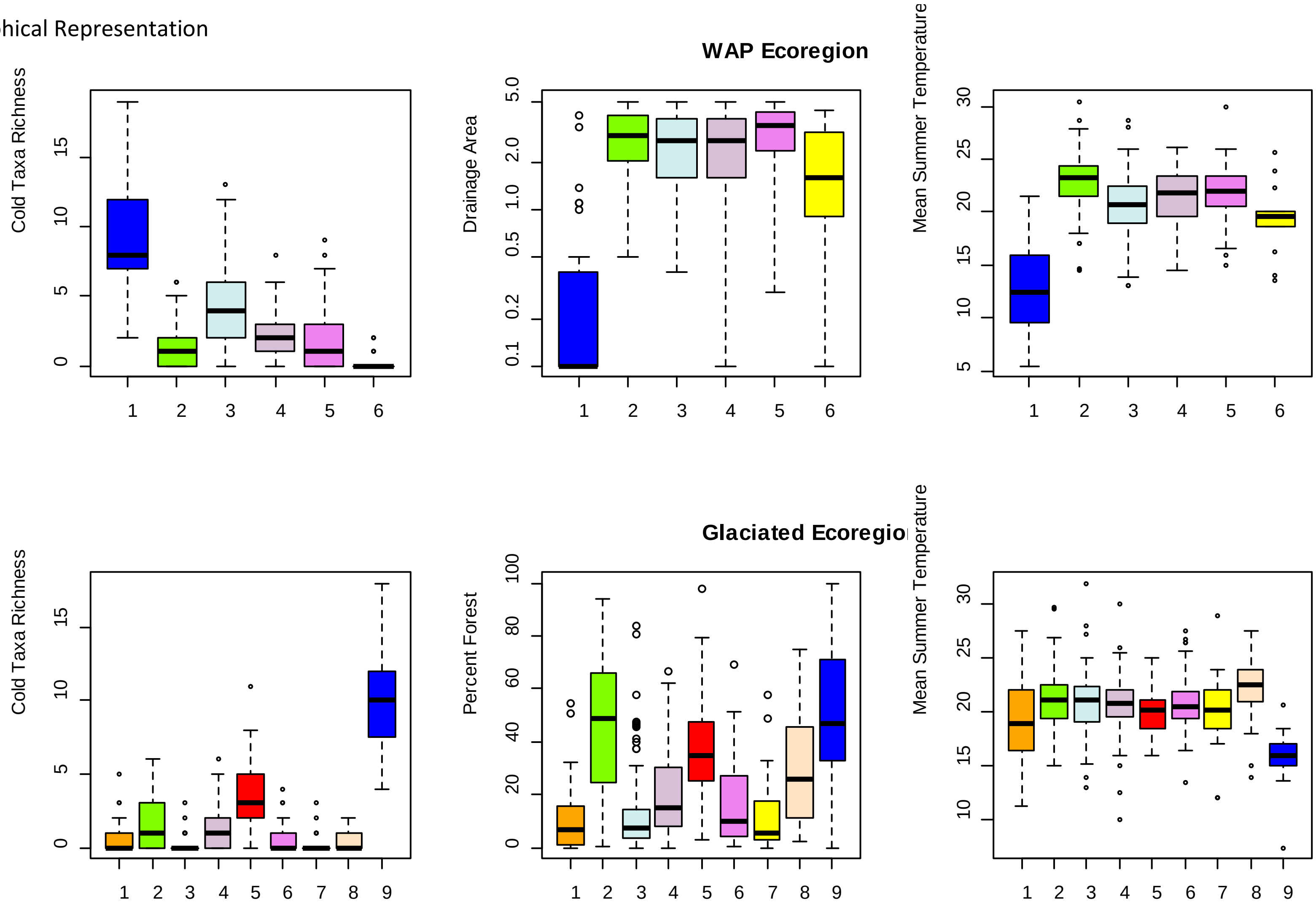

Figure 8. Distributions of environmental variables plotted by groups identified by hierarchical clustering.

The results from hierarchical clustering, NMDS and RDA all show a distinct group of sites that relate most strongly to a temperature gradient. Those sites (or their group membership) are colored blue in all of the previous plots. The distributions shown in Figure 8 show that the sites aligned with temperature (i.e., the blue distributions) also contain decidedly higher numbers of coldwater indicator taxa, and suggest that for the WAP ecoregion, mean summer temperature and drainage area efficiently classify those sites. And for the glaciated ecoregions, a combination of mean summer temperature and forest cover appears to classify the blue sites. However, those environmental measures were suggested based on linear associations (from the NMDS and RDA). Non-linear or interactive effects were not tested.

Boosted Regression

Boosted regression tree (BRT) analysis essentially combines two techniques, recursive partitioning and machine learning. Recursive partitioning, or tree models, partition data between response and predictor variables such that relatively homogeneous groupings of the response variable are binned by ranges of one or more predictors. In this regard, tree models are a method of deviance reduction. The machine learning, or boosting, aspect of BRT examines a series of tentative tree models constructed from a training subset of data, and seeks to reduce residual deviance by iteratively fitting new trees from a sampled fraction of withheld data (Elith et al., 2008). The predictive performance of a final model is judged using cross-fold validations. BRT is particularly well suited to complex environmental data sets because it examines and models regions of the data and interactions without a priori specification, unlike in general linear models where stratification has to be coded up-front. However, when applied to a classification problem, BRT functions like logistic regression, and includes diagnostic measures like area under the receiver operator (ROC) curve. Another advantage to BRT is that it handles missing values by imputing a mean value from non-missing values within a node where that particular case was selected[1].

BRT models were run using candidate predictor variables suggested by the results from the NMDS and RDA analyses. A binary response variable for respective environmental matrices was created as 1, is a blue group site; and 0, is not a blue group site. Results from initial models are shown on the next pages, and include the relative influence (on deviance reduction) of predictor variables. Relative influence values less than ~ 4 indicate that the variable alone contributed little information. However, those need to be examined in terms of interactions with other variables before discounting. Without going into too much detail here, final candidate variables were chosen iteratively based on examining deviance explained by interactions with other variables, and running simplified models sequentially leaving out variables.

For either the WAP ecoregion or the glaciated ecoregions, two final predicator variables were identified. For the WAP, drainage area and mean summer water temperature classified sites into the blue/not blue group with a high degree of accuracy. For the glaciated ecoregions, mean summer water temperature and percent forest cover classified the blue/not blue sites. For the glaciate regions, streams with mean summer water temperatures less than 18^{o} C and with greater than 65% forested land cover are highly likely to support coldwater taxa and high percentages of taxa classed as sensitive (see Figure 11). For the Western Allegheny Plateau Ecoregion, streams with mean summer water temperatures less than 18^{o} C and smaller than 0.5 mi^{2} in drainage area are likely to support high numbers of coldwater taxa and high percentages of sensitive taxa.

---

[1] Full disclosure, this is as best I can glean from the documentation.

Glaciate Ecoregions

	var	rel.inf
meancelt	meancelt	36.39480346
PROB_1DAY	PROB_1DAY	12.33408831
totforest	totforest	10.95027976
mwdrained	mwdrained	6.55932991
cationx	cationx	5.48488424
RIFFLE	RIFFLE	5.03957993
QHEI	QHEI	4.47736324
soilslope	soilslope	3.40912430
SUBSTRATE	SUBSTRATE	2.46826486
balt5	balt5	2.44321658
toturb	toturb	2.26479478
poordrain	poordrain	2.19719710
lowurb	lowurb	1.80157396
totgrass	totgrass	1.55259095
orgmat	orgmat	0.94499991
GRADE	GRADE	0.71866197
bawx	bawx	0.58691391
rowcrop	rowcrop	0.32619605
flowcode	flowcode	0.04613679
illini	illini	0.00000000

fitting final gbm model with a fixed number of 5981 trees for cgx (i.e., cold group)

mean total deviance = 0.701
mean residual deviance = 0.349

estimated cv deviance = 0.386 ; se = 0.014

training data correlation = 0.905
cv correlation = 0.833 ; se = 0.02

training data AUC score = 0.995
cv AUC score = 0.982 ; se = 0.01

elapsed time - 0.04 minutes
Warning messages:
1: glm.fit: algorithm did not converge
2: glm.fit: fitted probabilities numerically 0 or 1 occurred
3: glm.fit: fitted probabilities numerically 0 or 1 occurred

Western Allegheny Plateau Ecoregion

	var	rel.inf
DA	DA	62.185151103
meancelt	meancelt	26.709777851
PROB_1DAY	PROB_1DAY	3.770742592
totforest	totforest	2.973172284
mwdrained	mwdrained	1.406791647
watercap	watercap	0.742999230
cationx	cationx	0.618867752
totgrass	totgrass	0.575800717
flowcode	flowcode	0.556544713
GRADE	GRADE	0.269357383
soilslope	soilslope	0.116631168
SUBSTRATE	SUBSTRATE	0.018298396
minecnt	minecnt	0.014516649
QHEI	QHEI	0.012099394
toturb	toturb	0.010808837
lowurb	lowurb	0.009087728
RIFFLE	RIFFLE	0.007359101
rowcrop	rowcrop	0.001993455
poordrain	poordrain	0.000000000

fitting final gbm model with a fixed number of 6092 trees for cgx

mean total deviance = 0.701
mean residual deviance = 0.346

estimated cv deviance = 0.387 ; se = 0.019

training data correlation = 0.904
cv correlation = 0.83 ; se = 0.039

training data AUC score = 0.995
cv AUC score = 0.979 ; se = 0.015

elapsed time - 0.04 minutes

Glaciate Ecoregions

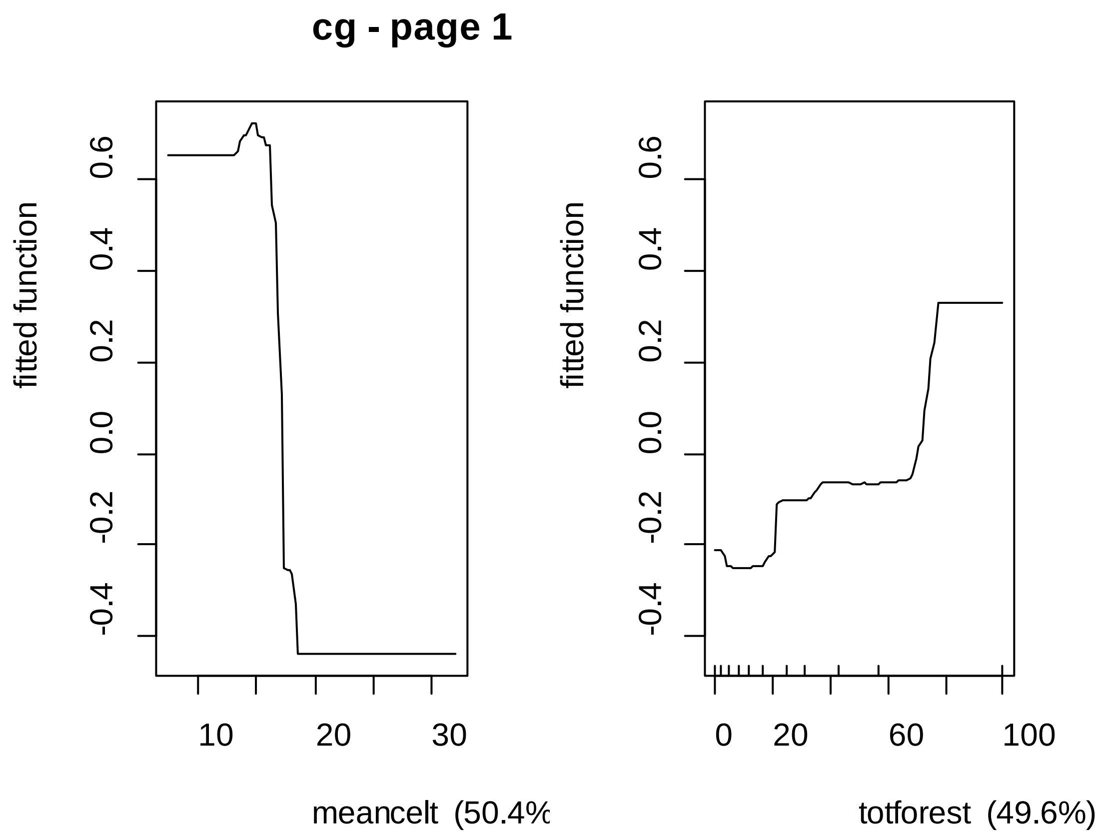

Figure 9. Fitted function from the BRT model for the glaciated ecoregions.

fitting final gbm model with a fixed number of 3008 trees for cg

mean total deviance = 0.377
mean residual deviance = 0.28

estimated cv deviance = 0.302 ; se = 0.019

training data correlation = 0.628
cv correlation = 0.527 ; se = 0.074

training data AUC score = 0.971
cv AUC score = 0.936 ; se = 0.017

elapsed time - 0.41 minutes
Warning messages:
1: glm.fit: algorithm did not converge
2: glm.fit: fitted probabilities numerically 0 or 1 occurred

Western Allegheny Plateau Ecoregion

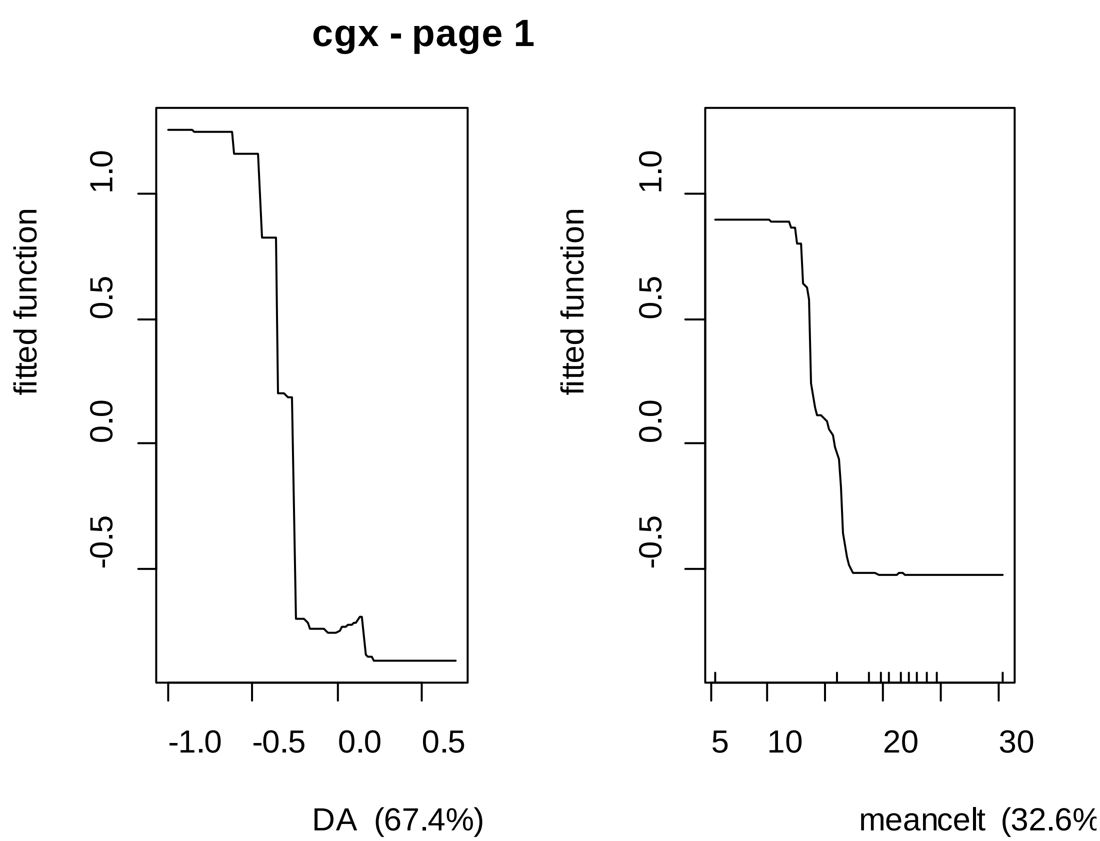

Figure 10. Fitted functions for the BRT model fit to the WAP ecoregion.

fitting final gbm model with a fixed number of 5963 trees for cgx

mean total deviance = 0.701
mean residual deviance = 0.371

estimated cv deviance = 0.397 ; se = 0.011

training data correlation = 0.856
cv correlation = 0.815 ; se = 0.023

training data AUC score = 0.989
cv AUC score = 0.968 ; se = 0.016

elapsed time - 0.03 minutes

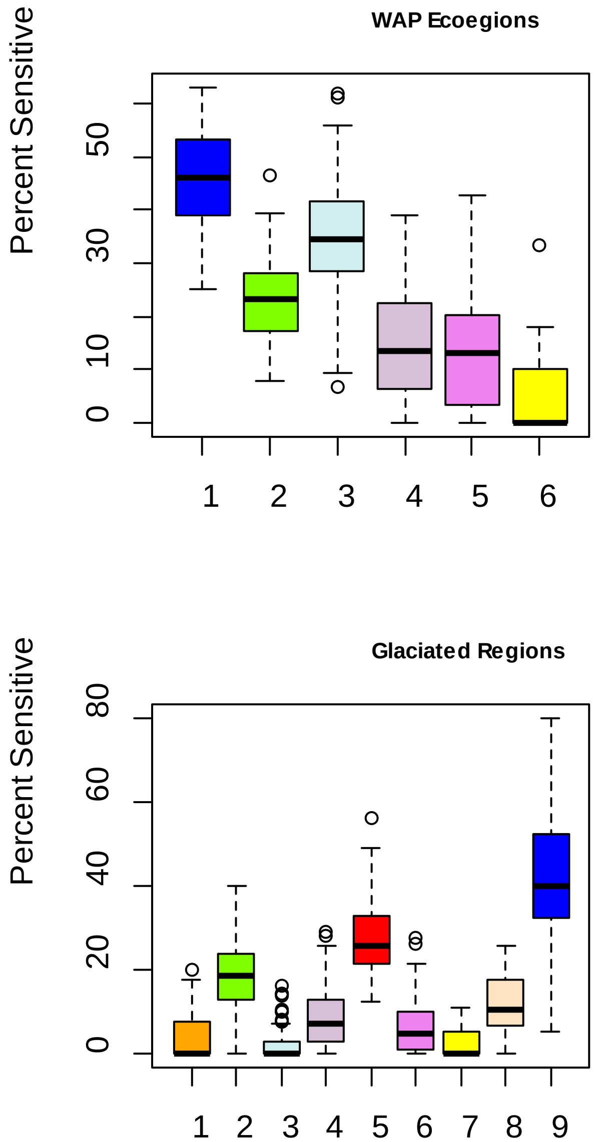

Figure 11. Plots showing distributions of the percent of taxa classed as sensitive at sites binned by groups identified by hierarchical clustering.

Environmental variables, including chemistry, fit to the NMDS for the glaciated ecoregions.
***VECTORS

	NMDS1	NMDS2	NMDS3	NMDS4	r2	Pr(>r)	
a	-0.25765	-0.66251	0.40155	-0.57745	0.0365	0.1835	
B	0.01188	0.32707	-0.80712	-0.49136	0.0297	0.2912	
Cc	0.78016	-0.15472	0.26397	0.54565	0.0768	0.0133	*
gm	-0.13997	-0.18577	-0.42994	-0.87238	0.0800	0.0106	*
illini	0.02154	0.23528	0.14611	0.96064	0.2662	0.0001	***
kames	0.86674	-0.48574	-0.05830	-0.09702	0.0447	0.1074	
L4	-0.80907	0.19063	-0.55209	-0.06527	0.0594	0.0393	*
Lclay	-0.42064	0.14874	0.88105	-0.15712	0.0984	0.0025	**
Lsilt	-0.28159	0.13049	-0.62132	-0.71947	0.0318	0.2665	
Lsand	-0.50605	0.66403	-0.25073	-0.49001	0.0284	0.3163	
em	0.17211	-0.61412	0.03667	-0.76934	0.0734	0.0156	*
outwash	0.00874	0.47618	0.11767	-0.87140	0.0306	0.2755	
hummoc	0.35219	0.62975	0.46359	-0.51426	0.0212	0.4796	
gaqyld	-0.27692	-0.43689	-0.20030	-0.83206	0.0264	0.3556	
bawx	-0.10689	-0.19151	-0.08605	-0.97185	0.1018	0.0023	**
bagt100	-0.06960	-0.31835	-0.15831	-0.93207	0.0388	0.1641	
balt5	0.14180	0.13126	-0.02725	0.98077	0.2253	0.0001	***
bat25	-0.03934	-0.00862	0.06699	-0.99694	0.1013	0.0016	**
bat5	-0.24000	-0.13363	0.07741	-0.95841	0.0824	0.0071	**
batnc	0.00000	0.00000	0.00000	0.00000	0.0000	1.0000	
exdrained	-0.13314	0.19065	-0.86219	-0.45006	0.0287	0.3171	
mwdrained	0.85916	0.10601	-0.07256	0.49532	0.2318	0.0001	***
poordrain	-0.84011	-0.31073	0.11489	-0.42950	0.2374	0.0001	***
notflooded	0.49021	-0.66320	0.53244	0.19072	0.0576	0.0470	*
minecnt	-0.48618	-0.15148	0.33177	-0.79411	0.0345	0.2178	
soilslope	0.86796	0.06411	0.13784	0.47280	0.2587	0.0001	***
orgmat	-0.36983	-0.50861	0.01588	-0.77736	0.2365	0.0001	***
pctclay	-0.02027	-0.77615	0.62601	-0.07270	0.0473	0.0954	.
cationx	0.13358	-0.42454	0.23631	0.86376	0.0849	0.0059	**
watercap	-0.22416	-0.41041	-0.31477	0.82598	0.1083	0.0011	**
PK2	0.49857	0.19302	0.39866	0.74514	0.0535	0.0601	.
Q7	-0.74837	-0.54378	0.15901	-0.34491	0.0056	0.9238	
Q8	0.05225	-0.78156	0.48206	0.39250	0.0041	0.9539	

Q9	0.47902	-0.54366	0.60405	0.33180	0.0051	0.9327	
CSL1085LFP	0.86522	0.34816	0.18223	0.31139	0.2275	0.0001	***
LC11IMP	-0.09937	0.93276	-0.31458	0.14535	0.2002	0.0001	***
LC11DEV	0.05383	0.96056	-0.26680	0.05690	0.1856	0.0001	***
LC92STOR	-0.17586	-0.50445	0.18161	-0.82560	0.0561	0.0505	.
PROB_1DAY	0.42090	-0.03503	0.05183	0.90495	0.1602	0.0001	***
PROB_7DAY	0.49013	-0.03854	0.08926	0.86621	0.1317	0.0002	***
PROB_30DAY	0.67256	-0.08392	0.29229	0.67468	0.0746	0.0159	*
FOREST	0.77500	-0.08003	0.58480	0.22577	0.1471	0.0001	***
PRECIP	0.37017	-0.05668	0.10789	0.92094	0.2154	0.0001	***
water	-0.33168	0.45260	0.53540	-0.63126	0.0490	0.0800	.
lawns	0.08608	0.95810	-0.21277	0.17135	0.3121	0.0001	***
popden	0.09128	0.92313	-0.20691	0.31095	0.2937	0.0001	***
lowurb	-0.00812	0.97856	-0.18014	0.09951	0.3048	0.0001	***
medurb	-0.17399	0.94098	-0.27592	0.09032	0.3072	0.0001	***
highurb	-0.30179	0.92380	-0.20849	0.10975	0.2076	0.0001	***
toturb	-0.07328	0.97075	-0.20612	0.09889	0.3277	0.0001	***
barren	-0.33092	0.52283	0.78536	-0.01870	0.0870	0.0073	**
deciduous	0.85590	-0.19779	0.41572	0.23555	0.2635	0.0001	***
pines	0.62852	0.17241	0.25027	0.71596	0.1341	0.0004	***
mixwoods	-0.29981	-0.15668	0.20746	0.91789	0.0117	0.7522	
scrub	0.86181	-0.42185	0.23614	-0.15352	0.1122	0.0011	**
totforest	0.85556	-0.19377	0.41041	0.24906	0.2662	0.0001	***
herbs	0.81729	-0.23940	0.30762	-0.42437	0.0924	0.0039	**
hay	0.52637	-0.71780	0.33384	-0.31023	0.1201	0.0006	***
totgrass	0.57762	-0.66514	0.33683	-0.33241	0.1383	0.0001	***
rowcrop	-0.71479	-0.65246	-0.19650	-0.15738	0.3073	0.0001	***
woodywet	0.13767	0.46375	0.19923	-0.85223	0.0845	0.0068	**
emergwet	0.23873	-0.04775	0.08866	-0.96585	0.0571	0.0458	*
totwet	0.14401	0.44027	0.19489	-0.86454	0.0868	0.0060	**
mindo	0.73663	0.54190	-0.23212	-0.33142	0.3128	0.0001	***
maxdo	0.41847	0.72105	-0.53888	0.12079	0.1591	0.0001	***
meando	0.60554	0.63879	-0.44849	-0.15533	0.2657	0.0001	***
mincelt	-0.40617	-0.11613	-0.48153	0.76790	0.0762	0.0116	*
maxcelt	-0.46949	0.30906	-0.55704	0.61136	0.0948	0.0038	**
meancelt	-0.47854	0.11689	-0.54801	0.67603	0.1353	0.0001	***
mincond	-0.65729	0.52557	-0.41512	-0.34555	0.0175	0.5863	
maxcond	-0.64083	0.70504	-0.00063	-0.30374	0.0393	0.1636	
meancond	-0.66903	0.61712	-0.14906	-0.38646	0.0322	0.2556	
minmnat	0.17347	-0.83540	-0.52155	0.00002	0.0405	0.1496	
maxmnat	-0.69682	0.14741	-0.53945	-0.44911	0.0327	0.2389	
meanmnat	-0.29751	-0.43364	-0.63031	-0.57110	0.0396	0.1562	
minmxat	-0.10705	-0.28177	-0.20393	0.93143	0.1219	0.0003	***
maxmxat	-0.37527	-0.14064	-0.47092	0.78590	0.0781	0.0110	*
meanmxat	-0.25532	-0.28647	-0.35885	0.85087	0.1230	0.0002	***
minph	0.74477	0.40735	-0.29856	0.43617	0.1761	0.0001	***
maxph	0.41823	0.41051	-0.63899	0.49825	0.1818	0.0001	***
meanph	0.59570	0.45994	-0.54210	0.37380	0.2222	0.0001	***

minprec	-0.65826	0.06924	-0.74954	-0.00996	0.0244	0.4019	
maxprec	0.23817	-0.27220	0.04188	-0.93136	0.0420	0.1274	
meanprec	0.18575	-0.57960	0.35770	-0.70825	0.0484	0.0856	.
meanfar	0.06280	0.63714	-0.72464	-0.25495	0.0198	0.5171	
al	-0.28214	0.32011	-0.14222	0.89314	0.1529	0.0002	***
alk	0.27746	-0.63460	-0.66672	0.27529	0.0121	0.7381	
as	-0.44312	0.28707	-0.20503	0.82414	0.1685	0.0001	***
ba	-0.23361	0.34417	-0.20702	0.88550	0.1428	0.0002	***
ca	-0.17166	0.41166	-0.17800	0.87714	0.1478	0.0002	***
cl	-0.40763	0.33027	-0.71387	0.46384	0.0155	0.6300	
cod	-0.83305	0.08509	-0.34187	0.42650	0.0131	0.7093	
fe	-0.36926	0.28960	-0.17388	0.86577	0.1424	0.0004	***
hard	-0.18640	0.39923	-0.19281	0.87675	0.1434	0.0002	***
k	-0.30017	0.37443	-0.12516	0.86835	0.1799	0.0001	***
mg	-0.22649	0.38086	-0.24489	0.86236	0.1273	0.0005	***
mn	-0.43298	0.21257	-0.13567	0.86541	0.1598	0.0001	***
nh3	-0.88670	-0.38227	0.00500	0.26001	0.1288	0.0001	***
no2	-0.87985	-0.21309	-0.40547	0.12666	0.0770	0.0093	**
nox	-0.38813	0.29619	-0.65292	-0.57908	0.0279	0.3293	
so4	0.20741	-0.66000	-0.71402	-0.10750	0.0153	0.6448	
sodium	-0.23191	0.56102	-0.21713	0.76442	0.1681	0.0001	***
sr	-0.26964	0.40146	-0.28985	0.82590	0.1251	0.0005	***
tds	-0.19747	-0.29205	-0.79994	0.48560	0.0055	0.9231	
tkn	-0.89337	-0.00801	-0.34880	0.28313	0.0683	0.0220	*
tp	-0.92354	0.02027	0.06793	0.37688	0.2111	0.0001	***
tss	-0.48946	-0.55128	-0.33116	0.58894	0.0336	0.2395	
zn	-0.24599	0.40649	-0.13280	0.86984	0.1558	0.0001	***
QHEI	0.86461	0.35780	-0.01383	0.35248	0.2694	0.0001	***
SUBSTRATE	0.66132	0.31054	-0.04445	0.68136	0.2984	0.0001	***
RIFFLE	0.83814	0.45155	0.07415	-0.29686	0.2047	0.0001	***
POOL	0.44254	0.32843	-0.12839	-0.82450	0.0809	0.0068	**
CHANNEL	0.71756	0.26103	0.10067	0.63783	0.3402	0.0001	***
GRADE	0.73583	0.26034	0.33173	0.52984	0.2277	0.0001	***
HWMI	0.86369	-0.16461	-0.47551	0.02902	0.7809	0.0001	***
DA	-0.56778	-0.16324	-0.80507	-0.05330	0.1279	0.0003	***

Signif. codes: 0 ‘***’ 0.001 ‘**’ 0.01 ‘*’ 0.05 ‘.’ 0.1 ‘ ’ 1

Permutation:free

Number ofpermutations: 9999

Environmental variables, excluding chemistry, fit to the NMDS for the glaciated ecoregions.

***VECTORS

	NMDS1	NMDS2	NMDS3	NMDS4	r2	Pr(>r)	
a	0.05360	-0.09550	0.71285	-0.69271	0.0654	0.0001	***
B	0.42996	0.25286	0.36978	-0.78387	0.0193	0.0878	.
Cc	0.57650	-0.12379	-0.10815	0.80039	0.1095	0.0001	***
gm	-0.46813	0.12543	-0.61837	-0.61866	0.0541	0.0002	***
illini	0.19128	-0.00698	-0.07676	0.97850	0.1647	0.0001	***
kames	0.64989	-0.23320	0.05926	-0.72094	0.0332	0.0103	*
L4	-0.92904	-0.14279	-0.23154	0.25077	0.0384	0.0030	**
Lclay	-0.51581	-0.25736	0.71252	-0.40003	0.0656	0.0002	***
Lsilt	0.42719	0.01506	-0.13507	-0.89389	0.0188	0.1037	
Lsand	-0.27491	0.19745	0.93073	-0.13852	0.0779	0.0001	***
em	0.05537	-0.11104	0.01033	-0.99222	0.0353	0.0058	**
outwash	0.04144	-0.12756	-0.18624	-0.97331	0.0159	0.1663	
hummoc	0.58944	0.42434	0.47058	-0.50105	0.0276	0.0229	*
gaqyld	-0.75015	-0.16128	0.11550	-0.63081	0.0623	0.0001	***
bawx	-0.49615	-0.10829	-0.20326	-0.83714	0.1055	0.0001	***
bagt100	-0.69877	-0.19488	-0.45528	-0.51620	0.0542	0.0003	***
balt5	0.34845	-0.05157	-0.03108	0.93539	0.1891	0.0001	***
bat25	-0.07671	-0.01751	0.15049	-0.98548	0.0773	0.0001	***
bat5	-0.33258	0.22618	0.16682	-0.90022	0.0584	0.0002	***
batnc	0.00000	0.00000	0.00000	0.00000	0.0000	1.0000	
exdrained	0.78882	0.22205	0.38269	-0.42663	0.0333	0.0101	*
mwdrained	0.89866	0.13373	-0.06748	0.41227	0.2526	0.0001	***
poordrain	-0.88636	-0.32775	0.00954	-0.32689	0.2587	0.0001	***
notflooded	0.67429	-0.23900	0.67784	0.16956	0.0768	0.0001	***
minecnt	-0.15104	-0.20587	-0.00581	-0.96684	0.0169	0.1318	
soilslope	0.82235	0.16264	-0.02161	0.54481	0.2841	0.0001	***
orgmat	-0.34999	-0.51161	0.26639	-0.73811	0.1560	0.0001	***
pctclay	-0.50291	-0.62189	-0.32109	0.50719	0.0955	0.0001	***
cationx	-0.13784	-0.36359	-0.40377	0.82812	0.1335	0.0001	***
watercap	-0.24079	-0.57622	-0.62505	0.46829	0.1193	0.0001	***
PK2	0.56461	0.07497	-0.33530	0.75045	0.0738	0.0001	***
Q7	0.19185	-0.13574	-0.77060	-0.59241	0.0085	0.4787	

Q8	0.74880	-0.13326	-0.62643	-0.17066	0.0148	0.1896	
Q9	0.84273	-0.07821	-0.49524	-0.19604	0.0211	0.0691	.
CSL1085LFP	0.76343	0.34902	0.30163	0.45210	0.2798	0.0001	***
LC11IMP	-0.26157	0.93706	0.02918	-0.22945	0.3084	0.0001	***
LC11DEV	-0.21666	0.92471	0.08703	-0.30066	0.2945	0.0001	***
LC92STOR	0.37608	-0.48473	0.75078	-0.24482	0.0690	0.0001	***
PROB_1DAY	0.35609	-0.15176	-0.09191	0.91745	0.1801	0.0001	***
PROB_7DAY	0.39891	-0.14021	-0.07335	0.90323	0.1530	0.0001	***
PROB_30DAY	0.51266	-0.15626	0.01754	0.84407	0.0740	0.0001	***
FOREST	0.82734	0.01948	0.46402	0.31595	0.2160	0.0001	***
PRECIP	0.58940	-0.07421	-0.08973	0.79941	0.2455	0.0001	***
water	-0.53370	-0.28014	0.55729	-0.57106	0.0319	0.0105	*
lawns	-0.06918	0.94394	0.12188	-0.29890	0.2792	0.0001	***
popden	-0.15323	0.98325	0.04988	-0.08521	0.3125	0.0001	***
lowurb	-0.13338	0.95298	0.06302	-0.26471	0.3620	0.0001	***
medurb	-0.25964	0.92825	-0.05736	-0.26011	0.2991	0.0001	***
highurb	-0.22824	0.95442	-0.03711	-0.18869	0.1943	0.0001	***
toturb	-0.17400	0.95020	0.02404	-0.25741	0.3813	0.0001	***
barren	-0.58760	0.26960	0.72914	-0.22451	0.0186	0.1012	
deciduous	0.84671	-0.31805	0.21925	0.36587	0.4165	0.0001	***
pines	0.62061	-0.02324	0.16733	0.76570	0.1032	0.0001	***
mixwoods	0.23782	-0.41967	-0.07411	0.87283	0.0529	0.0003	***
scrub	0.84252	-0.52841	0.07446	-0.07352	0.1621	0.0001	***
totforest	0.84287	-0.31986	0.21341	0.37645	0.4211	0.0001	***
herbs	0.90529	-0.34332	0.22203	0.11526	0.1190	0.0001	***
hay	0.74905	-0.62666	-0.16984	0.13182	0.1771	0.0001	***
totgrass	0.78138	-0.59814	-0.11983	0.13160	0.2139	0.0001	***
rowcrop	-0.63037	-0.74626	-0.20430	0.06327	0.3035	0.0001	***
woodywet	0.48573	-0.36345	0.33191	-0.72236	0.0403	0.0020	**
emergwet	0.42981	-0.70394	0.47949	-0.29970	0.0367	0.0055	**
totwet	0.48693	-0.39194	0.34614	-0.69963	0.0422	0.0016	**
meancelt	-0.29728	-0.46051	-0.74871	0.37281	0.0827	0.0001	***
QHEI	0.91526	0.30010	-0.18037	0.19928	0.4173	0.0001	***
SUBSTRATE	0.76581	0.39064	-0.22529	0.45845	0.3747	0.0001	***
RIFFLE	0.81750	0.50711	-0.25680	-0.09268	0.3057	0.0001	***
POOL	0.74386	0.25032	-0.25958	-0.56270	0.1605	0.0001	***
CHANNEL	0.84371	0.22850	-0.05707	0.48237	0.3726	0.0001	***
GRADE	0.65841	0.34609	0.36006	0.56310	0.2745	0.0001	***
HWMI	0.89013	-0.16033	-0.41228	0.10946	0.7777	0.0001	***
DA	-0.11103	-0.51261	-0.79469	-0.30556	0.1535	0.0001	***

Signif. codes: 0 '***' 0.001 '**' 0.01 '*' 0.05 '.' 0.1 ' ' 1

Permutation:free

Number ofpermutations: 9999

164observations deleted due tomissingness

Environmental variables, including chemistry, fit to the NMDS for the WAP ecoregion.
***VECTORS

	NMDS1	NMDS2	NMDS3	r2	Pr(>r)	
mwdrained	0.07650	0.99269	-0.09332	0.3344	0.0001	***
poordrain	0.60372	-0.60610	0.51785	0.3351	0.0001	***
notflooded	-0.34992	0.76881	0.53525	0.0432	0.0251	*
minecnt	0.41296	-0.61882	0.66823	0.2487	0.0001	***
soilslope	-0.38694	0.48157	-0.78636	0.1376	0.0001	***
orgmat	0.76999	0.28664	-0.57005	0.0718	0.0016	**
pctclay	0.99155	0.12288	0.04149	0.0827	0.0003	***
cationx	0.98089	-0.16375	-0.10504	0.0881	0.0004	***
watercap	-0.49250	0.80650	0.32710	0.1136	0.0001	***
Q7	0.01559	-0.92981	0.36772	0.0484	0.0306	*
Q8	-0.06297	-0.92967	0.36297	0.0476	0.0339	*
Q9	-0.06540	-0.93245	0.35532	0.0471	0.0347	*
CSL1085LFP	-0.94137	-0.17783	0.28669	0.0178	0.2819	
LC11IMP	0.52172	-0.05128	0.85157	0.1208	0.0001	***
LC11DEV	0.36935	-0.02497	0.92895	0.0605	0.0076	**
LC92STOR	0.18437	-0.97279	0.14030	0.0904	0.0013	**
PROB_1DAY	-0.56121	0.32451	-0.76141	0.1510	0.0001	***
PROB_7DAY	-0.64617	0.31023	-0.69730	0.1323	0.0001	***
PROB_30DAY	-0.74824	0.19608	-0.63379	0.0576	0.0211	*
FOREST	-0.46211	-0.87371	-0.15196	0.0122	0.4712	
PRECIP	-0.23534	0.02200	-0.97166	0.0681	0.0027	**
water	0.06585	-0.96259	-0.26282	0.0917	0.0010	***
lawns	0.17748	0.09906	0.97913	0.1550	0.0001	***
popden	0.11285	0.12439	0.98580	0.1870	0.0001	***
lowurb	0.50330	0.03134	0.86354	0.1776	0.0001	***
medurb	0.34359	-0.02407	0.93881	0.1516	0.0001	***
highurb	0.33596	-0.12775	0.93317	0.1181	0.0001	***
toturb	0.45207	0.00479	0.89197	0.1739	0.0001	***
barren	0.76263	-0.64681	0.00474	0.0742	0.0034	**
deciduous	-0.74107	0.28228	-0.60920	0.2710	0.0001	***
pines	0.67324	-0.18809	-0.71510	0.0690	0.0031	**

mixwoods	0.10320	0.31505	-0.94345	0.0651	0.0045	**
scrub	-0.35409	0.31392	-0.88095	0.1025	0.0003	***
totforest	-0.66911	0.27599	-0.69002	0.3031	0.0001	***
herbs	0.88490	-0.33653	-0.32203	0.0535	0.0125	*
hay	0.75315	-0.15507	0.63931	0.1328	0.0001	***
totgrass	0.86779	-0.22057	0.44530	0.1640	0.0001	***
rowcrop	0.39814	-0.55097	0.73343	0.1183	0.0001	***
woodywet	0.82036	-0.57099	0.03134	0.0215	0.2004	
emergwet	0.73037	-0.65051	0.20829	0.0441	0.0512	.
totwet	0.79131	-0.60362	0.09730	0.0456	0.0301	*
meancelt	0.88967	0.37894	-0.25474	0.1131	0.0001	***
mincond	0.30376	-0.56220	0.76920	0.2338	0.0001	***
maxcond	0.27602	-0.51289	0.81287	0.2399	0.0001	***
meancond	0.28241	-0.53764	0.79447	0.2437	0.0001	***
minmnat	0.56873	0.37711	-0.73098	0.1354	0.0001	***
maxmnat	0.34349	0.83152	-0.43657	0.0826	0.0008	***
meanmnat	0.50494	0.55554	-0.66062	0.1065	0.0001	***
minmxat	-0.72253	0.67878	-0.13120	0.0373	0.0524	.
maxmxat	-0.92049	0.39056	0.01284	0.1154	0.0001	***
meanmxat	-0.87048	0.49115	-0.03223	0.0783	0.0005	***
minph	0.67478	0.61751	0.40416	0.3929	0.0001	***
maxph	0.62932	0.62677	0.45948	0.3598	0.0001	***
meanph	0.66003	0.61544	0.43081	0.4015	0.0001	***
minprec	0.79892	0.04957	-0.59939	0.0156	0.3211	
maxprec	-0.58508	0.61100	0.53325	0.0338	0.0680	.
meanprec	0.49280	0.79966	0.34306	0.0067	0.7005	
meanfar	0.92902	-0.29054	-0.22916	0.0696	0.0023	**
al	0.00662	0.01139	-0.99991	0.0606	0.0051	**
alk	0.90968	0.29622	-0.29109	0.0476	0.0252	*
as	0.15631	0.19521	-0.96823	0.0709	0.0020	**
ba	0.21918	0.32932	-0.91843	0.0893	0.0005	***
ca	0.49741	0.12557	-0.85838	0.0497	0.0144	*
cl	0.88983	-0.02060	-0.45582	0.0615	0.0032	**
cod	0.59302	0.00230	-0.80519	0.0834	0.0005	***
fe	0.54160	-0.23903	-0.80594	0.0574	0.0072	**
hard	0.35904	0.18502	-0.91480	0.0515	0.0117	*
k	0.30644	0.20009	-0.93062	0.0569	0.0071	**
mg	0.31924	0.18683	-0.92908	0.0383	0.0437	*
mn	0.59931	-0.38836	-0.70000	0.1693	0.0001	***
nh3	0.70717	-0.52357	-0.47517	0.1509	0.0001	***
no2	0.68143	-0.03811	-0.73089	0.0935	0.0006	***
nox	0.95326	0.27718	-0.12024	0.0155	0.3524	
so4	0.86091	-0.20316	-0.46643	0.0261	0.1371	
sodium	0.64282	0.13024	-0.75486	0.0253	0.1450	
sr	0.41515	0.16733	-0.89423	0.0392	0.0395	*
tds	0.66564	-0.08249	-0.74170	0.0871	0.0006	***
tkn	0.85540	-0.26229	-0.44665	0.1181	0.0002	***
tp	0.94571	-0.10242	0.30846	0.1254	0.0002	***

tss	0.68888	-0.19448	-0.69830	0.0803	0.0009	***
zn	-0.16007	-0.06455	-0.98499	0.0871	0.0003	***
QHEI	-0.05802	0.92061	-0.38616	0.1382	0.0001	***
SUBSTRATE	-0.68125	0.71852	-0.14011	0.4258	0.0001	***
RIFFLE	-0.64861	0.60420	0.46287	0.1751	0.0001	***
POOL	0.56988	0.55008	-0.61045	0.0162	0.3374	
CHANNEL	-0.21545	0.72858	-0.65019	0.0949	0.0003	***
GRADE	-0.85445	0.24916	0.45589	0.2296	0.0001	***
DA	0.62515	0.77985	-0.03190	0.1145	0.0001	***
HWMI	-0.15565	0.95982	-0.23351	0.7099	0.0001	***

Signif. codes: 0 '***' 0.001 '**' 0.01 '*' 0.05 '.' 0.1 ' ' 1

Permutation:free

Number ofpermutations: 9999

227observations deleted due tomissingness

Environmental variables, excluding chemistry, fit to the NMDS for the WAP ecoregion.

***VECTORS

	NMDS1	NMDS2	NMDS3	r2	Pr(>r)	
mwdrained	-0.25225	0.95810	-0.13572	0.3258	0.0001	***
poordrain	0.58452	-0.60570	0.53987	0.2884	0.0001	***
notflooded	-0.84302	0.50910	0.17359	0.0938	0.0001	***
minecnt	0.31592	-0.70121	0.63913	0.2584	0.0001	***
soilslope	-0.38848	0.23209	-0.89175	0.1335	0.0001	***
orgmat	0.63746	0.21984	-0.73845	0.0335	0.0116	*
pctclay	0.97362	0.20270	-0.10481	0.0593	0.0002	***
cationx	0.98438	-0.14562	-0.09895	0.0648	0.0001	***
watercap	-0.37250	0.84808	0.37684	0.0768	0.0001	***
Q7	0.04595	-0.97035	0.23728	0.0249	0.0551	.
Q8	-0.02529	-0.97614	0.21568	0.0251	0.0552	.
Q9	-0.02964	-0.97968	0.19837	0.0251	0.0552	.
CSL1085LFP	-0.99243	-0.08042	0.09284	0.0441	0.0037	**
LC11IMP	0.60791	-0.05248	0.79227	0.0812	0.0003	***
LC11DEV	0.38122	0.01777	0.92432	0.0376	0.0059	**
LC92STOR	-0.20124	-0.88565	0.41848	0.0848	0.0001	***
PROB_1DAY	-0.58226	0.12924	-0.80266	0.1452	0.0001	***
PROB_7DAY	-0.65116	0.08117	-0.75459	0.1231	0.0001	***
PROB_30DAY	-0.72342	-0.08418	-0.68526	0.0545	0.0019	**
FOREST	-0.96271	-0.12137	-0.24178	0.0382	0.0041	**
PRECIP	-0.16745	-0.15081	-0.97428	0.0893	0.0001	***
water	0.08459	-0.98851	-0.12531	0.0826	0.0002	***
lawns	0.29590	0.15725	0.94219	0.1188	0.0001	***
popden	0.27283	0.09756	0.95710	0.1374	0.0001	***
lowurb	0.57710	0.06768	0.81387	0.1133	0.0001	***
medurb	0.45746	-0.01077	0.88917	0.1127	0.0001	***
highurb	0.46656	-0.10291	0.87848	0.0815	0.0001	***
toturb	0.54001	0.03471	0.84094	0.1167	0.0001	***
barren	0.77180	-0.63237	0.06649	0.1009	0.0001	***
deciduous	-0.69128	0.16205	-0.70418	0.2193	0.0001	***
pines	0.72636	-0.17448	-0.66480	0.0630	0.0004	***
mixwoods	0.42700	0.03145	-0.90371	0.0576	0.0005	***

scrub	-0.60952	0.06765	-0.78988	0.0793	0.0002	***
totforest	-0.63381	0.14181	-0.76038	0.2509	0.0001	***
herbs	0.79195	-0.48002	-0.37736	0.0547	0.0009	***
hay	0.58417	0.07586	0.80808	0.1084	0.0001	***
totgrass	0.72995	-0.03467	0.68262	0.1225	0.0001	***
rowcrop	0.42260	-0.39587	0.81529	0.0806	0.0001	***
woodywet	0.80445	-0.53352	-0.26119	0.0233	0.0561	.
emergwet	0.69646	-0.71570	0.05202	0.0315	0.0459	*
totwet	0.78309	-0.59911	-0.16685	0.0415	0.0073	**
meancelt	0.93746	0.16378	-0.30715	0.1528	0.0001	***
QHEI	-0.47491	0.76999	-0.42612	0.1693	0.0001	***
SUBSTRATE	-0.77656	0.61571	-0.13361	0.4240	0.0001	***
RIFFLE	-0.80382	0.49440	0.33081	0.1966	0.0001	***
POOL	0.44979	0.48843	-0.74774	0.0121	0.2545	
CHANNEL	-0.56742	0.64715	-0.50914	0.1299	0.0001	***
GRADE	-0.91289	0.20659	0.35205	0.1993	0.0001	***
DA	0.93495	0.27575	-0.22321	0.1681	0.0001	***
HWMI	-0.20200	0.94992	-0.23842	0.6783	0.0001	***

Signif. codes: 0 ‘***’ 0.001 ‘**’ 0.01 ‘*’ 0.05 ‘.’ 0.1 ‘ ’ 1

Permutation: free

Number of permutations: 9999

96 observations deleted due to missingness