Manaaki Whenua
Landcare Research

Wetland plant foliage nutrients as indicators of soil nutrients

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Wetland plant foliage nutrients as indicators of soil nutrients

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Summary

Project and client

- Hawke's Bay Regional Council, via an Envirolink, contracted Manaaki Whenua – Landcare Research to assess the suitability of wetland plant foliage to indicate soil nutrient status, as an alternative to sampling soil directly.
- Current soil sampling is undertaken 5-yearly, and one alternative approach (subject to the findings in this report) is for soil sampling be undertaken 10-yearly, with foliage-only sampling at the intervening 5-year interval. Supplementary soil sampling might then be initiated if foliage-only sampling in some wetlands gives cause for concern in terms of nutrient change.
- Hawke's Bay Regional Council, along with other councils, implements its monitoring using the *Wetland Monitoring Handbook* (Clarkson et al. 2004), which recommends sampling total nitrogen and total phosphorus in soil, along with the foliage of the dominant plant species.

Objectives

1 Quantify the degree of correlation between soil nutrient (particularly nitrogen and phosphorus) and plant nutrient data.

2 Create a preliminary list of 'strong' and 'weak' homeostatic plant species, and:

   a a list of the subset of species to be targeted for collecting and informing nutrient status

   b if there is a strong enough correlation between soil and foliage nutrients, establish preliminary limits of foliage nutrients for those species.

3 Scope and make recommendations for future work to develop limits (e.g. 10th and 90th percentiles) or baselines for soil nutrients for all wetland classes (including marshes, currently a knowledge gap), which reflect the 'natural' state.

Methods

We:

- collated data from the New Zealand Wetland Database, Hawke's Bay Regional Council, Greater Wellington, Waikato Regional Council, and the Department of Conservation
- assessed the overall correlation between soil and foliage nutrients, and analysed, for selected species, the correlation between soil nitrogen (N) and foliage N, soil phosphorus (P) and foliage P, and between the soil N:P ratio and the foliage N:P ratio
- used orthogonal regression and Pearson correlation coefficients to determine whether soil–foliage nutrient correlation was strong enough to establish preliminary limits for foliage nutrients
- scoped future work to develop limits for soil nutrients in all wetland classes.

Results

- We collated over 1,600 records of foliage nutrients and 1,095 associated unique soil samples. Standards of wetland naming (or wetland ID), wetland class and species were found to be inconsistent, and future syntheses, including setting nutrient limits, would benefit from a unified approach to data collection and management.
- Overall there was a poor correlation between soil N and foliage N, and between soil P and foliage P. There were two distinct trends in the relationship between soil N:P and foliage N:P: a lack of response by foliage to increasing soil N:P, and a steep increase in foliage N:P at low values of soil N:P.
- Twelve species were sufficiently numerous to be analysed for species-specific correlations between soil and foliage nutrients. We found no species were good indicators for soil N, nor for soil P. However three species deserve further investigation as soil P indicators: *Leptospermum scoparium, Coprosma tenuicualis,* and *Gleichenia dicarpa* (*G. dicarpa* with further sampling at higher levels of soil P).
- Three species were reasonable indicators for the soil N:P ratio (*Empodisma minus, L. scoparium, Schoenus pauciflorus*) and two species are recommended for further investigation *(Typha orientalis* and *Machaerina rubiginosa*).
- Marsh wetland types were poorly represented by the species that were considered candidate indicator species. However, two of the most numerous species that had been sampled in marshes were tested in our species-specific analyses.
- Marshes remain relatively poorly sampled overall, and we consider further sampling needs to be conducted before interim (or final) limits for nutrients can be set, as has already occurred for other wetland types.
- At the national level, a future sampling strategy to update the interim limits for soil nutrients would ideally include:
    - quantification of the national extent of wetlands by wetland class (preferably including wetlands smaller than those mapped under Freshwater Ecosystems of New Zealand or the Land Cover Database)
    - quantification of the area of wetlands already sampled, proportionate to wetland class area, and by geographical region and estimated degree of degradation
    - a power analysis to calculate the number of samples required
    - stratified sampling (with the number of samples determined as above) by region and estimated degree of degradation.

Conclusions

- Nine species are recommended as having potential for indicators of soil nutrient status: the seven above-listed species, and *Chionochloa rubra* and *Carex diandra* which showed promise in earlier investigations but insufficient data excluded them from the current analysis. Foliage analyses for these species would potentially be suitable as complementary measures of nutrient changes, along with monitoring of species composition and some proxy of biomass. All or any of these variables may change when nutrients change, and therefore plant foliage is just one 'tool' in the toolbox and should not be relied on alone.

- That said, soil sampling may not indicate change after nutrient inputs, where that input has been taken up by the plant or microbial communities. Therefore, foliage monitoring may be informative even if it does not reflect soil nutrient status.
- Nitrogen (at least total nitrogen, as used in this study) is not suitable for monitoring with plant foliage, and we found no species to recommend for further investigation, unlike soil P. As an alternative, we suggest sampling anthropogenic N where necessary using mineral N (NH_4^+ and NO_3^-) as an indicator.

Recommendations

1 Undertake an analysis of the correlation between abundance weighted mean foliar N, P, and N:P; and soil N, P, and N:P, as an alternative method for indicating soil nutrient changes.

2 Conduct further fertilisation experiments across a range of wetlands to assess the utility of the N:P ratio as an indicator of wetland vulnerability to relative nutrient input change. A previous pilot study indicated that the N:P ratio in foliage nutrients is a useful indicator of which nutrient is limiting the vegetation community.

3 Carry out targeted sampling of the potential 'indicator species' where available to confirm utility, and also to demonstrate sensitivity to change over time, because current work assesses different individuals across a gradient, not sensitivity to change within individuals. These species are:

   a *Empodisma minus*
   b *Leptospermum scoparium*
   c *Schoenus pauciflorus*
   d *Typha orientalis*
   e *Machaerina rubiginosa*
   f *Coprosma tenuicualis*
   g *Gleichenia dicarpa*
   h *Chionochloa rubra*
   i *Carex diandra*

4 Carry out further work to identify indicator species for marshes, and further stratified sampling for the purpose of setting baselines to finalise the interim limits.

5 Adopt a tripartite approach to monitoring potential nutrient impacts, particularly where soil sampling is dropped, by: (a) ensuring a sufficient baseline of soil samples exists for a wetland; (b) including plot composition and estimated biomass in change detection, in addition to plant foliage nutrients; (c) monitoring identified indicator species (even if not dominant). This tripartite method may require methodological development for estimating biomass in wetlands. Consideration should also be given to monitoring forms of nutrients that reflect anthropogenic inputs, in addition to total N and total P, where concern exists about anthropogenically-induced increases in nutrients.

6 Establish a set of national data quality standards for wetland monitoring to enable more accurate data syntheses. Specific opportunities for improving the consistency of data include wetland names/identifiers and wetland classes, including transitional classes.

1 Introduction

Wetlands are at risk from multiple drivers of decline, one of which is nutrient enrichment. Although often considered the 'kidneys' of the earth (Mitsch & Gosselink 2015), in that they are able to filter (nutrients in the case of wetlands, other elements in the case of kidneys), excess nutrients reaching wetlands allow invasion by nutrient-exploiting species (e.g. Zhao et al. 2015). Detection of increased nutrient input is necessary in order to take ameliorative action.

Typically, plants obtain their nutrients from the soil, and so measuring soil nutrients is a direct way of measuring the nutrients that might be available to plants. Alternatively, measuring the plant foliage directly may indicate the nutrients that are available to the plant via the soil. The plant foliage ratio of nitrogen (N) to phosphorus (P) (the ratio is referred to as N:P), for example, is a well-documented indicator of whether N or P is limiting biomass production (Koerselman & Meuleman 1996; Güsewell et al. 2003; Burge et al. 2020).

Compared to sampling soil, sampling plant foliage is easier and quicker, and the material is lighter, simplifying transport. Plus, the analytical costs compared to soil are less. However, just how well nutrient ratios (and concentrations) in plants reflect the nutrient status of the soil depends on the degree to which plants regulate such nutrient ratios in comparison to their availability. This is referred to as 'homeostasis' (Sterner & Elser 2002).

Homeostasis is maintained by negative feedbacks, so that as an element (such as N) increases in plant tissue, plant uptake and storage of that element decrease. Within wetland plants there is known variability in the degree to which different species control homeostasis (Güsewell & Koerselman 2002). Plant species that maintain *weak* homeostasis will be the best bio-indicators of ecosystem (or soil) eutrophication, because they do not restrict their uptake of nutrients as the supply of nutrients increases. Weak homeostasis with regard to phosphorus is the relatively well-documented phenomenon of 'luxury' uptake (Boeye et al. 1997; Güsewell & Koerselman 2002).

In this report we determine the degree to which New Zealand wetland species maintain homeostasis over a range of soil nutrient conditions by analysing existing data sets from regional councils, the Department of Conservation, and MWLR. From this analysis we develop a preliminary list of plant species with weak homeostasis which might be suitable for bio-monitoring, and assess whether their relationship to soil nutrients is strong enough to act as proxies for soil nutrient status and to be assessed against the interim limits for soil nutrients (hereafter 'the Interim Limits' report) (Clarkson et al. 2015). Given that the soil limits remain 'interim' and were developed some years ago, we also assess whether there are now enough wetland sites across all wetland types to update these limits, and the areas and methods by which an update might best be achieved in the future.

This report is based on the hypothesis that substantial change in wetland nutrient supply should be reflected in plant nutrients. However the literature also suggests that sustained increases in plant nutrients will lead to a shift in species composition, towards species that are better adapted to high fertility conditions, usually expressed through an increase in

biomass (Güsewell et al. 2003). Thus although it is beyond the scope of this report, monitoring vegetation composition and estimates of biomass are important and complementary to foliage and soil nutrient data and will provide important information about the long-term effects of eutrophication.

2 Background

This report is funded by Envirolink contract number C09X2003 with Hawke's Bay Regional Council. As soil sampling is technically more difficult than foliage sampling, and therefore more expensive, we have been asked to investigate whether foliage nutrients are a reasonable substitute sampling method for soil nutrient sampling, and if so, which species are most useful.

3 Objectives

In light of the above background, we have been asked to:

- quantify the degree of correlation between soil nutrient (particularly N and P) and plant nutrient data
- create a preliminary list of 'strong' and 'weak' homeostatic plant species, which involves creating:
  - a list of the subset of species to be targeted for collecting and informing nutrient status
  - a list of preliminary limits of foliage nutrients linked to the interim soil limits for weak homeostatic plant species – *if* there is a strong enough correlation between soil and foliage nutrients
- scope and make recommendations for future work to develop limits (e.g. 10th and 90th percentiles) or baselines for soil nutrients for all wetland classes, which reflect the 'natural' state (this includes the wetland-type marshes, a current knowledge gap).

4 Methods

4.1 Data

Data for soil and foliage nutrients were sourced from the New Zealand Wetland Database, held by Manaaki Whenua – Landcare Research, including data collected on behalf of regional councils (e.g. Bay of Plenty Regional Council). Further data were provided by the Department of Conservation, and regional councils: Greater Wellington, Hawke's Bay Regional Council, and Waikato Regional Council. Field data collection of soils and foliage followed the protocols in the *Wetland Monitoring Handbook* (Clarkson et al. 2004).

Most data contributors used the Manaaki Whenua Soil Chemistry Laboratory. We did not have the information necessary to identify the samples that were analysed externally. We

compare the gravimetric concentrations of total N and total P to foliage N and P; not all data points included bulk density, which would be required to calculate volumetric concentrations. Where methods were not specified, we assumed that both total N and P were calculated using the Kjeldahl method for both soil and foliage (Blakemore et al. 1987).

In combining the data we relied on wetland names to distinguish individual wetlands or wetland complexes. However, this may arbitrarily separate parts of wetlands that were given a name such as 'Awarua burnt' or similar. We tried to account for this by intersecting the plot data as spatial points, with the Wetlands Of National Importance wetlands layer (sometimes referred to as a WONI layer), but some plots fell outside the layer, and some plots had no spatial coordinates.

In combining data we also found some duplicates that were not initially obvious due to differences in institutional naming conventions for the same wetlands, and differences in rounding. We implemented a routine to pick up duplicates by checking for similar values of nutrients that were sampled on the same day, on the same plot, but not necessarily in the same wetland. We checked and confirmed that this technique did not exclude samples taken from the same day and plot number but from different wetlands. In the future it is hoped a national data set of wetland extent will be available in order to attribute plots to wetlands in a systematic manner, and plots to wetland types, within wetland mosaics. In the interim we left the names as is.

We checked data for outliers in terms of N and P concentrations, and for N:P. We found only a small set of data from 2002 where the soil total N seemed implausibly low (<0.1%); this affected four soil samples from ephemeral wetlands, which were linked to 36 foliage samples. This small set of data was excluded. There were other high or low values in our data set that might be considered to be 'outliers', but we could not justify excluding them based on the information available to us (e.g. soil organic carbon, dry bulk density, wetland class).

We included some data linked to a published field study (Burge et al. 2020) on soil N and P after 4 years of quarterly fertilisation. Burge et al. (2020) note that foliage N:P ratios correctly indicated that biomass should increase with N fertilisation: biomass increased under the highest N (70 kg/ha/year of each of NH_4^+ and NO_3^-) treatments. We present summary statistics (mean and standard error) from soil samples taken after 4 years of fertilisation, from three vegetation communities, all of which were dominated by one species (*Chionochloa rubra*, *Carex diandra*, *Schoenus pauciflorus*). These data demonstrate that fertilisation and subsequent vegetation change (increased biomass) are not necessarily accompanied by changes in soil nutrients (N in this case). It is from this data set that we recommend monitoring *Carex diandra* and *Chionochloa rubra*, in addition to the species identified in the analyses set out in this report – which include *Schoenus pauciflorus.*

4.2 Species-level analysis

We quantified how well plant N, P, and the plant N:P ratio reflected the N and P content of soil samples taken nearby (all samples taken on plots that are typically 2 × 2 m to 10 × 10 m), at the same time. We used orthogonal regression, which, unlike other methods, accounts for errors in the measurements of both soil and plant foliage (i.e. both the response and the predictor). Orthogonal regression allows calculation of a Pearson correlation coefficient, which we report. Pearson values of r = 0.5 or better are considered to be a good correlation for the purposes of this report.

We selected species that had foliage sampled at a minimum of 10 wetlands, and which had had their foliage sampled at least 20 times. We included the minimum sample size for wetlands to attempt to ensure geographical spread in the modelled populations. This resulted in 12 species being selected. To reduce pseudo-replication, we chose the most recent soil and foliage samples where a plot within a wetland had been sampled more than once for soil and foliage.

We do not list species with 'strong' homeostasis as we consider this would best be confirmed using fertilisation experiments (i.e. within-plant changes).

4.3 Update to the interim limits

We calculated how many wetlands within each wetland class (e.g. bog, fen) were represented in the combined data set to test the viability of an update of the Interim Limits report. Based on this tabulation we do not consider there to be sufficient wetlands to update the guidelines, and therefore in the conclusions section we discuss a roadmap for updating the guidelines in the future.

5 Results

5.1 Available data

In addition to data from the New Zealand Wetland Database, we received data from: Waikato Regional Council, the Department of Conservation, Greater Wellington, and Hawke's Bay Regional Council.

We collated 1,618 records of foliage N and P. Of these, 1,592 records had accompanying soil total N, 1,573 had accompanying total P data, and 1,573 had both (i.e. all records with soil total P had soil total N records). Note that foliage is often sampled from two species at a single plot, so although there are 1,618 unique foliage records, there are fewer unique soil samples in the data set.

Wetland class was given for 1,553 of the 1,618 records, and the numbers for each class are set out below. We note that in some cases small areas within wetland mosaics may be attributed to the larger class surrounding them, and so there is some 'noise' expected in the wetland classification of plots within large wetlands.

Table 1. Number of data records for plant foliage nutrient samples for each wetland class, ordered from most to least numerous.

Wetland class	Count
Swamp	731
Bog	327
Fen	263
Marsh	83
Saltmarsh	59
Marsh/swamp	38
Seepage	10
Swamp/salt marsh	10
Ephemeral	5
Young bog	3
Shallow water	2
Fen/young bog	1
Swamp/fen	1

Notes: Combined classes are given where the field ecologists considered the wetland to be a transition between classes; ephemeral wetlands are typically placed within marshes. Because some sites had many data records, the number of unique sites per wetland class is lower than the numbers shown; for example, only 25 marsh 'sites' were sampled, despite 83 data records being situated within the marsh class.

5.2 Relationship between soil and foliage nutrients

We created visualisations of the overall relationships between soil and foliage. Although we include a regression line in the panels in Figure 1, it is clear that it would be ill advised to predict or further interpret the relationships given the spread in the data. Overall there was a poor relationship between soil N and foliage N, and between soil P and foliage P (Figure 1).

There appeared to be *two* trends within the data set for the relationship between foliage N:P and soil N:P. The first, highlighted by a linear regression line (Figure 1(C), green line), illustrates a general trend for plant foliage to have lower N:P ratios than soils, indicated by points below the 1:1 line. This indicates that they have relatively more P in their tissue than N compared to the soil. The second relationship was the case of plant samples having a greater N:P ratio than that found in the soil (i.e. above the 1:1 line in Figure 1(C)), indicating that plants had relatively less P than N in their tissues relative to the soil.

We investigated the strength of relationships between foliage and soil nutrients further by testing within species, where the number of samples per species was adequate (see Methods).

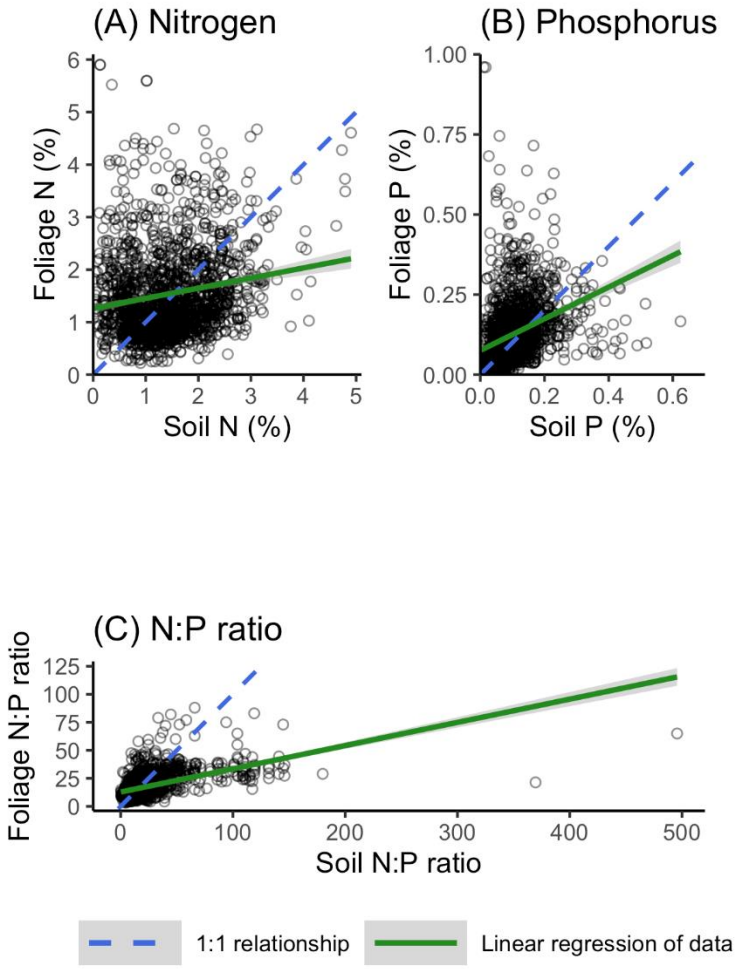

Figure 1. (A) Relationship between soil and foliage nitrogen. (B) Relationship between soil P and foliage P. (C) Relationship between foliage N:P ratio and soil N:P ratio.

Notes: (A) Overall, a poor relationship between soil and foliage N. The simple linear regression fit (green line, grey shading indicates 95% CI) indicates that as soil N increases, little increase in foliage N is seen. The 1:1 line is shown in blue for visual reference. (B) A poor relationship between soil P and foliage P. Note: top left of the figure, relatively low soil P values but high foliage P, and bottom right of the figure, high values of soil P but relatively low values of foliage P. (C) The N:P relationship between plants and soil varies. It appears there are in two trends in the data: the one demonstrated by the linear regression, which would indicate relatively strong homeostasis (plants consistently maintain a lower N:P ratio than that in the soil), and a second, where plants are closer (although slightly above) the ratio in the soil. We note two outliers in the figure, which we investigated but found no reason to exclude them. They had very low total soil P (<0.005%).

5.3 Species-level analysis

For foliage nutrients to be a good indicator of soil nutrients it is desirable that:

1 as soil nutrients increase, foliage nutrients increase (a positive correlation); and

2 there is a relatively strong relationship between soil nutrients and foliage nutrients (a high correlation [r > 0.5]).

Overall there was a poor correlation between soil N and foliage N: foliage N appeared to be relatively insensitive to changes in soil N (Figure 2). In Figure 2 the equation is given as an intercept (e.g. 2.44 for *Typha*), which is where the regression line would intercept the y-

axis when the x-axis is zero, and a slope equation (e.g. -0.16 * SoilTotalN), which is used to calculate what the value of foliage N will be for any given value of soil N.

A good example of foliage N being insensitive to soil N is *Schoenus pauciflorus*, which maintains a foliage N of around 1% regardless of the soil N level. Effectively, *Schoenus* is maintaining tight control of its foliar N composition relative to soil total N. Pearson's r ranged from negative numbers (that is, as soil N increased there was a trend towards declining foliage N), to a maximum of 0.319 for *Empodisma robustum*, followed by 0.290 for *Carex geminata*.

Overall, there was a stronger relationship between soil P and foliage P, compared to N (above). All relationships between soil P and foliage P were positive, except for *Typha orientalis*, which had both an 'outlier' (unusually high soil P) and multiple high foliage P values at relatively low soil P values. Unlike for N, where the maximum Pearson's r value was 0.319, the maximum r value was 0.457 (for *Leptospermum scoparium*). We highlight that there was a number of high foliage P values for *Leptospermum scoparium* at relatively low soil P values. If only foliage had been sampled in the field without any soil sampling, and the equation presented in Figure 2 used, these values and the equation would have predicted a value of soil P perhaps three times higher than it was in reality. Nevertheless, *Leptospermum scoparium* covered the bulk of the range of soil phosphorus values in our samples, making it a potentially useful 'general' indicator of soil P. *Typha orientalis* included an outlier, where the soil P sampled was 0.72% (Toreparu wetland). With this outlier removed, the relationship between soil P and foliage P for *Typha* was still strongly negative. Further analysis of *Gleichenia dicarpa* (r = 0.354) and *Coprosma tenuicaulis* (r = 0.348) with more samples would be useful, as the sample size for these species was relatively small. Other species (e.g. *Empodisma robustum*) had higher r values than *C. tenuicaulis* and *G. dicarpa* but more replicates, and we do not consider these as high a priority.

The relationship between the soil N:P ratio and the foliage N:P was the strongest of the three relationships measured. Foliage N:P ratios were well correlated (r > 0.5) with soil N:P ratios for three species: *Empodisma minus*, *Leptospermum scoparium*, and *Schoenus pauciflorus*. Also, *Machaerina rubiginosa* and *Typha orientalis* (r = 0.416 and r = 0.455, respectively) would be worth considering after further sampling, particularly because they are abundant enough to be sampled across a wide range of N:P ratios.

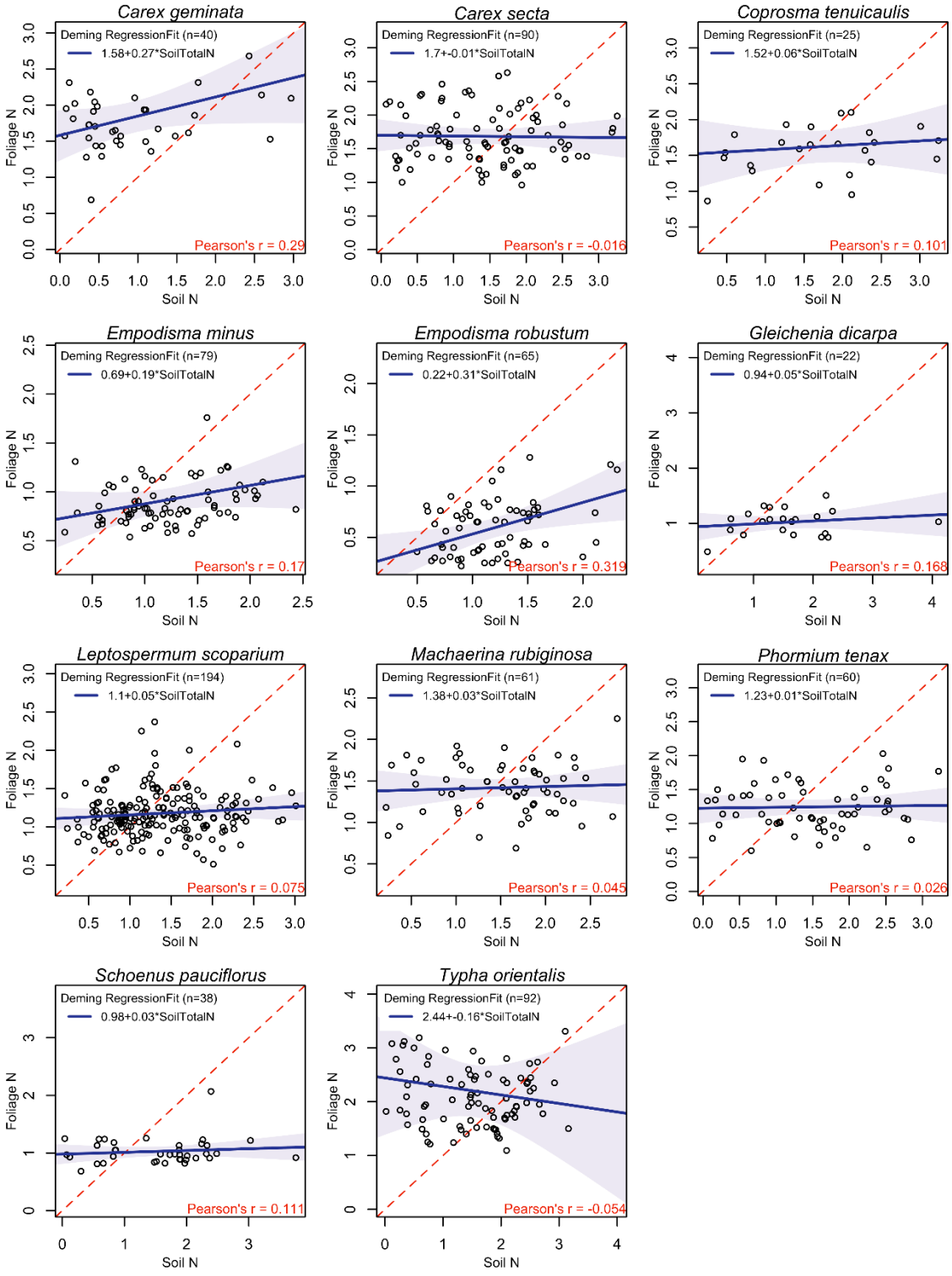

Figure 2. Relationship between foliage N and soil N for selected species.

Notes: Foliage N is not well correlated with soil N. For most species the correlation is near 0 (uncorrelated) or negative (as soil N increases, foliage N decreases). Pearson's r gives an indication of the strength of the linear correlation between soil N and foliage N. The blue line indicates the regression line, with shading to represent the 95% CI; the red dashed line represents a 1:1 relationship for visual reference. The regression is fitted with a 'Deming' regression (another name for orthogonal regression); the n in brackets is the number of records used in fitting the regression.

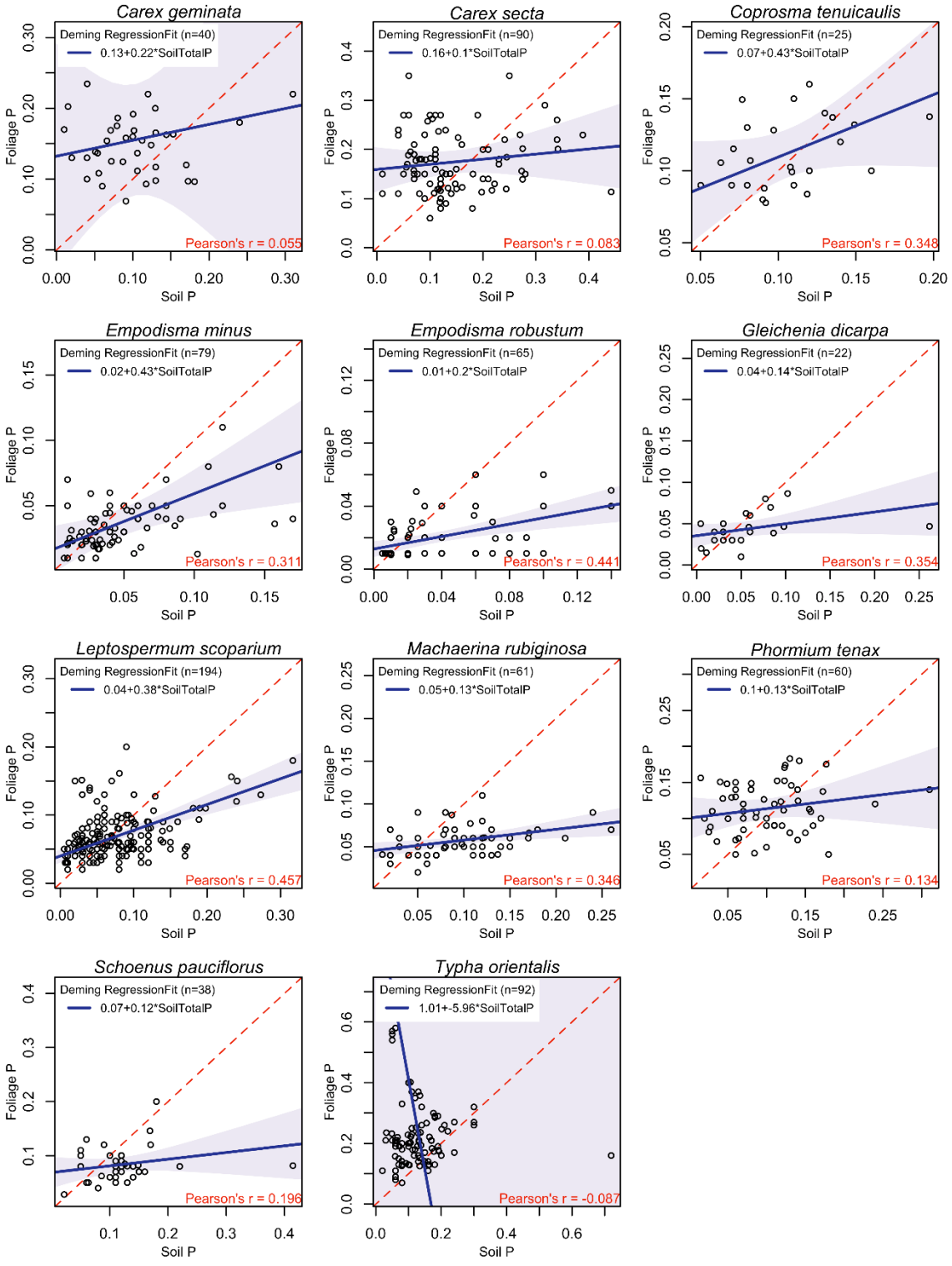

Figure 3. Relationship between foliage P and soil P.

Notes: Foliage P is not well correlated with soil P, although the relationship is stronger than that for N. For most species the correlation is positive but less than a 1:1 relationship. Pearson's r gives an indication of the strength of the linear correlation between soil P and foliage P. The blue line indicates the regression line, with shading to represent the 95% CI; the red dashed line represents a 1:1 relationship for visual reference.

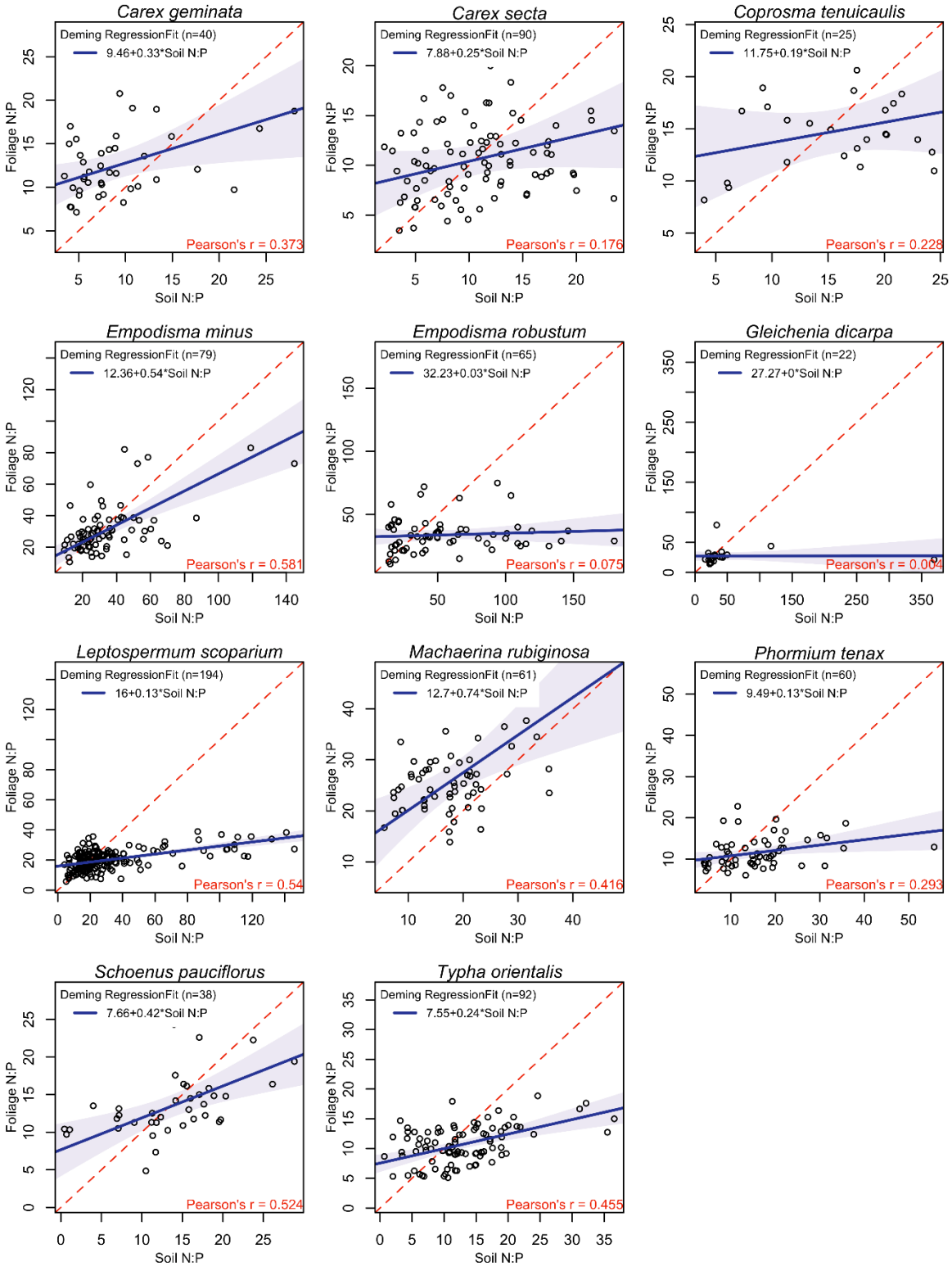

Figure 4. Relationship between foliage N:P ratio and soil N:P ratio.

Notes: Foliage N:P ratios are well correlated ($r \geq 0.5$) with soil N:P ratios for three species: *Empodisma minus*, *Leptospermum scoparium*, and *Schoenus pauciflorus*. *Typha orientalis* shows a much stronger relationship between soil and foliage N:P than for either N or P alone. The blue line indicates the regression line, with shading to represent the 95% CI; the red dashed line represents a 1:1 relationship for visual reference.

The number of samples for each of the species identified as candidates for monitoring change in soil P, or soil N:P ratio, is given in Table 2. Note that marshes are poorly represented by the species included as potential 'indicator species'. However, we investigated further and found that *Schoenus pauciflorus* was the most well-represented species sampled from marshes, with seven samples from marsh wetlands. *Schoenus pauciflorus* was tested in all species-specific analyses. The next most numerous species in the foliage sampling were *Carex geminata* (n = 5 in marshes; included in our analyses) and *Carex maorica* (also n = 5 in marshes; n = 44 in all wetland sites; only sampled in three wetlands).

Table 2. Number of observations in total, and in each wetland class, for species with a good correlation (≥ 0.5 Pearson correlation; indicated by a "Yes" in the column 'Indicator Type') to either soil P or soil N:P ratio, or where the relationship deserves further investigation (indicated by "More info" in the column 'Indicator Type').

Notes: Species codes: LEPSCO = *Leptospermum scoparium*; GLEDIC = *Gleichenia dicarpa*; COPTEC = *Coprosma tenuicaulis*; EMPMIN = *Empodisma minus*; SCHPAU = *Schoenus pauciflorus*; TYPORI = *Typha orientalis;* MACRUB = *Machaerina rubiginosa*. Total N = total number of observations.

Species	Indicator for:	Indicator type	Correlation	Total N	Bog	Ephemeral	Fen	Fen/ young bog	Marsh	Saltmarsh	Seepage	Swamp	Swamp/ salt marsh	Young bog	NA
LEPSCO	Soil P	More info	0.457	194	60	2	56	1	4	2	2	47	0	0	20
GLEDIC	Soil P	More info	0.354	22	6	0	15	0	0	0	0	0	0	0	1
COPTEC	Soil P	More info	0.348	25	0	0	4	0	1	1	2	16	0	0	1
EMPMIN	Soil N:P ratio	Yes	0.581	79	54	0	16	0	0	0	0	0	0	3	6
LEPSCO	Soil N:P ratio	Yes	0.540	194	60	2	56	1	4	2	2	47	0	0	20
SCHPAU	Soil N:P ratio	Yes	0.524	38	0	0	2	0	6	0	0	28	0	0	2
TYPORI	Soil N:P ratio	More info	0.455	92	0	0	4	0	3	7	0	71	5	0	2
MACRUB	Soil N:P ratio	More info	0.416	61	0	0	28	0	0	2	1	23	0	0	7

5.4 Soil N after 4 years of fertilisation

The Ō tū Wharekai (Ashburton Lakes) data set demonstrates the issues with detecting eutrophication in wetlands: there was no consistent or clear treatment effect of fertilisation on soil total N after 4 years, compared to non-fertilised plots within the Ō tū Wharekai data set. Data from the same study indicated that the plant foliage N:P ratio indicated the plant community was N limited and N did in fact increase the phytomass (live biomass) of the most highly fertilised (70 kg/ha/year $NH4^+$-N [referred to as 70AN in the figure below] and 70 kg/ha/year NO_3^--N [referred to as 70NN in the figure below]) plots. These data serve as a reminder that nutrient additions – which may have been large enough to have effects on the vegetation community – may not be detected with soil sampling alone.

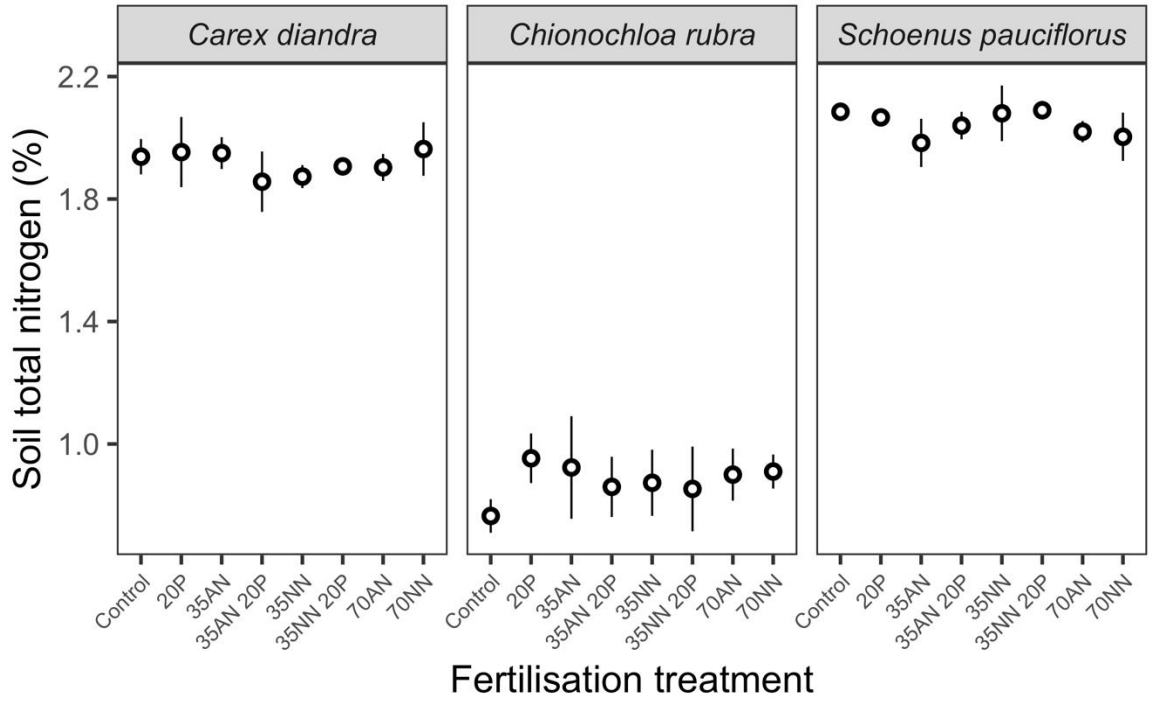

Figure 5. Soil total nitrogen (mean +/- standard error) after 4 years of fertilisation in vegetation dominated by three species – *Carex diandra*, *Chionochloa rubra,* and *Schoenus pauciflorus*.

Notes: Differences between treatments are dwarfed by differences among vegetation communities within Ō tū Wharekai (Ashburton Lakes) wetland complex. 20P = 20 kg/ha/year phosphorus; 35AN & 35NN = 35 kg/ha/year $NH4^+$-N and NO_3^--N, respectively (with suffix 20P to indicate a combined addition of nitrogen and phosphorus); 70AN & 70NN = 35 kg/ha/year $NH4^+$-N and NO_3^--N, respectively.

5.5 On deriving anthropogenic input from soils analysis

Plant uptake of N occurs mainly through ammonium (NH_4^+) and nitrate (NO_3^-) in the soil solution. In the natural environment the majority of these molecules are initially supplied by micro-organisms that can convert atmospheric N_2 into ammonia (NH_3, also plant-available), which is usually rapidly converted first into ammonium by protonation, and further into nitrate by microbially mediated nitrification if sufficient oxygen is supplied.

Agricultural amendments (i.e. fertilisers) target NH_4 and NO_3^- either by supplying them directly (e.g. as ammonium nitrate, NH_4NO_3) or in a form that is readily converted into these compounds by micro-organisms (e.g. urea fertiliser via urease, protonation, nitrification). In addition, indirect delivery of NH_3 and NO_x compounds can occur via dry or wet atmospheric deposition. In particular, the aerial deposition of NH_3 is strongly linked to modern agriculture.

Like atmospheric N, NH3, $NH4^+$, and NO_3^- -are all mineral N forms, in contrast to organic N compounds that also contain carbon–hydrogen bonds. Organic N is usually not directly plant-available and has to be first converted into ammonia through mineralisation of plant/animal material (i.e. ammonification).

In terms of an indicator for the anthropogenic eutrophication of wetlands, it is therefore advisable to focus on the two main plant-available *mineral* forms of N (i.e. NH_4^+ and NO_3^-). KCl-extractions have been routinely used to quantify NH_4^+ and NO_3^- in soils. Under the impact of agricultural fertilisation these values are expected to be higher than the natural background levels. Artificially elevated ecosystem N levels may not be reflected clearly through total N (organic and mineral N; e.g. by dry combustion or Kjeldahl digestion), since the response of total N to N fertilisation can be masked by enhanced N cycling of biota (i.e. plants and micro-organisms).

More sophisticated methods based on stable N isotopes that can help to quantitatively partition fertiliser-derived N from natural background N are widely used in science, but are probably beyond the scope of routine measurements. Incubation methods that determine the rates of N mineralisation (i.e. the conversion of organic N into mineral N by microbes) can also be used as an indicator of artificial N supply. However, incubation is also more time-consuming (a 56-day incubation period is a common standard). We consider that measuring KCl-extractable NH_4^+/NO_3^- (e.g. as in Blakemore et al. 1987) is a simple and appropriate indicator of both 'labile' N (i.e. highly environmentally mobile and accessible to biota) and anthropogenic N enrichment to assess N eutrophication in wetlands.

5.6 Roadmap to update the interim limits

In order to determine the best way to update the interim limits, we first assessed how many wetlands were represented in our data set and how many wetlands were represented by wetland class. We had 1,100 soil samples, representing some 300 wetland sites. This is less than the number of foliage samples discussed earlier; this is because typically the foliage of more than one species is collected on each plot. Some 'pseudo-turnover' in wetland site name was apparent; that is, what appeared to be the same wetland had different names. As not all samples had spatial coordinates recorded there was no way to easily verify this. Table 3 sets out the number of unique sites within each wetland class *where a site had at least three samples taken* as a minimum number to represent something of the natural variability within a wetland.

In the Interim Limits report, on progressing towards quantitative limits for wetlands, there were 15 marsh sites sampled. When we apply a minimum of three samples per wetland, we only have 10 marsh sites (and 12 bog sites) within the data set (Table 3). The data set contained 25 marshes (and 35 bogs) if no minimum number was specified.

Table 3. Number of wetlands that have at least three soil samples, by wetland class

Wetland class	Number of sites	Median number of plots per site
Swamp	47	4
Fen	19	4
Bog	12	11
Marsh	10	3
Saltmarsh	6	3
NA	5	4
Ephemeral	1	3
Marsh/swamp	1	38
Swamp/salt marsh	1	10
Young bog	1	3

Notes: The median number of plots is a per site-class combination. For example, there was one ephemeral wetland that had at least three samples, and, as indicated by the median (of one value), it had three samples. NA stands for no wetland class specified: there were five wetland sites where at least three soil cores had been undertaken where wetland class was not specified. We retained transitional classes where these were noted in the data (e.g. marsh/swamp).

As indicated by Table 3, marshes remain relatively poorly sampled, and we consider further sampling needs to be conducted before interim (or final) limits for nutrients can be set. A future sampling strategy at the national level should include:

- quantification of the national extent of wetlands by wetland class (preferably including smaller wetlands than those mapped under Freshwater Ecosystems of New Zealand (FENZ) or the Land Cover Database)
- quantification of the area of wetlands already sampled, proportionate to wetland class area, and by geographical region and inferred degree of degradation
- a power analysis to calculate the number of samples required
- stratified sampling (with the number of samples determined as above) by region and inferred degree of degradation to identify sites.

Such an approach might highlight where additional sample sites are needed in under-sampled regions, or under-sampled pristine (or under-sampled poor condition) wetlands, across all wetland classes.

6 Conclusions

Increased nutrients entering a wetland may show up in many forms, and no particular method will be a 'fail safe' measure of increased nutrients. This is amply demonstrated by our results. No plant species were good indicators of soil N alone, nor soil P alone. However, there were several plant species that exhibited 'weak' homeostasis and showed a good correlation with the N:P ratio in the soil, and therefore are likely to indicate important changes in the N:P ratio. Several additional species are suggested as being worthy of further monitoring as indicators of soil P, or soil N:P.

Soil and plant foliage nutrients are just two ways in which changes in incoming nutrients might be reflected in wetlands: plant community biomass, microbial volatilisation, and species turnover are other indicators. Therefore, we recommend employing several measures as indicators of nutrient enrichment.

6.1 Species-specific analysis

Three species were identified as having a reasonable correlation to soil nutrient status (specifically to the N:P ratio). This means they would be suitable as complementary measures of nutrient changes, along with monitoring of species composition and some proxy of biomass. No species were suitable for monitoring N or P alone, although some species are suggested for further investigation for P. The species suggested for monitoring the N:P ratio cover a range of wetland types, aside from marshes. Note that a shift in the N:P ratio will only be useful where only *one* of N or P changes in supply: if both change in supply, the N:P ratio is unlikely to be sensitive to change.

We suggest that where foliage is sampled in the future, dominant species continue to be tested. This is because some species excluded from our analyses because of insufficient replication might be good candidates, once they are sampled enough.

A potential limitation of our analysis is that these results are based on sampling different plots across a gradient of nutrient levels. However, the power to detect nutrient change within a wetland (and within plots) with foliage analyses, relies on shifts *within individuals* being detectable. We were unable to assess this, because we did not have the data to test repeated measures within the same individuals after a change in nutrient levels.

Foliar total N is unsuitable for monitoring change in wetland nutrient status, where single species are analysed (as here); nor did we find species worthy of further investigation. We make recommendations for targeted soil sampling of anthropogenic N as an alternative. Despite this, the N:P ratio has been successfully used to indicate N limitation in a New Zealand wetland (Burge et al. 2020), meaning the mere lack of a good indicator of soil total N does not mean it is not possible to diagnose nutrient vulnerability in New Zealand wetlands.

An alternative to the single-species analysis conducted here would be to test abundance weighted means of foliar N and P, and test the correlation between the weighted mean and soil N and P. The abundance weighted mean takes into account the average value of foliar N and P for each species in the plot, and then calculates a weighted mean for the plot, with the weighting for each species linked to its abundance in the plot (more abundant species have a higher 'weight'). Weighted foliar P reflected shifts in soil P (correlated with site age) in a terrestrial chronosequence in New Zealand (Richardson et al. 2004). There are two key benefits of the weighted mean approach: firstly, where a change in nutrients has caused plant composition to shift so much that the dominant species is no longer present in a plot, it will still be possible to see an effect, unlike single-species analysis; and secondly, once sufficient species in the New Zealand wetland flora have been sampled, there is no need to conduct foliage sampling unless circumstances require – such as where it is desirable to know whether the wetland community is principally limited by N, or P, or both, which can be derived from the N:P ratio (see above).

6.2 On deriving anthropogenic input from soils analysis

We have suggested quantifying mineral N where there is a need to monitor anthropogenic N. Fertiliser-derived N is usually in the form of soluble ions that are highly available to biota (ammonium and nitrate). These forms of mineral N change readily, making them suitable indicators for recent and rapid changes in nutrient inputs, for instance, after receiving pulses of runoff or subsurface drainage water. The flipside of this is that mineral N can vary across short temporal scales (e.g. pulsed inputs) and spatial scales (e.g. close to pathways of delivery). Repeated measurements are necessary to detect long-term changes in the levels of biota-available N or persistent anthropogenic input.

Therefore, mineral N sampling is relatively intensive in terms of effort, and would be best used in a targeted fashion where there are concerns about increasing nutrients, or to develop baselines for particular sites. Laboratory costs are currently approximately $31 per sample (excluding the base costs for drying and grinding soil samples). Sampling effort is not much higher than for typical soil sampling: refrigeration of samples after sampling is advised to minimise biological activity in the samples, which can alter soil N, and samples should be sent to the laboratory for analysis as soon as it is practical to do so.

6.3 Representation of wetland types

Data quality limits our ability to make inferences about where to sample further to increase the representativeness of the wetland data available. Key improvements would be for all samples to have spatial coordinates and a wetland class recorded (and as a 'transitional class', if this is more appropriate). If all spatial coordinates were recorded, then issues such as confusion over wetland name could be avoided by using spatial position.

We undertook initial efforts to classify wetlands that did not have a wetland class ascribed to them, using the FENZ database layers (Ausseil et al. 2011), but not all wetlands fell within the FENZ layers. Having a more precise map of wetland extent would allow analysis of how many wetlands have been sampled (total and by wetland type), which would allow for future targeted sampling to be carried out. These kinds of analyses require that any maps of wetland extent collected by local authorities would be available for national-scale analysis.

Although we did not attempt to rerun a previous analysis for setting baseline limits, it is worth noting that the number of marshes sampled has not increased greatly since the Interim Limits report. The previous report also indicated that marshes had the greatest spread for certain attributes such as soil nutrients – due to differences in landscape context. Most marshes were relatively nutrient rich and high in pH. However oligotrophic marshes are found in sand dune ecosystems, driving a larger, natural source of variability than other wetland classes. We suggest, therefore, that marshes be made a priority for data collection, where possible, and that some form of landscape context be recorded, such that marshes could be disaggregated into subcategories for a potentially more refined analysis. Substrate is one possibility for a supplementary data field.

7 Recommendations

No soil or foliage chemical analysis is a 'perfect' measure of nutrient inputs, particularly when sampled on a 5-yearly or longer basis. We do not recommend single species foliage sampling as a method to predict soil N or P, based on the current data. On this basis we make the following recommendations.

1 Undertake an analysis of the correlation between abundance weighted mean foliar N, P, and N:P; and soil N, P, and N:P, as an alternative method for indicating soil nutrient changes.

2 Conduct further fertilisation experiments across a range of wetlands to assess the utility of the N:P ratio as an indicator of wetland vulnerability to relative nutrient input change. A previous pilot study indicated that the N:P ratio in foliage nutrients is a useful indicator of which nutrient is limiting the vegetation community.

3 Carry out targeted sampling of the potential 'indicator species' where available to confirm utility, and also to demonstrate sensitivity to change over time, because current work assesses different individuals across a gradient, not sensitivity to change within individuals. These species are:

   a *Empodisma minus*

   b *Leptospermum scoparium*

   c *Schoenus pauciflorus*

   d *Typha orientalis*

   e *Machaerina rubiginosa*

   f *Coprosma tenuicualis*

   g *Gleichenia dicarpa*

   h *Chionochloa rubra*

   i *Carex diandra*

4 Carry out further work to identify indicator species for marshes, and further stratified sampling for the purpose of setting baselines to finalise the interim limits.

5 Adopt a tripartite approach to monitoring potential nutrient impacts, particularly where soil sampling is dropped, by: (a) ensuring a sufficient baseline of soil samples exists for a wetland; (b) including plot composition and estimated biomass in change detection, in addition to plant foliage nutrients; (c) monitoring identified indicator species (even if not dominant). This tripartite method may require methodological development for estimating biomass in wetlands. Consideration should also be given to monitoring forms of nutrients that reflect anthropogenic inputs, in addition to total N and total P, where concern exists about anthropogenically induced increases in nutrients.

6 Establish a set of national data quality standards for wetland monitoring to enable more accurate data syntheses. Specific opportunities for improving the consistency of data include wetland names/identifiers and wetland classes, including transitional classes.

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