

Water regimes, aquatic ecosystem metabolism and microbial diversity in floodplain wetlands

Final summary report on water regimes, aquatic ecosystem metabolism and microbial diversity in floodplain wetlands of the Macquarie Marshes.

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Executive Summary

Microbial communities provide vital metabolic processes in inland floodplain wetlands. Gross primary productivity (GPP) and planktonic respiration (PR) – together comprising aquatic ecosystem metabolism – fluctuate spatially and over time in these systems due to variations in inundation, nutrients, microbial diversity, and other environmental factors. The balance of GPP/PR determines whether an aquatic ecosystem exhibits net autotrophy (i.e. consumption of carbon) or net heterotrophy (i.e. production of carbon).

We studied patterns of aquatic ecosystem metabolism, soil carbon and nutrients, and microbial diversity in 50 soil samples from two wetlands in the Macquarie Marshes, New South Wales, Australia. Soil samples were collected from sites on transects with known inundation history and incubated in the laboratory to assess GPP and PR. Carbon, nutrients and geochemistry were determined by standard methods, and microbial diversity was assessed by Illumina sequencing of the bacterial 16S rRNA gene.

GPP and PR were highly variable, although both Horseshoe Lagoon and Willancorah Swamp were predominantly autotrophic with mean GPP/PR ratios of 2.1 ± 0.27 and 1.9 ± 0.27 , respectively. Comparison of GPP/PR patterns with inundation patterns allowed identification of three common trophic zones: 1) an autotrophic zone with low inundation frequency, 2) a heterotrophic zone with increasing inundation frequency, and 3) an autotrophic zone with high inundation frequency.

Actinobacteria and Proteobacteria were dominant relative to other phyla in the microbial community identified from the inundated samples, and principal component analysis demonstrated that the microbial communities in Horseshoe and Willancorah were distinct at the phylum level. Overall, Willancorah had greater microbial diversity than Horseshoe.

Further analysis of inundation regimes, microbial communities, aquatic metabolism, carbon dynamics and nutrient availability should be undertaken to further explain processes of carbon cycling and transformation in floodplain wetlands such as the Macquarie Marshes, and to understand the likely impacts of changes to hydrological, geomorphological and biogeochemical processes related to climate change.

1. Introduction

Floodplain wetland ecosystems respond dynamically to variations in hydrology, geomorphology and climate. At the base of aquatic food webs, microbial diversity and aquatic ecosystem metabolism (i.e. gross primary productivity [GPP] and planktonic respiration [PR]) determine rates of carbon production and use, and sustain biodiversity at higher trophic levels (Young et al. 2008). Autotrophic systems ($GPP/PR > 1$) produce more carbon through photosynthesis than they consume by respiration. In contrast, heterotrophic systems ($GPP/PR < 1$) consume more carbon through respiration than they produce by photosynthesis (Wetzel and Likens 1991). Therefore, carbon storage in wetland soils results from an imbalance in the provision and degradation of carbon *in situ* and contributed from other upstream sources. All of these factors are influenced by water availability in the form of inundation, although relationships between water regimes, soil carbon, microbial diversity, and aquatic metabolism remain unquantified in many rivers and wetlands.

Previous research has shown that a discontinuum of ecological patterns and processes related to hydrological, geomorphological and biogeochemical factors occurs in rivers and wetlands (e.g. Kobayashi et al. 2011). Furthermore, the short-term response of nutrients, carbon and planktonic microbial communities to inundation can be highly variable (Kobayashi et al. 2009). There is a pressing need to investigate and characterise the variability and relationships between hydrological conditions, geomorphological attributes, ecological processes and their abiotic controls in floodplain wetlands.

This project investigated water regimes, aquatic metabolism and microbial biodiversity in the Macquarie Marshes, a high conservation value aquatic ecosystem. Understanding the role of inundation and the links between aquatic metabolism, microbial diversity and carbon storage are critical for ecosystem and water management in light of predicted changes to climate and hydrology in the Murray-Darling Basin.

The aims of the research were to:

- 1) Quantify soil carbon, microbial diversity and aquatic metabolism across a gradient of inundation in floodplain wetlands of the Macquarie Marshes;
- 2) Identify links between inundation, aquatic metabolism, microbial diversity, and other environmental parameters (e.g. soil carbon and nutrients).
- 3) Develop a conceptual model of aquatic ecosystem response (incorporating microbial diversity and aquatic metabolism) to changing inundation regimes.

Our hypothesis was that microbial diversity, soil carbon and nutrients, and aquatic primary production and respiration would vary in the wetlands due to their recent historical inundation (i.e.

water regime). Broadly, we expected to find more carbon and nutrients in the regularly inundated zone, and higher microbial diversity, primary productivity and respiration in the intermediate zone between zones of low and high inundation frequency. We examined microbial diversity with molecular-based techniques, and measured carbon, nutrients and geochemistry in wetland soils at two wetlands in the Macquarie Marshes, and compared these with measurements of GPP and PR from mesocosm inundation experiments for the same sites.

2. Regional setting

The Macquarie River in central New South Wales drains an area of ~26,000 km² upstream of Narromine. Downstream of Narromine, the river debouches onto a semiarid alluvial floodplain-fan in the lower reaches to form the Macquarie Marshes (Figure 1). The Macquarie Marshes are the largest freshwater wetlands in the Murray-Darling Basin, extending for a distance of ~120 km between Warren and Carinda and spreading up to 30 km wide. The core wetlands in the southern, northern and eastern parts of the system are comprised of extensive reed beds, grass plains, woodlands and forests that rely on overbank and overland flooding from many small anastomosing and distributary channels (Ralph and Hesse 2010). Mean annual rainfall in the region of the Macquarie Marshes is ~500 mm, while mean annual evaporation is ~2000 mm. Therefore, the wetlands have a significant net deficit rainfall balance and are sustained by flooding from the upstream catchment. Discharge in the Macquarie River and inundation in the Macquarie Marshes are seasonally and annually variable, and are affected by interannual and interdecadal climatic trends, related to ENSO and IPO (Ralph and Hesse, 2010). Floodplain connection and inundation is greatest where channels become small and shallow, allowing water to flood out onto adjacent alluvial surfaces forming wetlands with a range of aquatic habitats determined by hydrogeomorphic and biogeochemical attributes.

3. Sites and methods

3.1. Wetlands and soil sampling

Two wetlands in the Southern Macquarie Marshes were selected on the basis of their habitat and vegetation types. Horseshoe Lagoon is situated in Buckiinguy Swamp at the end of Buckiinguy Creek, and has an open, sparsely vegetated lagoon surrounded by a zone of moderately vegetated mixed marsh, and then a dry and sparsely vegetated floodplain (Figures 2a). Willancorah Swamp is located in the terminal reach of Monkeygar Creek and is characterised by a densely vegetated Phragmites reed bed, surrounded by a zone of densely vegetated mixed marsh, and then a dry and sparsely vegetated floodplain (Figure 2b).

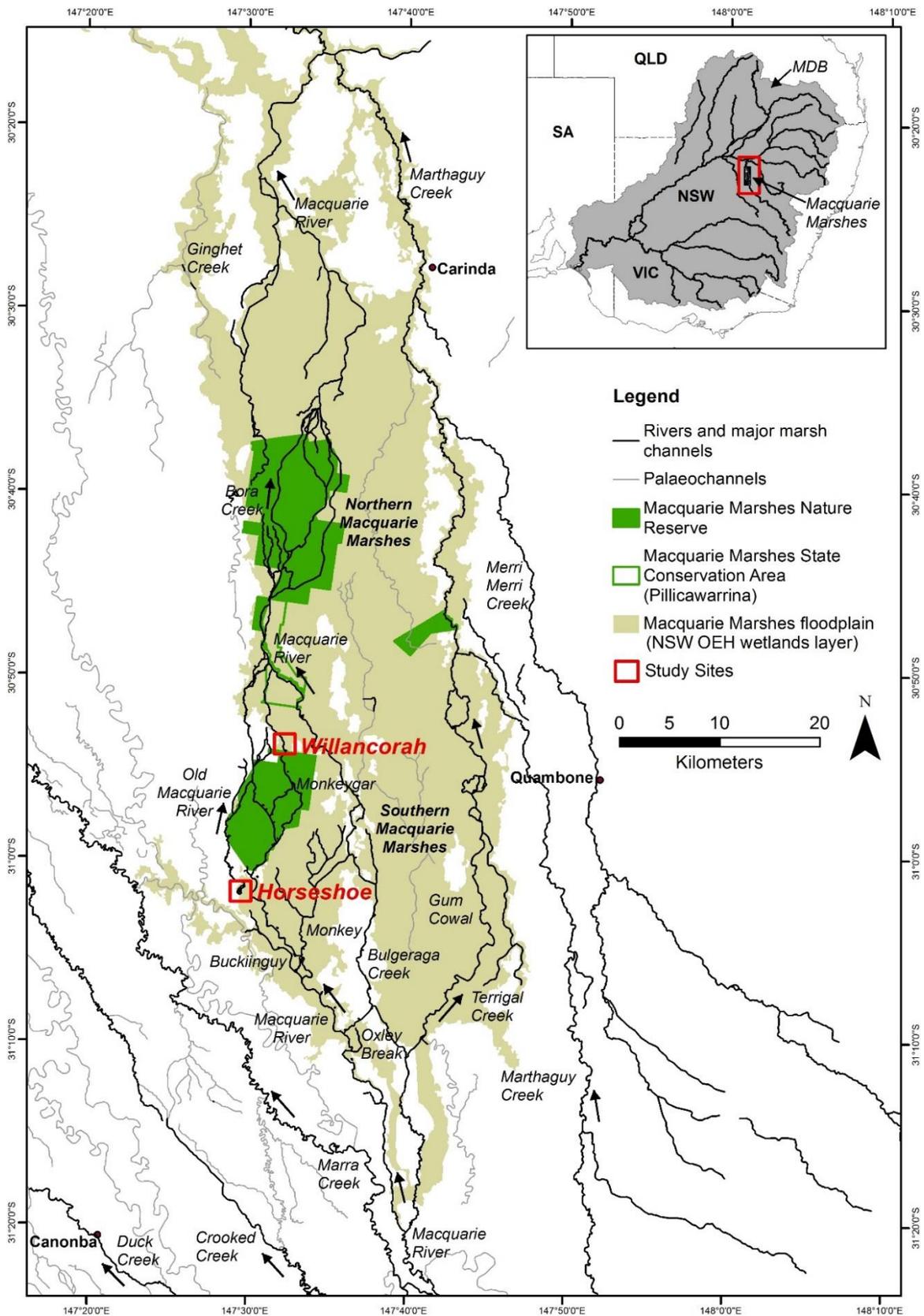


Figure 1. Location of the Macquarie River and Macquarie Marshes in the Murray-Darling Basin (inset map) and sites Horseshoe Lagoon and Willancorah Swamp in the Macquarie Marshes (main map).

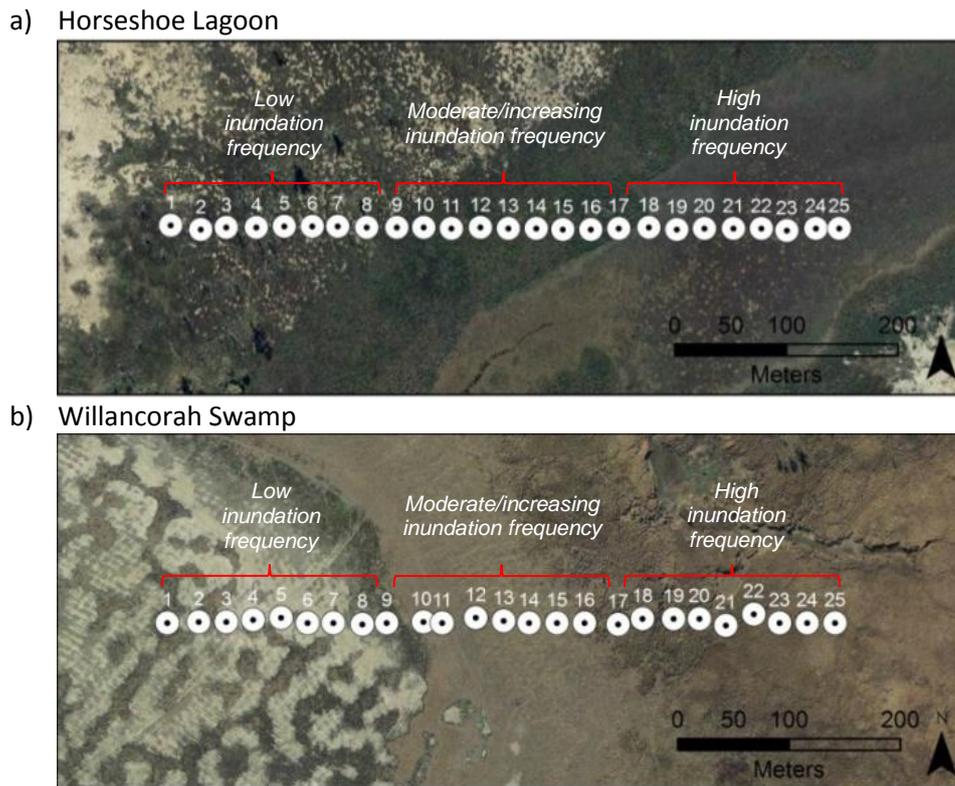


Figure 2. Aerial photographs showing vegetation communities for a) Horseshoe Lagoon and b) Willancorah Swamp. The patterns of vegetation reflect wetland inundation history, with consistently low inundation frequency from 0-175 m (sites 1-8), increasing inundation frequency from 200-375 m (sites 9-16), and consistently high inundation frequency from 400-600 m (sites 17-25).

The transition from sparsely vegetated floodplain with infrequent inundation to densely vegetated wetland with frequent inundation occurs over a distance of ~ 300 m on the western margins of both Horseshoe Lagoon and Willancorah Swamp (Figure 2a and 2b). Therefore, wetland soils were sampled across 600 m long transects following the gradient of inundation in each of the wetlands (sampling occurred between 27 February and 3 March 2015). The inundation frequency at the sites ranged from zero inundation (0 spring floods in 28 years), to annual inundation (28 spring floods in 28 years; for the period 1979-2006, see Thomas et al. 2011).

Nine soil cores, each 10 cm deep, were taken from within a series of consecutive 25x25 m cells on each wetland transect, and these nine samples were combined to form one bulk sample for analysis to account for variability within each cell ($n=25$ bulk samples for each wetland transect). Field observations were made of vegetation communities at each sampling point. Soil samples were transferred to the laboratory where mesocosm inundation experiments were established to assess GPP of phytoplankton and PR under glasshouse conditions. Representative sub-samples were extracted for geochemical analysis, carbon and nutrient measurements, and microbial DNA analysis.

3.2. Geochemistry

Soil samples were sent to the Department of Primary Industries Diagnostic and Analytical Services Environmental Laboratory at Wollongbar, New South Wales, for geochemistry analysis. All major elements including total phosphorus (TP) were determined by inductively coupled plasma atomic emission spectroscopy (ICP-AES), while total carbon (TC) and total nitrogen (TN) were determined by the Dumas combustion method.

3.3. Gross primary productivity of phytoplankton and planktonic respiration

GPP of phytoplankton ($\mu\text{g C L}^{-1} \text{h}^{-1}$) and PR ($\mu\text{g C L}^{-1} \text{h}^{-1}$) were estimated from dissolved oxygen (DO) concentrations in biological oxygen demand (BOD) bottles (300 mL volume) measured *in situ* at the beginning and the end of light and dark bottle mesocosm incubation experiments (Wetzel and Likens 1991). DO (mg L^{-1}) and water temperature ($^{\circ}\text{C}$) were measured using a YSI Model 5100 Dissolved Oxygen/Temperature Meter (YSI Inc., USA). A single light and dark bottle were placed in each mesocosm ($n=25$ pairs of BOD bottles for each wetland). Each bottle was rinsed and filled with sample water without air bubbles (Wetzel and Likens 1991) and incubated for ~ 24 hours. GPP and PR were estimated for each sample based on the formulae in Wetzel and Likens (1991, page 210).

3.4. Microbial diversity profiling

Subsamples of wetland soils were sent to the Australian Genome Research Facility (AGRF) for nucleic acid extraction and diversity profiling using Illumina sequencing of the 16S rRNA gene with universal prokaryote primers (341Forward-806Reverse).

3.5. Richness and diversity

Richness for all phyla and species in each bulk sample were calculated as the total number of OTUs at the phylum or the species level. Microbial diversity was calculated as the inverse of Simpson's D index using the standardised formula (Levins 1968; Maurer and McGill 2011).

3.6. Statistical analyses

Ordinary least-squares regression and ANOVA analyses were performed using standard methods. Principal component analysis (PCA) with a covariance bi-plot was used to examine the similarities or dissimilarities between Horseshoe Lagoon and Willancorah Swamp based on the covariance matrices for the proportions of microbial OTUs at the phylum level. Prior to PCA analysis, all proportional data were transformed by arcsine square-root (Fox and Weisberg 2011).

4. Results

4.1. Wetland inundation

Long-term inundation frequency for the period 1979 to 2006 (28 years) varied from zero to 26 floods at Horseshoe Lagoon and from zero to 28 floods at Willancorah Swamp (data from Thomas et al. 2011). The vegetation communities at both sites (see Figure 2) reflect a general trend of consistently low inundation frequency at sites from 0-175 m, increasing inundation frequency at sites from 200-375 m, and consistently high inundation frequency at sites from 400-600 m along each of the wetland transects.

4.2. Soil carbon and nutrients

TC in soil was variable along each of the wetland transects, but gradually increased with distance into each wetland as inundation frequency increased (Figure 3a and 3b). TN also increased with distance into the wetlands (Figure 3c and 3d) and the more densely vegetated sections of Willancorah from 250-600 m on the transect had the highest and most variable TC and TN content overall. TP was consistently low and less variable than TC or TN in both wetlands (Figure 3e and 3f). Significant positive linear relationships occur for TC and TN in both wetlands ($R^2=0.99$ and $p<0.0001$ for Horseshoe, $R^2=0.97$ and $p<0.0001$ for Willancorah; Figure 4).

4.3. Other major elements

Major elements in soil including aluminium (Al), copper (Cu), iron (Fe), sulfur (S) and sodium (Na) were variable along each of the wetland transects. In general, however, Al, Cu, Fe and S increased with distance into each wetland (Figure 5a, 5b, 5c, 5d, 5e, 5f, 5g and 5h) while Na decreased into each wetland (Figure 5i and 5j). As with TC and TN, the more densely vegetated sections of Willancorah from 400-600 m on the transect had the highest and most variable element concentrations of all the sites.

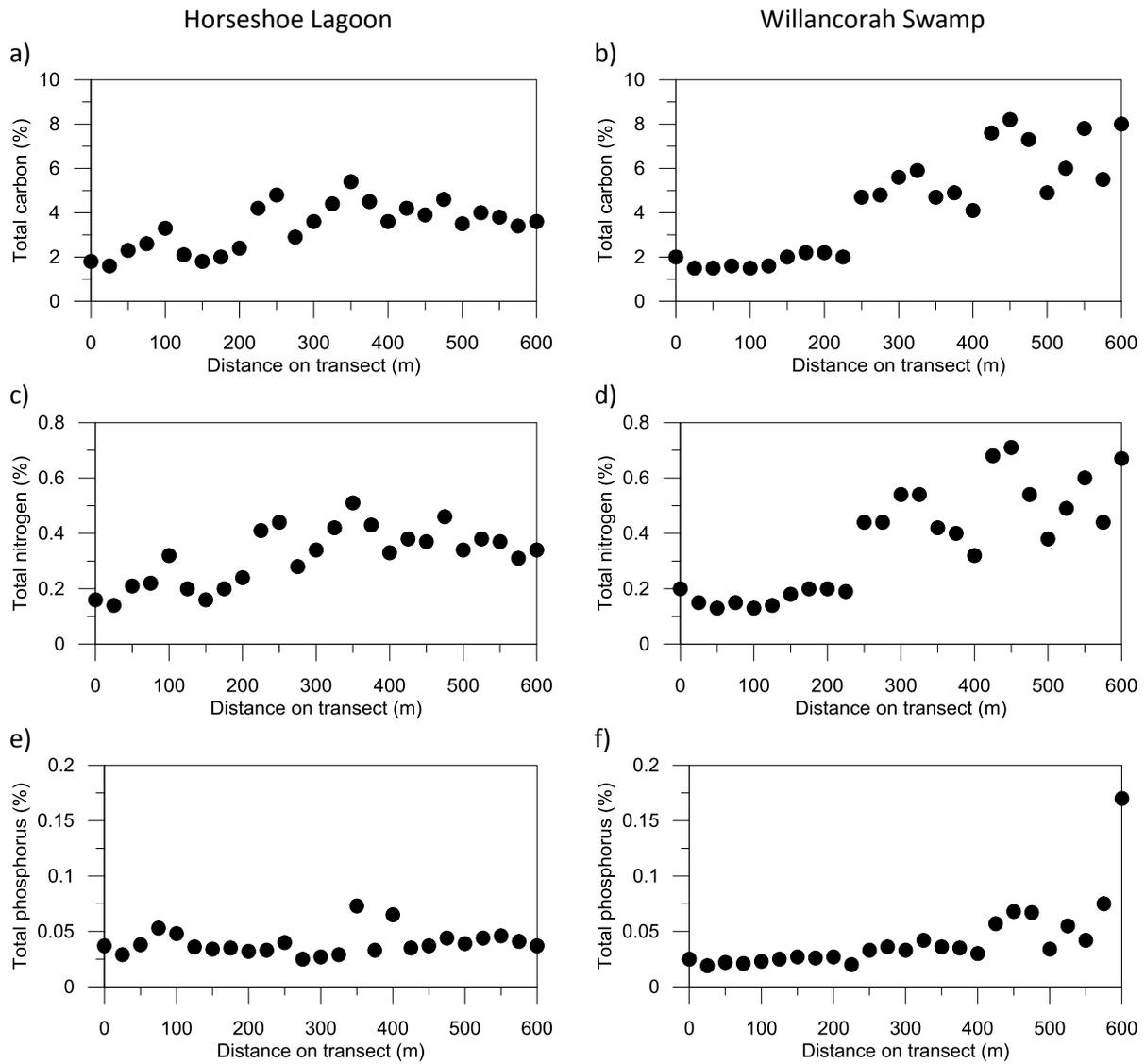


Figure 3. TC, TN and TP in soils from Horseshoe Lagoon and Willancorah Swamp.

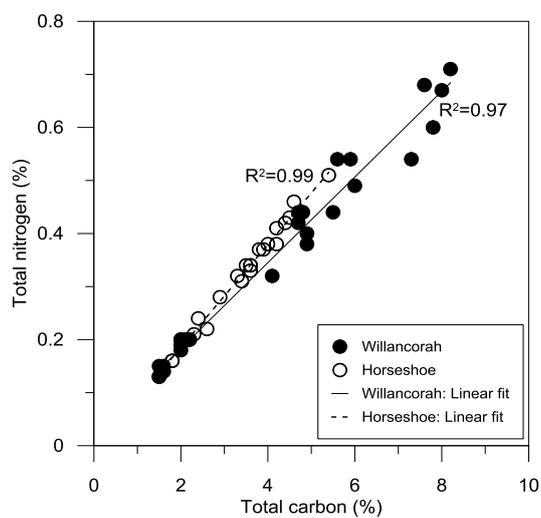


Figure 4. Linear regressions for TN and TP in soils from Horseshoe Lagoon and Willancorah Swamp.

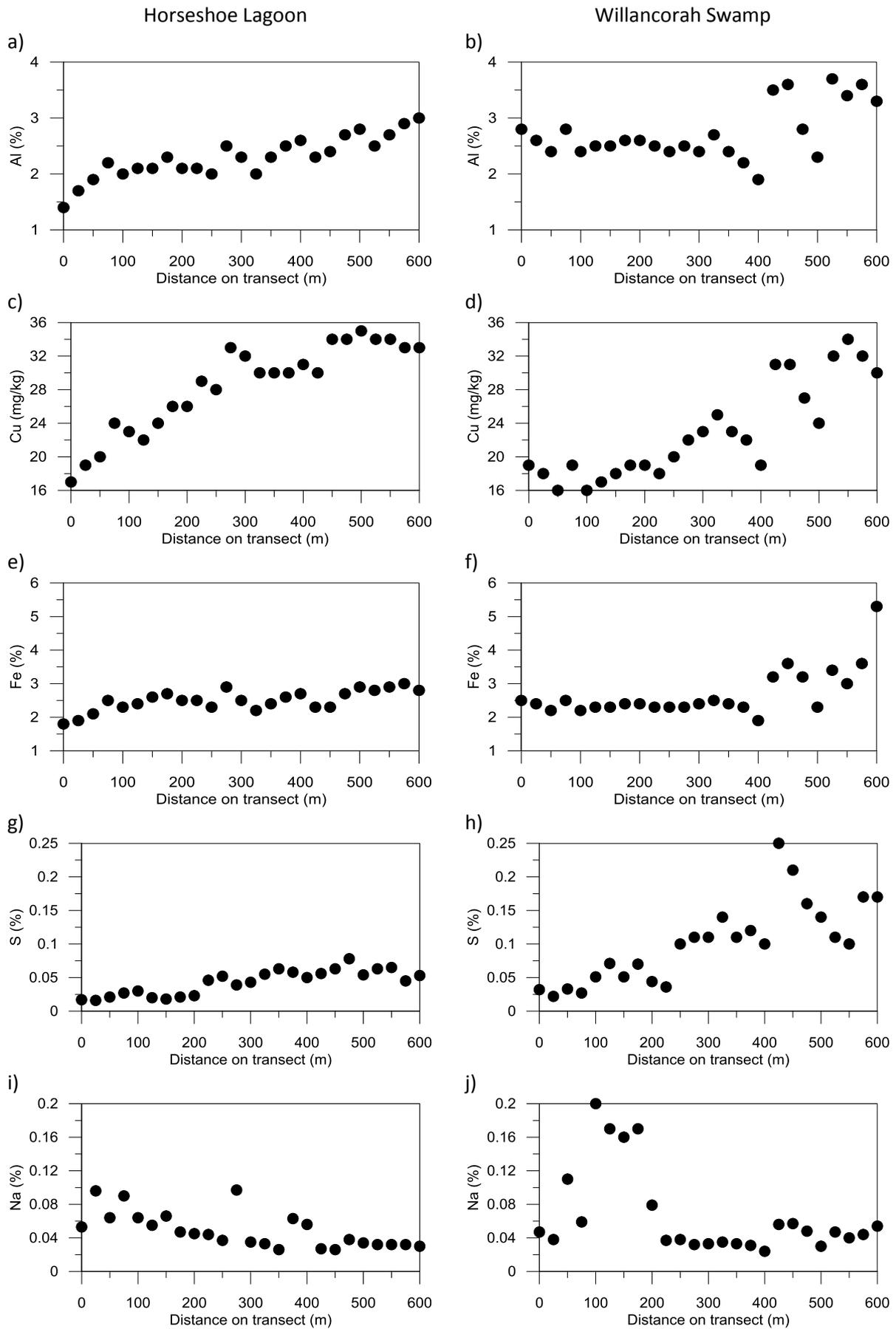


Figure 5. Key elements in soils from Horseshoe Lagoon and Willancorah Swamp.

4.4. Aquatic ecosystem metabolism

GPP was highly variable in mesocosm inundation experiments using samples from each of the wetland transects, ranging from 10.4-108.7 $\mu\text{g C L}^{-1} \text{h}^{-1}$ at Horseshoe (Figure 6a) and from 1.2-53.8 $\mu\text{g C L}^{-1} \text{h}^{-1}$ at Willancorah (Figure 6b). PR was also highly variable, ranging from 6.9-62.5 $\mu\text{g C L}^{-1} \text{h}^{-1}$ at Horseshoe (Figure 6c) and from 3.8-42.9 $\mu\text{g C L}^{-1} \text{h}^{-1}$ at Willancorah (Figure 6d). PR was slightly higher from 200-400 m on each transect, in the areas with increasing inundation frequency between the low and high frequency inundation sections at either end of the transects. Consequently, both wetlands had lower GPP/PR in the central parts of each transect, where GPP/PR was closer to, or below, 1 (Figure 6e and 6f).

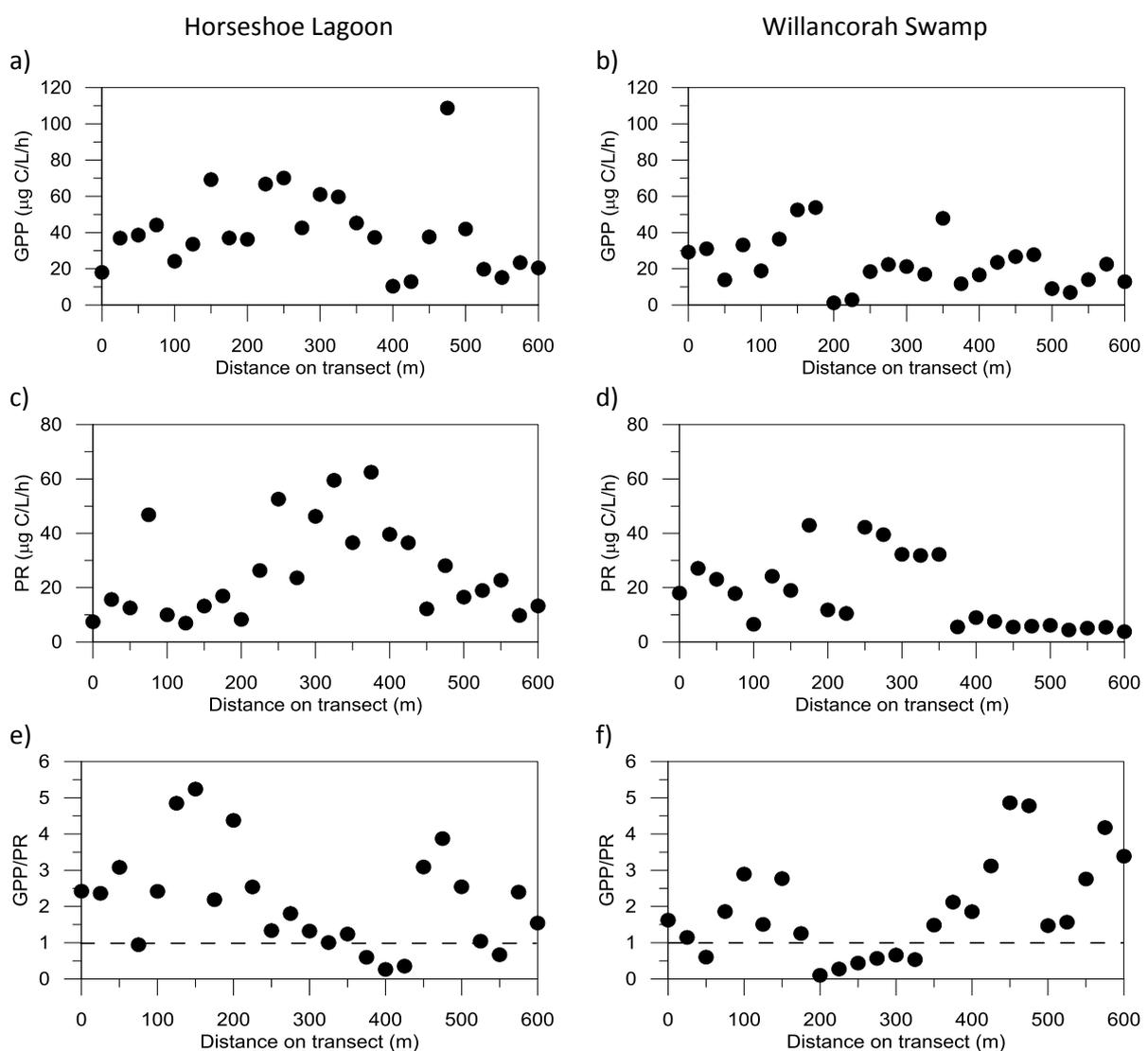


Figure 6. GPP, PR and GPP/PR from mesocosm inundation experiments using soil samples from Horseshoe Lagoon and Willancorah Swamp. The dashed horizontal lines in e) and f) indicate the autotrophic:heterotrophic threshold of 1 (i.e. GPP/PR=1).

Mean GPP and PR (\pm standard error) were higher at Horseshoe (GPP $40.4 \pm 4.5 \mu\text{g C L}^{-1} \text{ h}^{-1}$; PR $25.7 \pm 3.4 \mu\text{g C L}^{-1} \text{ h}^{-1}$) than at Willancorah (GPP $22.8 \pm 2.8 \mu\text{g C L}^{-1} \text{ h}^{-1}$; PR $17.4 \pm 2.6 \mu\text{g C L}^{-1} \text{ h}^{-1}$). However, Horseshoe and Willancorah have mean GPP/PR ratios of 2.1 ± 0.27 and 1.9 ± 0.27 , respectively, making the balance of GPP and PR in both wetlands very similar. Both wetlands are predominantly autotrophic (GPP/PR >1). Just five samples at Horseshoe and seven samples at Willancorah fall below the autotrophic: heterotrophic threshold of 1 (Figure 7a). Comparison of all the GPP/PR results with distance across the floodplain shows that three common trophic zones can be identified related to inundation regime: 1) an autotrophy dominated zone with low inundation frequency (zone 1, from 0-175 m on both transects), 2) a heterotrophy dominated zone with increasing inundation frequency (zone 2, from 200-375 m on both transects), and 3) an autotrophy dominated zone with high inundation frequency (zone 3, from 400-600 m on both transects; Figure 8b). One way ANOVA showed that the three zones are significantly different ($F=4.062$, $p=0.02$) and Tukey's pairwise comparison showed that the greatest difference existed for zones 2 and 3 ($p=0.03$).

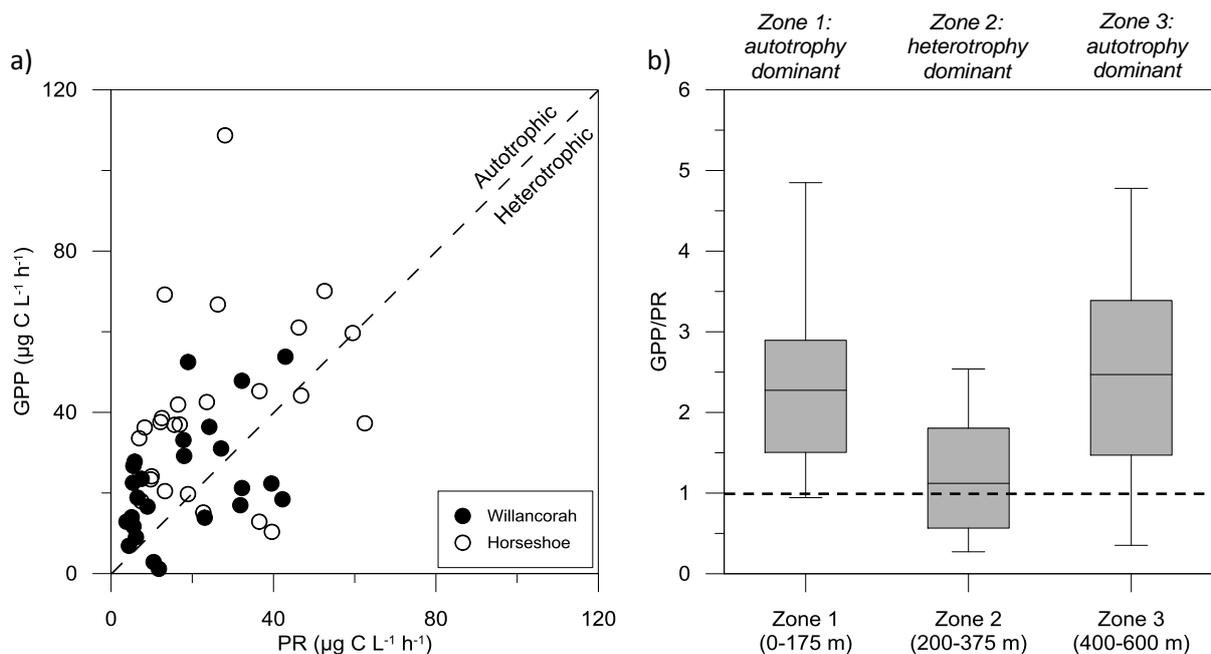


Figure 7. a) Relationship between GPP and PR for samples from Horseshoe Lagoon and Willancorah Swamp (dashed diagonal line indicates the autotrophic: heterotrophic threshold of 1), and b) comparison of GPP/PR for all samples across three common trophic zones (horizontal lines within the boxes indicate the mean values, whiskers indicate the 10th and 90th percentiles, and the dashed horizontal line indicates the GPP/PR threshold of 1).

4.5. Microbial diversity

Results from 16S rRNA genetic sequencing show that Horseshoe Lagoon is dominated by Actinobacteria while Willancorah has a higher occurrence of Proteobacteria followed by

Actinobacteria (Figure 8). Firmicutes, Chloroflexi, Acidobacteria, Gemmatimonadetes and Bacteroidetes have a higher occurrence in Willancorah than in Horseshoe, while the remainder of phyla detected had a limited occurrence. PCA shows that the microbial communities in Horseshoe and Willancorah are distinct (Figure 9). In total, 69 phyla were identified (see Appendix A1 and A2), made up of 1343 identifiable species.

Although microbial phylum richness (Figure 10a and 10b) and diversity (Figure 10c and 10d) were variable across both wetlands, there was a general trend of increasing phylum richness and diversity with distance into Horseshoe, and greater phylum richness and diversity in the middle of the transect at Willancorah. Overall, Willancorah had a greater range of microbial phyla than Horseshoe (Figure 9). Similarly, microbial species richness (Figure 10e and 10f) and diversity (Figure 10g and 10h) were variable in the wetlands, but there was a trend of increasing species richness and diversity with increasing inundation in Horseshoe, and less diversity in Horseshoe overall, while species richness and diversity were greater in the intermediate zone between the low and high inundation frequency zones in Willancorah.

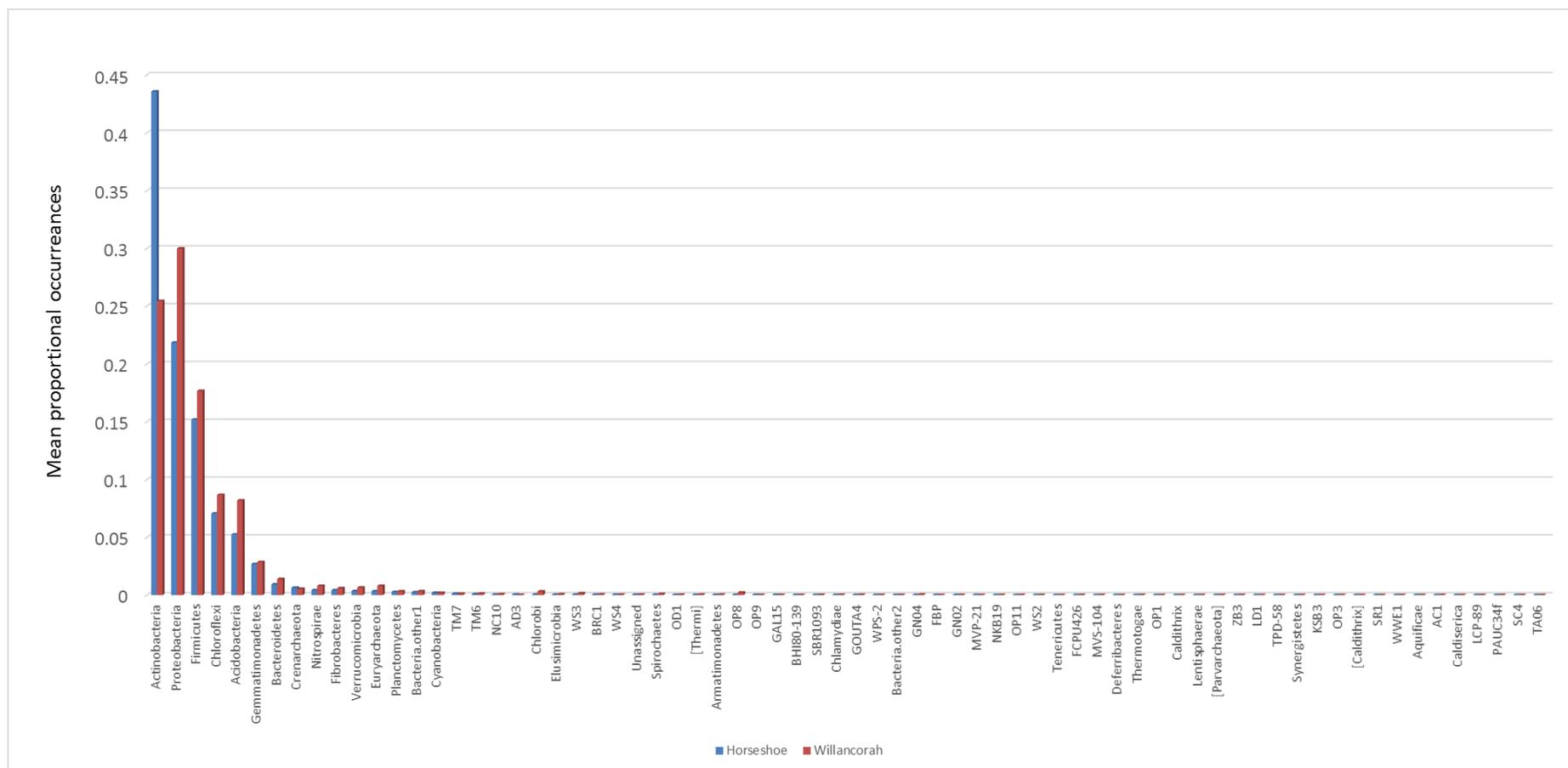


Figure 8. Mean proportional occurrence of microbial phylum in soils from Horseshoe Lagoon and Willancorah Swamp.

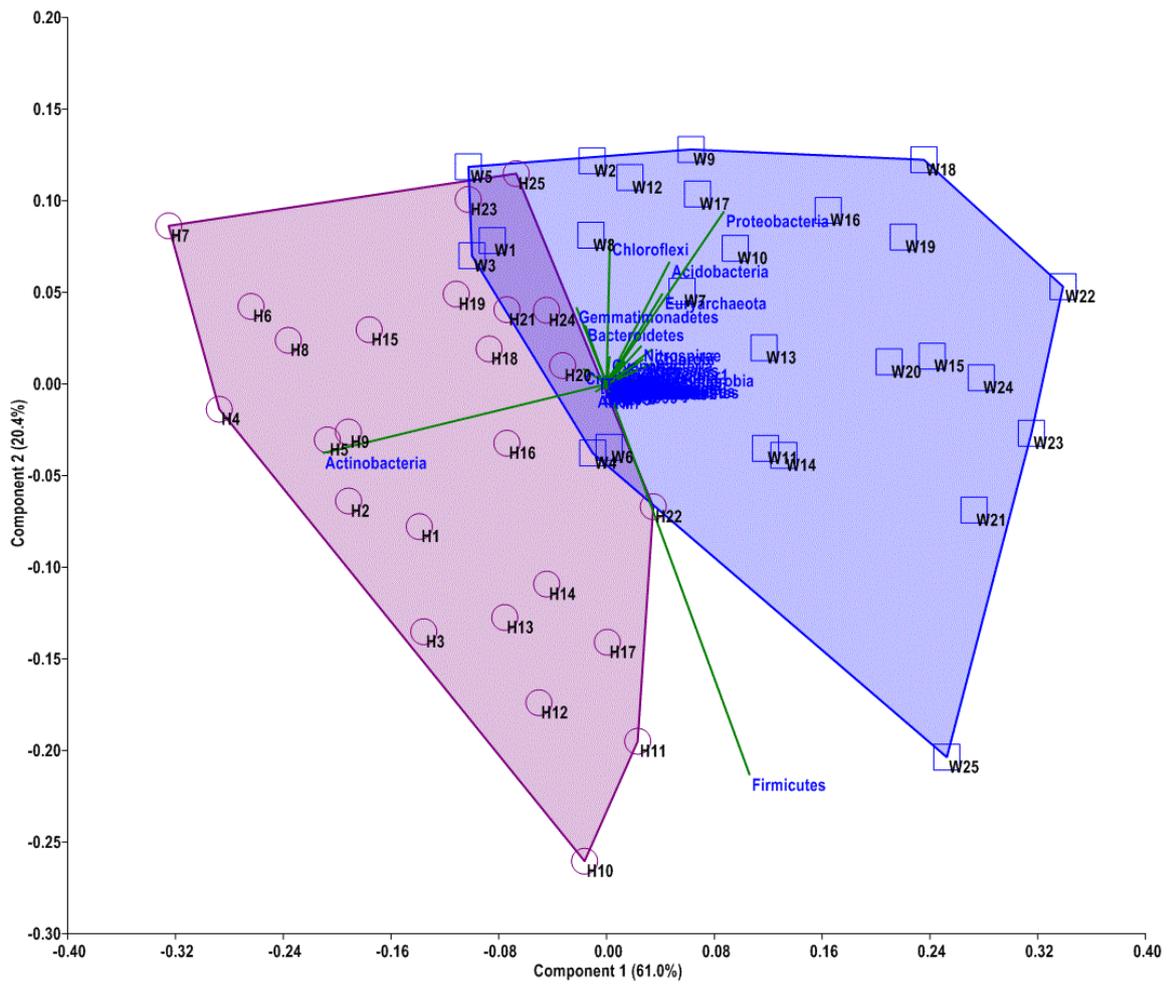


Figure 9. PCA of microbial phylum in soils from in Horseshoe Lagoon and Willancorah Swamp.

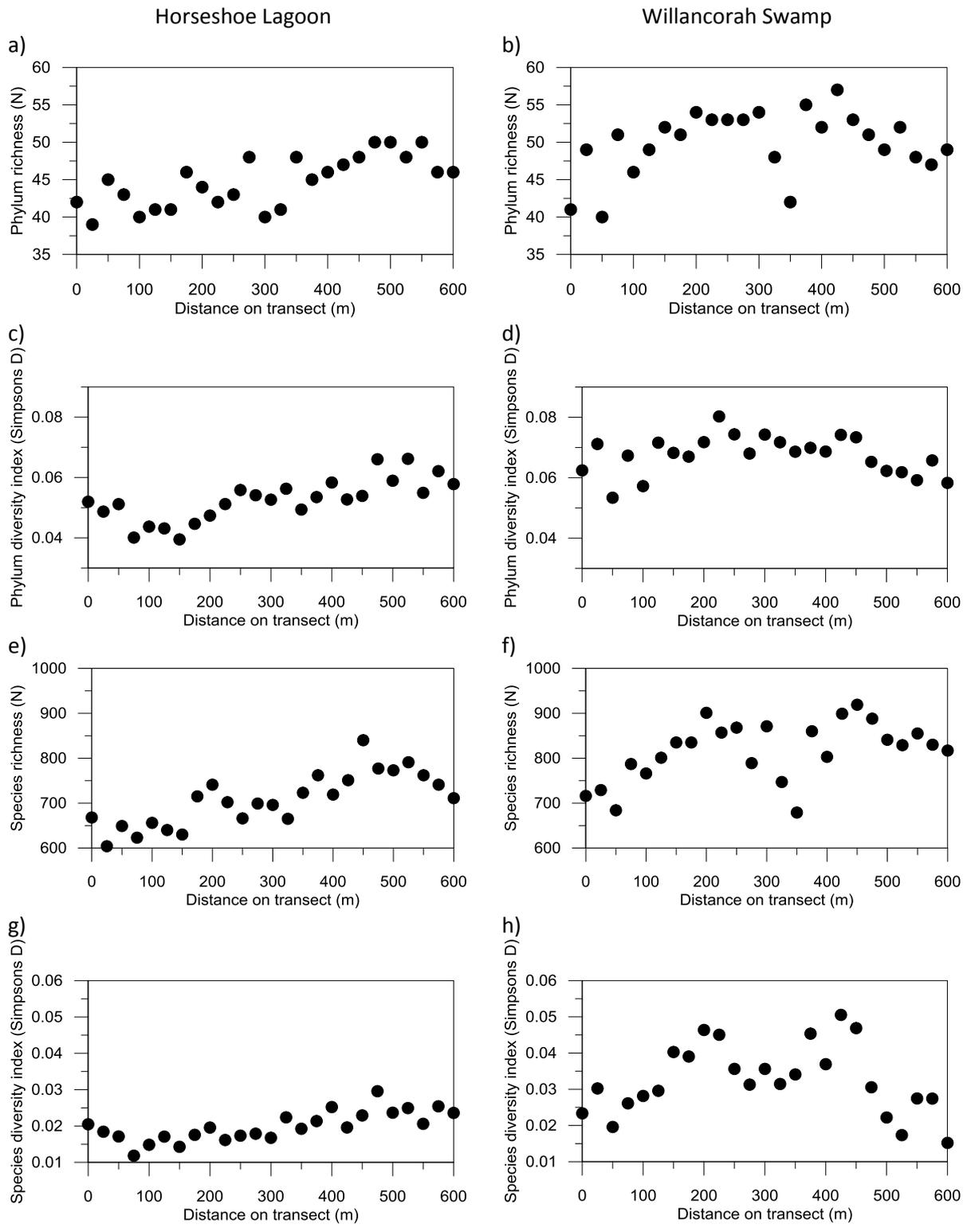


Figure 10. Microbial phylum richness and diversity, and species richness and diversity in soils from Horseshoe Lagoon and Willancorah Swamp.

4.6. Relationships between soil carbon, aquatic ecosystem metabolism, and microbial diversity

A significant positive linear relationship occurs between TC and phylum diversity at Horseshoe ($R^2=0.31$, $p=0.004$), but not at Willancorah (Figure 11a). A significant negative relationship occurs between TC and GPP/PR at Horseshoe ($R^2=0.26$, $p=0.009$) and a significant positive relationship occurs for TC and GPP/PR at Willancorah ($R^2=0.28$, $p=0.006$) (Figure 11b). No significant relationships occur between GPP/PR and phylum diversity at Horseshoe or Willancorah (Figure 11c). As expected, strong positive relationships occurred between phylum diversity and species diversity at Horseshoe ($R^2=0.77$, $p<0.0001$) and at Willancorah ($R^2=0.58$, $p<0.0001$) (Figure 11d).

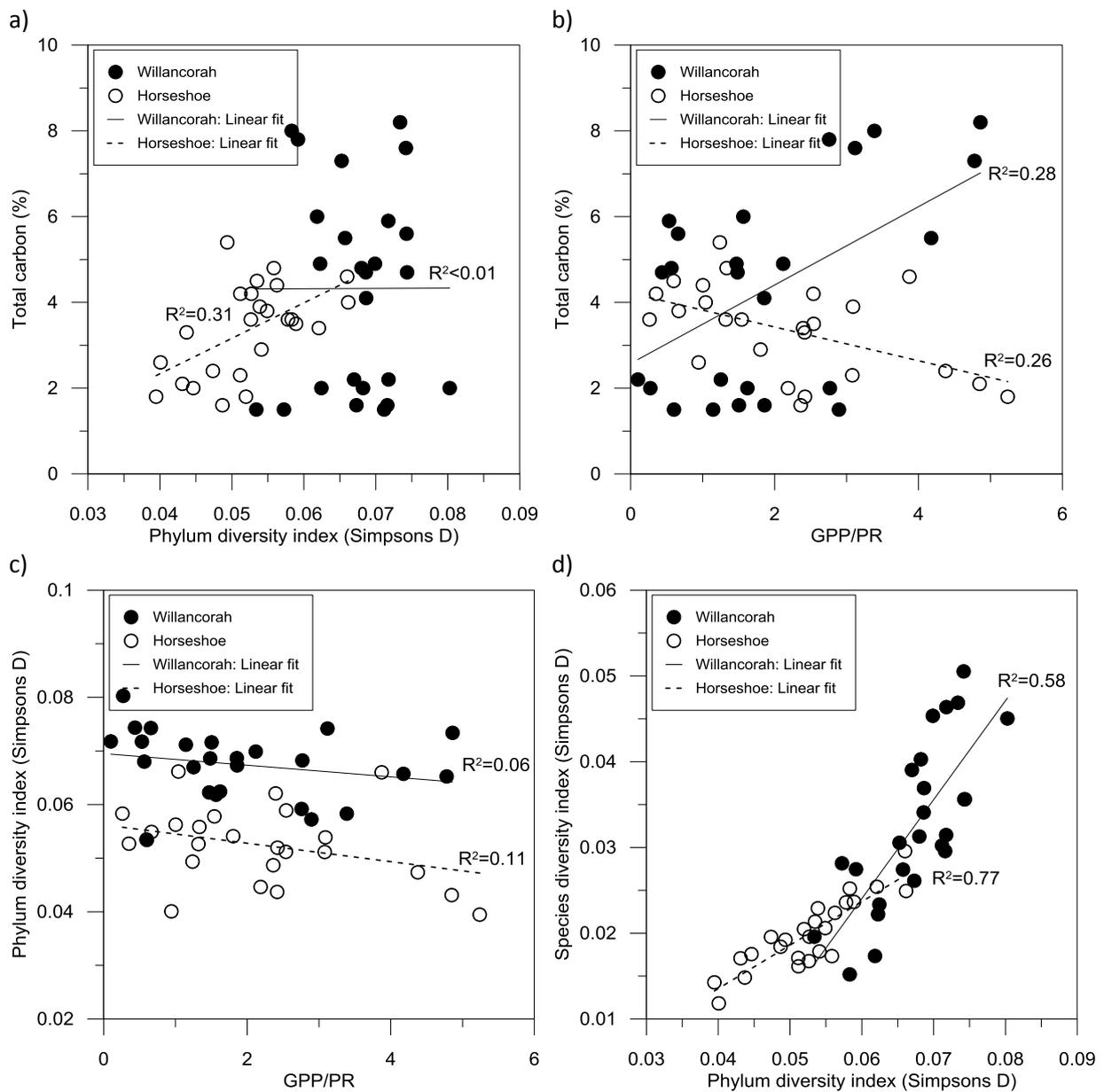


Figure 11. Linear regressions for total carbon, microbial phylum diversity, GPP/PR, and species diversity at Horseshoe Lagoon and Willancorah Swamp.

5. Discussion and conclusions

5.1. Patterns and thresholds of aquatic metabolism, microbial diversity and soil carbon

Microbial communities contribute to vital metabolic processes in floodplain wetlands. Our findings show that aquatic metabolism, microbial diversity and soil carbon in two wetlands of the Macquarie Marshes vary in response to differences in habitat and inundation, as well as nutrients and other environmental factors. Although GPP and PR were highly variable, both Horseshoe and Willancorah were predominantly autotrophic, with mean GPP/PR ratios of 2.1 ± 0.27 and 1.9 ± 0.27 , respectively. Therefore the balance of GPP/PR shows that these ecosystems are dominated by consumption of carbon, rather than production of carbon. Comparison of GPP/PR patterns with inundation frequency allowed identification of three trophic zones that were common to both wetlands: 1) an autotrophic zone with low inundation frequency, 2) a heterotrophic zone with increasing inundation frequency, and 3) an autotrophic zone with high inundation frequency. Distinct thresholds occurred between the zones, as GPP/PR shifted from above to below the autotrophic: heterotrophic ratio of 1. Significant positive relationships were found between soil carbon and nitrogen in both wetlands, although only Horseshoe had significant relationships between carbon, nitrogen, GPP/PR, inundation and microbial diversity at phylum and species levels. Of the microbial community identified, Actinobacteria and Proteobacteria were dominant in both wetlands, and PCA demonstrated that the microbial communities in Horseshoe and Willancorah were distinct at the phylum level. Microbial phylum and species richness and diversity were variable in the wetlands, but there were general trends of increasing richness and diversity with increasing inundation in Horseshoe, and greater richness and diversity between the low and high inundation zones at Willancorah. Overall, Willancorah Swamp had greater microbial diversity than Horseshoe Lagoon.

Our hypothesis was that microbial diversity, GPP/PR and soil carbon and nutrients would vary proportionally in the wetlands due to inherited traits related to their water regimes. We sampled across a gradient of habitats and inundation and we expected to find more carbon and nutrients in regularly inundated zones, and higher microbial diversity and GPP/PR in the intermediate zone between zones of low and high inundation frequency. However our results challenge these simple assumptions, since neither of the wetlands showed the same spatial patterns or relationships between all key measures.

Previous research has shown that during inundation, the ecological patterns and processes of channels and floodplains are complex, and that channels can influence floodplains and wetlands by providing inputs of dissolved inorganic and organic matter, and of aquatic organisms (Jenkins and Boulton, 2003). We did not consider channel contributions in our study, instead assuming that

inundation affecting the soil character and microbial communities was likely unidirectional and inundation frequency declined away from the centre of each wetland. This limitation could be addressed by future research that incorporates specific short-term inundation data. Furthermore, knowledge of metabolic process rates (e.g. nutrient uptake and dissolved organic matter release by autotrophic plankton, and rates of grazing and nutrient release by heterotrophic plankton) in rivers and wetlands in the Macquarie Marshes would improve our understanding of ecosystem patterns and processes during inundation (see Kobayashi et al. 2011). Overall, ecological changes in wetlands due to flow changes and regulation have been well documented (e.g. Poff et al. 1997; Bunn and Arthington 2002; Rood et al. 2005), as have those related to other anthropogenic modifications (e.g. Kingsford 2000; Steinfeld and Kingsford 2013), but considerable further research is required before we can understand the likely impacts of changes to hydrological, geomorphological and biogeochemical processes related to climate change.

5.2. Conceptual model

One of our aims was to assess trends and thresholds of change for aquatic ecosystem response (i.e. metabolism and microbial diversity) in the context of changing inundation regimes. To this end, we propose a simple conceptual model of aquatic ecosystem response to changing inundation frequency based on our main findings. The conceptual model outlines how key components of the aquatic ecosystem may change should inundation regimes in the floodplain wetlands decline (Figure 12). With a hypothetical decline in inundation of ~25%, aquatic metabolism and microbial diversity would be slightly suppressed across the wetland, and carbon and nitrogen in soils would decline over time (variations to this trend and lags associated with a declining hydrological regime would also occur over time). With a hypothetical decline in inundation of ~50%, aquatic metabolism and microbial diversity would be more greatly suppressed across the wetland, and carbon and nitrogen in soils would decline further. The overall impact of a reduction in inundation would likely be a loss of microbial diversity, more erratic aquatic metabolism associated with boom and bust cycles of flooding, and a general reduction in soil carbon, available nutrients and habitat heterogeneity.

Due to the limitations of this study, however, we restrict our conceptual model to general reductions in inundation until further site- and system-specific data is available. Therefore the likely impacts of climate change on flood inundation are not specified in the conceptual model, nor are the possible effects of other facets of climate change such as increased CO₂ and temperature.

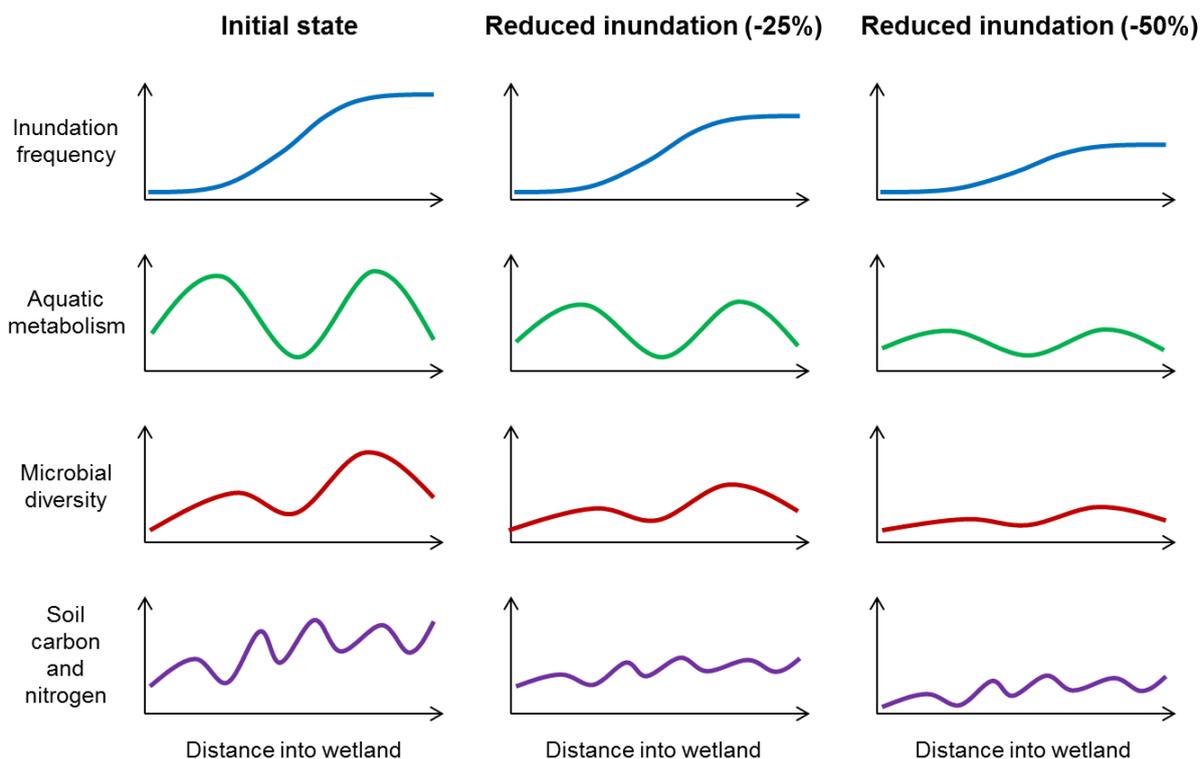


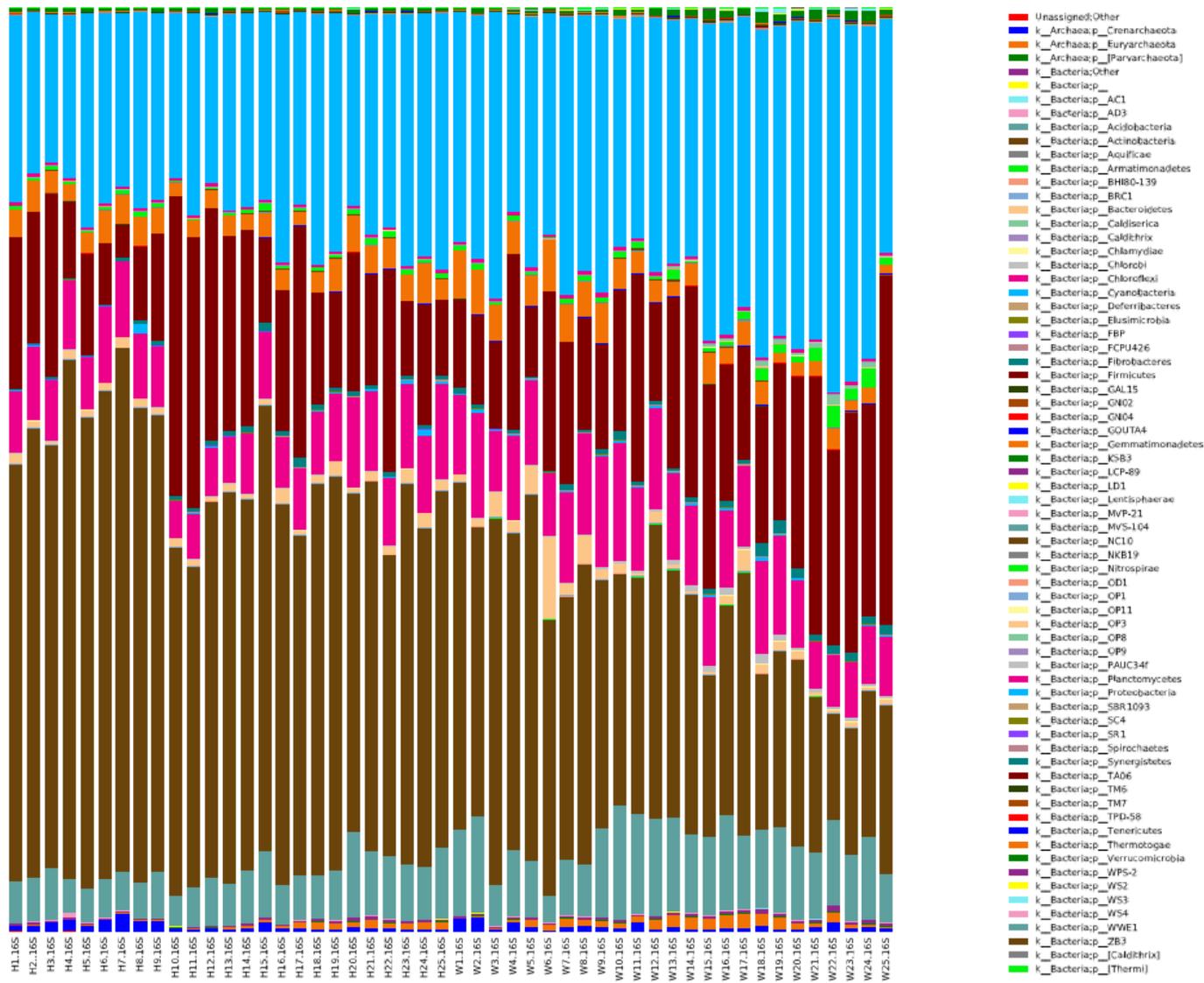
Figure 12. Conceptual model showing potential responses of aquatic metabolism, microbial diversity and soil carbon and nitrogen to declining wetland inundation frequency.

5.3. Conclusions

The large variations in aquatic metabolism and microbial diversity discovered during this research and the different relationships between key environmental parameters mean that our findings cannot easily be extrapolated across the Macquarie Marshes or to other wetland systems at this stage. Every wetland system is likely to have its own balance of aquatic metabolism and microbial diversity related to site-specific inundation regimes. Therefore further analysis of microbial communities, aquatic metabolism, inundation response, carbon, nutrients and other elements should be undertaken to explain processes of carbon cycling and transformation in floodplain wetlands such as the Macquarie Marshes, and to understand the likely impacts of changes to hydrological, geomorphological and biogeochemical processes related to climate change.

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Appendix A2. Microbial diversity at the phylum level (16S, 341F-806R) in soil samples from the Macquarie Marshes.