Soil Carbon and Nitrogen Dynamics in the Riparian Zones of Wyaralong Dam in Southeast Queensland, Australia

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When in eternal lines to time thou grow’st,
So long as men can breathe, or eyes can see,
So long lives this, and this gives life to thee.

William Shakespeare
Declaration of Originality

The experimentation, analyses, presentation and interpretation of results in this thesis represent original work that has not been previously submitted for a degree or diploma in any university. To the best of knowledge and belief, this thesis contains no material previously published or written by another person except where due reference is made within the thesis itself.

Qi Jiang
Abstract

Riparian zones are considered to be dynamic boundaries between terrestrial and aquatic systems, and are of primary importance in maintaining the vitality of landscapes and of surface water bodies. As an important part of the ecosystem, riparian zones are important for ecosystem functions and services, including biodiversity, water quality and recreation. However, riparian areas are often profoundly modified and degraded, with significant losses of ecological significance and functioning. The construction of dam is well known to change the local ecological patterns, especially by reducing the magnitude and frequency and changing duration of flood events.

Soil moisture plays a key role in determining the vitality and activity of soil microorganisms by controlling water and oxygen availability in the soils, therefore affecting microbial mediated carbon (C) and nitrogen (N) transformations in the riparian areas. Net accumulation of soil organic matter (SOM) in submerged soils often occur compared with those in the aerobic soils, due to the decrease in SOM decomposition. Nitrogen turnover in riparian soils is especially sensitive to soil moisture regimes. Autotrophic nitrification mainly occurs under aerobic conditions; while ammonification and immobilization of ammonium (NH₄⁺) could happen under both aerobic and anaerobic conditions. Soil N could be lost as nitrous oxide (N₂O) through nitrification under aerobic conditions, and through denitrification under anaerobic conditions. Rewetting dry soil always leads to a pulse of respiration and perhaps N mineralisation.

Therefore, soil C pools and N availability may be affected by the water fluctuation induced by seasonal climate conditions or the dam operation. However, soil C and N cycling processes in the riparian zone are complicated and influenced by different factors. The overall objective of this project was to quantify the sizes and key processes of soil C pools and N dynamics that would have been altered in the riparian zone after the construction of Wyaralong Dam in Southeast Queensland, Australia.

A series of experiments was conducted in order to assess the spatial and seasonal variations of soil C and N dynamics in the riparian areas of Wyaralong Dam. The experimental site (27°54'28.77"S, 152°52'53.59"E) is located on the Teviot Brook in eastern Beaudesert, Southeast Queensland. This area
has a subtropical climate with the mean minimum temperature of 13.1 °C (-3.7°C to 25.5 °C), mean maximum temperature of 26.5°C (13.3 °C to 40.1°C), and mean annual rainfall of 965.9 mm (841.0 mm to 1136.0 mm) (since 2007). Two sites in the riparian zones, which were located around the catchment dominated by grassland, but were of different soil types and slope positions, had been selected as study areas because they represented two main kinds of riparian soils in this area.

The first and second experiments were based on field work over two years (from February 2013 to August 2014). The first experiment (Chapter 3) examined the spatial and seasonal influence of soil moisture on soil labile C (hot-water extractable organic C (HWEOC) and microbial biomass C (MBC)) and N (hot-water extractable total N (HWETN) and microbial biomass N (MBN)) pools. The results showed that soil labile C and N pools decreased along the transects in both soil types, with the highest and lowest values detected at the upland slope and the riparian zone, respectively. Soil moisture played a significant role in regulating the seasonal variation of labile C and N pools. The aim of the second experiment (Chapter 4) was to explore whether soil N isotope composition (δ¹⁵N) and C isotope composition (δ¹³C) could be used as sensitive indicators for soil N and C dynamics in the subtropical riparian areas. The results showed that significant portions of the spatial and seasonal variations in soil δ¹⁵N could be explained by microbial activities driven by soil moisture. The third experiment (Chapter 5) was based on a laboratory incubation to evaluate the effect of soil moisture contents on net and gross N transformation rates in soils from two sites in the riparian zone. The results showed that significant influences of soil moisture could be found on soil net and gross N transformation rates in the riparian soils. The last experiment was designed to examine the structural characterization of SOM in whole soil and humic acid (HA). The results showed that variations in the structural characteristics of SOM in the upland soil, riparian soil, and sediment were small within each year; while most of the intensity of HAs was attributed to the primary aromatic spinning side band instead of authentic carboxylate in this study. However, the degree of humification increased from year 2013 to year 2015 at both sites in the riparian zone of the Wyaralong Dam.

From these results, it was concluded that soil moisture would be an important driving factor which influenced the spatial and seasonal variations of soil labile C and N pools. Soil δ¹⁵N and δ¹³C could be
used as sensitive indicators for soil N and C dynamics in the riparian areas, especially when soil δ^{15}N was combined with labile N pools, total N (TN) and soil C/N ratio. Under the laboratory incubation, gross N mineralization rates were low and increased with soil moisture both among and within each slope position treatment; while gross nitrification rates significantly decreased with the soil moisture within each slope position treatment. Gross nitrification rates were higher compared with the gross N mineralization rates. Most of the ^{15}\text{NH}_4^+ and ^{15}\text{NO}_3^- added were fixed in clay or immobilized into SOM (>87%) and considerable portions of the added ^{15}N were lost possibly through N gas emissions. Although little influence of soil moisture or slope position on the structural composition of SOM along the transects in the whole soil was detected in the riparian zone, a greater degree of decomposition of SOM in the upland soil than in the sediment was indicated by the humification index (HI) of the whole soil. Significant humification also occurred at both sites during the two years’ time.
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Table of Contents

Declaration of Originality ........................................................................................................ i
Abstract ................................................................................................................................... iii
Acknowledgement .................................................................................................................. vi
List of Figures ........................................................................................................................ xiii
List of Tables .......................................................................................................................... xvi
List of Supplemental Materials .............................................................................................. xvii
List of Abbreviations .............................................................................................................. xxi

Chapter 1 Introduction ............................................................................................................. 1

1.1 Riparian zone ..................................................................................................................... 1

1.1.1 Definition of riparian zone ......................................................................................... 1
1.1.2 Factors affecting the riparian zone .............................................................................. 2
1.1.3 The impacts of dam construction on the riparian zone .............................................. 3

1.2 Soil C cycling in riparian zones ....................................................................................... 3

1.2.1 The importance of soil organic C (SOC) ................................................................. 3
1.2.2 SOC pools .................................................................................................................. 4
1.2.3 Organic matter accumulation and decomposition in the inundation areas ..... 5

1.3 Soil N cycling in riparian zones ....................................................................................... 6

1.3.1 Soil N cycling ............................................................................................................. 6
1.3.2 N transformations in the riparian zone ...................................................................... 9

1.4 Dry-wet (D-W) effects on C and N mineralisation and fluxes in soils ...... 10

1.4.1 Birch effect ................................................................................................................. 11
1.4.2 Mechanisms involved in dry-wet effects ............................................................... 11
1.4.3 Relationship between dry-wet (D-W) effect and cumulative C and N mineralisation ................................................................. 12

1.5 Research questions and hypotheses ......................................................... 12

References .............................................................................................................. 16

Chapter 2 Material and Methods........................................................................... 28

2.1 Materials ......................................................................................................... 28

2.1.1 Study site ................................................................................................. 28

2.1.2 Experimental design ............................................................................... 29

2.2 Methods ......................................................................................................... 33

2.2.1 Soil processing ....................................................................................... 33

2.2.2 Soil analysis ......................................................................................... 33

Reference .............................................................................................................. 34

Chapter 3 Dynamics of soil labile carbon and nitrogen pools in riparian zone of Wyaralong Dam in Southeast Queensland, Australia .............................................. 35

3.1 Introduction .................................................................................................. 35

3.2 Materials and methods.............................................................................. 37

3.2.1 Study site and experimental design ...................................................... 37

3.2.2 Laboratory analyses ............................................................................. 37

3.2.3 Statistical analyses .............................................................................. 37

3.3 Results ......................................................................................................... 38

3.3.1 Monthly rainfall, temperature and dam levels ................................... 38

3.3.2 Soil basic chemical parameters ............................................................ 40

3.3.3 Effects of soil moisture ........................................................................ 50

3.3.4 Relationship among the soil C and N pools ...................................... 53

3.4 Discussion ................................................................................................... 53
3.4.1 Spatial variation of soil labile C and N pools ........................................... 53
3.4.2 Seasonal variation of soil labile C and N pools ........................................... 55

3.5 Conclusions ........................................................................................................ 58

References .................................................................................................................. 59

Chapter 4 Soil and sediment $\delta^{15}$N and $\delta^{13}$C provide insights into N and C dynamics of riparian zones in Southeast Queensland, Australia ......................................................... 84

4.1 Introduction .......................................................................................................... 84

4.2 Materials and methods ......................................................................................... 86
  4.2.1 Site selection and soil collection ................................................................. 86
  4.2.2 Laboratory analyses .................................................................................... 87
  4.2.3 Statistical analyses ..................................................................................... 87

4.3 Results .................................................................................................................. 87
  4.3.1 Soil $\delta^{15}$N and $\delta^{13}$C ........................................................................ 87
  4.3.2 Relationship among soil moisture and soil C and N pools ....................... 93
  4.3.3 Relationships of soil $\delta^{15}$N with labile N pools .................................... 96
  4.3.4 Relationships of soil $\delta^{13}$C with labile C pools .................................... 96
  4.3.5 Relationship of soil $\delta^{15}$N with soil C/N ratios and TN ....................... 96

4.4 Discussion .......................................................................................................... 100
  4.4.1 Spatial and seasonal patterns of $\delta^{15}$N .................................................. 100
  4.4.2 Spatial and seasonal patterns of $\delta^{13}$C .................................................. 104

4.5 Conclusion .......................................................................................................... 105

References .................................................................................................................. 107

Chapter 5 Effects of soil moisture on nitrogen transformations of subtropical riparian soils in Southeast Queensland, Australia ......................................................... 126

5.1 Introduction .......................................................................................................... 126
6.2.4 Solid-state $^{13}$C CPMAS NMR spectroscopy ........................................... 167

6.3 Results and discussions .......................................................................................... 168

6.3.1 $^{13}$C solid state NMR studies on the whole soils ............................................. 168

6.3.2 $^{13}$C solid state NMR studies on the isolated humic acids (HA) ..................... 177

6.4 Results and discussions .......................................................................................... 178

6.4.1 $^{13}$C solid state NMR studies on the whole soils ............................................. 178

6.4.2 $^{13}$C solid state NMR studies on the isolated humic acids (HA) ..................... 180

6.5 Conclusions ........................................................................................................... 187

Reference ...................................................................................................................... 188

Chapter 7 Summary, conclusions and future perspectives........................................... 194

7.1 General discussion and conclusions ........................................................................ 194

7.2 Future work ............................................................................................................. 197
List of Figures

Fig. 1.1 Nitrogen (N) cycle in ecosystems………………………………………………………………………………..7

Fig. 1.2 General model for N cycling in relation to the biogeochemical environment of the riparian
zone…………………………………………………………………………………………………………………………..10

Fig. 1.3 Potential effects of soil moisture on soil C and N pools in the riparian zones of Wyaralong
Dam………………………………………………………………………………………………………………….14

Fig. 1.4 A flowchart incorporated the research questions, hypotheses and thesis chapters of this
research work………………………………………………………………………………………………………………….15

Fig. 2.1 Soil sampling sites (S₁ and S₂) in the riparian zone of Wyaralong Dam………………….29

Fig. 2.2 Soil and sediment sampling points of Site 1 (S₁) and Site 2 (S₂) at Wyaralong Dam…………31

Fig. 3.1 Monthly rainfall, maximum temperature and minimum temperature for the Beaudesert
Drumley Street Station from January 2013 to December 2014……………………………………………………………39

Fig. 3.2 Water level fluctuation of Wyaralong Dam from January 2013 to December 2014………………39

Fig. 3.3 Relationship between distance from Sp₁ and soil moisture in the 0–10 cm soils a S₁ t (a)and S₂
(b)…………………………………………………………………………………………………………………..43

Fig. 3.4 Relationships between distance from Sp₁ and HWEOC (a), and between distance from Sp₁ and
HWETN (b) in the 0–10 cm soils at S₁…………………………………………………………………………45

Fig. 3.5 Seasonal patterns of HWEOC (a) and HWETN (b) in the first six slope positions in 0-10 cm
soil depth at S₁……………………………………………………………………………………………………………..46

Fig. 3.6 Relationships between distance from Sp₁ and MBC (a), and between distance from Sp₁ and
MBN (b) in the 0–10 cm soils at S₁…………………………………………………………………………………….48
Fig. 3.7 Seasonal patterns of MBC (a) and MBN (b) in the first six slope positions in 0-10 cm soil depth at S1, ..................................................................................................................................................................................49

Fig. 3.8 The effects of soil moisture on HWEOC (a) and HWETN (b) in the 0-10 cm soils at S1, ........51

Fig. 3.9 The effects of soil moisture on MBC (a) and MBN (b) in the 0–10 cm soils at S1, ............52

Fig. 4.1 Relationship between distance from Sp1 and δ\textsuperscript{15}N in the 0-10 cm soil depth at S1 (a) and S2 (b) .................................................................................................................................................................................................90

Fig. 4.2 Seasonal patterns of δ\textsuperscript{15}N within the first six slope positions in 0-10 cm soil depth at S1 (a) and S2 (b) .................................................................................................................................................................................................91

Fig. 4.3 The relationship between distance from Sp1 and δ\textsuperscript{13}C in the 0-10 cm soil depth at S1, ..........92

Fig 4.4 The effects of soil moisture on δ\textsuperscript{15}N at S1 (a) and S2 (b) in the 0–10-cm soil depth.................94

Fig. 4.5 The effects of soil moisture on δ\textsuperscript{13}C at S1 in the 0–10-cm (a) and 10-20 cm (b) soil depth.....95

Fig. 4.6 Relationships between soil δ\textsuperscript{15}N and HWETN at S1 (a) and S2 (b), and between soil δ\textsuperscript{15}N and MBN at S1 (c) and S2 (d) in 0-10 cm soil depth .................................................................................................................................................................................................97

Fig. 4.7 Relationships between δ\textsuperscript{13}C and HWEOC (a) and between soil δ\textsuperscript{13}C and MBC (b) in 0-10 cm soil depth at Site 1 (S1) .................................................................................................................................................................................................98

Fig. 4.8 Relationships between δ\textsuperscript{15}N and soil total C:N (a), and between δ\textsuperscript{15}N and TN (b) in 0-10 cm soil depth at Site 1 (S1) .................................................................................................................................................................................................99

Fig. 5.1 Net ammonification (NA), nitrification (NN) and N mineralization (NNM) rates during the 3-day lab incubation of the 0-10 cm soils from Site 1 (a) and Site 2 (c) in a subtropical riparian area. Gross N mineralization (GNM), and gross nitrification (GNN) rates during the 3-day lab incubation of the 0-10 cm soils from Site 1 (b) and Site 2 (d) in a subtropical riparian area. Values are the means ± standard errors (n=3) .................................................................................................................................................................................135
Fig. 6.1 Fractionation of soil organic matter (SOM) by classical methods

Fig. 6.2 Procedure for extraction, isolation, and purification of humic substances (HS)

Fig. 6.3 Solid-state $^{13}$C cross-polarization magic-angle nuclear magnetic resonance spectra for whole soils at $S_1$T$S_2$ at $S_1$ (a) and $S_2$ (b) in 2013, and $S_1$ (c) and $S_2$ (d) in 2015

Fig. 6.4 Exemplar $^{13}$C CPMAS spectra obtained for (a) humic sample $S_1T_3S$ obtained from the Wyaralong Dam site and (b) a biochar manufactured from sugar cane feedstock by pyrolysis under $N_2$ gas at 600°C

Figure 6.5 Exemplar simulation of the spectrum obtained for $^{13}$C CPMAS spectra of the biochar obtained from sugarcane at 600 °C, at 5300 Hz

Figure 6.6 Exemplar $^{13}$C CPMAS spectra obtained for (a) a humic acid sample obtained from the Wyaralong Dam site ($S_1T_3S$) and (b) a biochar manufactured from sugar cane feedstock by pyrolysis under $N_2$ gas at 600°C at a 4410Hz CPMAS spin rate

Figure 6.7 Exemplar $^{13}$C CPMAS spectra obtained for a biochar manufactured from sugar cane feedstock by pyrolysis under $N_2$ gas at 600°C at (a) a 5000Hz CPMAS spin rate and (b) a 4410Hz CPMAS spin rate

Figure 6.8 a 5000Hz CPMAS spin rate and (b) a 4410Hz CPMAS spin rate. The shift in the spacing of the spinning side bands with spin speed is in evidence

Figure 6.9 Exemplar $^{13}$C CPMAS spectra obtained for $S_1T_3S$ at CPMAS spin rates of (a) 3480Hz, (b) 4410Hz, and (c) 5000Hz, respectively

Fig. 6.10 Solid-state $^{13}$C cross-polarization magic-angle nuclear magnetic resonance spectra for humic acids (HA) at $S_1$ (a) and $S_2$ (b)
List of Tables

Table 1.1 Classification of soil organic carbon (SOC)………………………………………4

Table 2.1 Soil sampling sites (S₁ and S₂) information in the riparian zone of Wyaralong Dam……32

Table 3.1 Three-way analyses of variation (ANOVA) for soil moisture, hot-water extractable organic C (HWEOC), hot-water extractable total N (HWETN), microbial biomass C (MBC) and microbial biomass N (MBN) in 0-10 and 10-20 cm soil profiles at Site 1 (S₁) and Site 2 (S₂)…………………………41

Table 4.1 Table 4.1 Repeated measure analysis of variation (ANOVA) for total C (TC), total N (TN), stable C isotope composition (δ¹³C) and N isotope composition (δ¹⁵N) in 0-10 and 10-20 cm soil profiles at Site 1 (S₁) and Site 2 (S₂)………………………………………………………………………89

Table 5.1 Soil properties (0-10 cm) at study sites of S₁ (Site 1) and S₂ (Site 2) in the subtropical riparian areas of Wyaralong Dam……………………………………………………………………134

Table 5.2 Excess ¹⁵N removed from the added ¹⁵N-NH₄⁺ pool and transformed into other ¹⁵N pools of the incubation following addition of ¹⁵N-NH₄⁺ solution at different soil moisture levels……………138

Table 5.3 Excess ¹⁵N removed from the added ¹⁵N-NO₃⁻ pool and transformed into other ¹⁵N pools of the incubation following addition of ¹⁵N-NO₃⁻ solution at different soil moisture levels………………140

Table 6.1 Physical and chemical characteristics of soil organic matter (SOM) at Site 1 (S₁) and Site 2 (S₂) in 2013……………………………………………………………………………………170

Table 6.2 Physical and chemical characteristics of soil organic matter (SOM) at Site 1 (S₁) and Site 2 (S₂) in 2015……………………………………………………………………………………….171

Table 6.3 Relative intensities (%) of each chemical shift region and humification index (HI) in the 0-10 cm soil and sediment determined after integration and correlation for spinning side bands (SSB) of solid-state ¹³C CPMAS NMR spectra…………………………………………………………………172
List of Supplemental Materials

**Fig. S3.1** Relationship between distance from Sp$_1$ and soil moisture in the 10-20 cm soils at S$_1$ (a) and S$_2$ (b)...............................................................65

**Fig. S3.2** Seasonal patterns of soil moisture within the first six slope positions in 0-10 cm (a) and 10-20 cm (b) soil depth at S$_1$, and in 0-10 cm (c) and 10-20 cm (d) soil depth at S$_2$........................................66

**Fig. S3.3** Relationships between distance from Sp$_1$ and HWEOC (a), and between distance from Sp$_1$ and HWETN in the 10-20 cm soil depth at S$_1$ (b).................................................................67

**Fig. S3.4** Relationships between distance from Sp$_1$ and HWEOC in the 0-10 cm (a) and 10-20 cm (b) soil depth, and between distance from Sp$_1$ and HWETN in the 0-10 cm (c) and 10-20 cm (d) soil depth at S$_2$........................................................................68

**Fig. S3.5** Seasonal patterns of HWEOC (a) and HWETN (b) in the first five slope positions in 10-20 cm soil depth at S$_1$.................................................................69

**Fig. S3.6** Seasonal patterns of HWEOC in the first six slope positions in 0-10 cm (a) and 10-20 cm (b) soil depth, and seasonal patterns of HWETN in the first six slope positions in 0-10 cm (c) and 10-20 cm (c) soil depth at S$_2$.................................................................70

**Fig. S3.7** Relationships between distance from Sp$_1$ and MBC (a), and between distance from Sp$_1$ and MBN (b) in the 0–10 cm soils at S$_1$........................................................................71

**Fig. S3.8** Relationships between distance from Sp$_1$ and MBC in the 0–10 cm (a) and 10-20 cm (b) soil depth, and between distance from Sp$_1$ and MBN in the 0-10 cm (c) and 10-20 cm (d) soil depth at S$_2$........................................................................72

**Fig. S3.9** Seasonal patterns of MBC (a) and MBN (b) in the first five slope positions in 10-20 cm soil depth at S$_1$........................................................................73
**Fig. S3.10** Seasonal patterns of MBC in the first six slope positions in 0-10 cm (a) and 10-20 cm (b) soil depth, and seasonal patterns of MBN in the first six slope positions in 0-10 cm (c) and 10-20 cm (d) soil depth at S₂. ...........................................................................................................................................74

**Fig. S3.11** The effects of soil moisture on HWEOC (a) and HWETN (b) in the 10-20 cm soils at S₁...75

**Fig. S3.12** The effects of soil moisture on HWEOC in the 0-10 cm (a) and 10-20 cm (b) soils, and the effects of soil moisture on HWETN in the 0-10 cm (c) and 10-20 cm (d) soils at S₂. ...............76

**Fig. S3.13** The effects of soil moisture on MBC (a) and MBN (b) in the 10-20 cm soil depth at S₁. ...........................................................................................................................................77

**Fig. S3.14** The effects of soil moisture on MBC in the 0-10 cm (a) and 10-20 cm (b) soil depth, and the effects of soil moisture on MBN in the 0-10 cm (c) and 10-20 cm (d) soil depth at S₂. ...........................................................................................................................................78

**Fig. S3.15** Relationships between hot-water extractable C (HWEOC) and microbial biomass C (MBC) in 0-10 cm (a) and 10-20 cm (b) soil depth, and between hot-water extractable total N (HWETN) and microbial biomass N (MBN) in 0-10 cm (c) and 10-20 cm (d) soil depth at Site 1 (S₁) of Wyaralong Dam.............................................................................................................................................79

**Fig. S3.16** Relationships between hot-water extractable C (HWEOC) and microbial biomass C (MBC) in 0-10 cm (a) and 10-20 cm (b) soil depth, and between hot-water extractable total N (HWETN) and microbial biomass N (MBN) in 0-10 cm (c) and 10-20 cm (d) soil depth at Site 2 (S₂) of Wyaralong Dam.............................................................................................................................................80

**Fig. S4.1** Relationships between distance from Sp₁ and δ¹⁵N in the 10-20 cm soil depth at S₁ (a) and S₂ (b). .................................................................................................................................................115

**Fig. S4.2** Seasonal patterns of δ¹⁵N within the first five slope positions in 10-20 cm at S₁ (a) and S₂ (b). .................................................................................................................................................116
Fig. S4.3 Relationships between distance from Sp₁ and δ¹³C in the 10-20 cm soil depth at S₁ (a) and S₂ (b)…………………………………………………………………………………………………......117

Fig. S4.4 Seasonal patterns of δ¹³C within the first six slope positions in 0-10 cm (a) and 10-20 cm (b) soil depth at S₁, and in 0-10 cm (c) and 10-20 cm (d) soil depth at S₂……………………………………118

Fig. S4.5 The effects of soil moisture on δ¹⁵N at S₁ (a) and S₂ (b) in the 10-20-cm soil depth………………………………………………………………………………………………119

Fig S4.6 The effects of soil moisture on δ¹³C at S₂ in the 10-20 cm soil depth…………………………120

Fig. S4.7 Relationships between soil δ¹⁵N and HWETN (a), and between soil δ¹⁵N and MBN (b) in 10-20 cm soil depth at S₁………………………………………………………………………………………………121

Fig. S4.8 Relationships between soil δ¹⁵N and HWETN (a), and between soil δ¹⁵N and MBN (b) in 10-20 cm soil depth at S₂………………………………………………………………………………………………122

Fig. S4.9 Relationships between soil δ¹³C and HWEOC (a), and between soil δ¹³C and MBC (b) in 10-20 cm soil depth at S₁………………………………………………………………………………………………123

Fig. S4.10 Relationships between soil δ¹³C and HWEOC (a), and between soil δ¹³C and MBC (b) in 10-20 cm soil depth at Site 2 (S₂)………………………………………………………………………………………………124

Fig. S4.11 Relationships between δ¹⁵N and soil total C:N (a), and between δ¹⁵N and TN (b) in 0-10 cm soil depth at Site 2 (S₂)………………………………………………………………………………………………125

Table S3.1 Two-way analyses of variation (ANOVA) for hot-water extractable organic C (HWEOC), hot-water extractable total N (HWETN), microbial biomass C (MBC) and microbial biomass N (MBN) in 0-10 and 10-20 cm soil profiles at Site 1 (S₁) and Site 2 (S₂)…………………………………………………………81

Table S5.1 Net ammonification (NA), net nitrification (NN), net mineralization (NNM), gross N mineralization (GNM) and nitrification (GN) rates in the 0-10 cm soils during the 3-day incubation of soils collected at S₁ (Site1) and S₂ (Site 2) in a riparian areas of Wyaralong Dam…………………………159
Table S5.2 Pearson’s correlation analyses of nitrogen (N) transformation rates and other soil properties at the S₁ site (n=21)……………………………………………………………………………………161

Table S5.3 Pearson’s correlation analyses of nitrogen (N) transformation rates and other soil properties at the S₂ site (n=21)……………………………………………………………………………………161
### List of Abbreviations

<table>
<thead>
<tr>
<th>Abbreviation</th>
<th>Meaning</th>
</tr>
</thead>
<tbody>
<tr>
<td>N</td>
<td>Nitrogen</td>
</tr>
<tr>
<td>C</td>
<td>Carbon</td>
</tr>
<tr>
<td>SOC</td>
<td>Soil organic carbon</td>
</tr>
<tr>
<td>MBC</td>
<td>Microbial biomass carbon</td>
</tr>
<tr>
<td>MBN</td>
<td>Microbial biomass nitrogen</td>
</tr>
<tr>
<td>HWEOC</td>
<td>Hot-water extractable organic carbon</td>
</tr>
<tr>
<td>HWETN</td>
<td>Hot-water extractable total nitrogen</td>
</tr>
<tr>
<td>HS</td>
<td>Humic substances</td>
</tr>
<tr>
<td>SOM</td>
<td>Soil organic matter</td>
</tr>
<tr>
<td>WHC</td>
<td>Water holding capacity</td>
</tr>
<tr>
<td>SON</td>
<td>Soil organic nitrogen</td>
</tr>
<tr>
<td>DON</td>
<td>Dissolved organic nitrogen</td>
</tr>
<tr>
<td>SM</td>
<td>Soil moisture</td>
</tr>
<tr>
<td>EC</td>
<td>Electrical conductivity</td>
</tr>
<tr>
<td>WHC</td>
<td>Water holding capacity</td>
</tr>
<tr>
<td>TN</td>
<td>Total nitrogen</td>
</tr>
<tr>
<td>Abbreviation</td>
<td>Description</td>
</tr>
<tr>
<td>--------------</td>
<td>-------------</td>
</tr>
<tr>
<td>TC</td>
<td>Total carbon</td>
</tr>
<tr>
<td>$\delta^{15}$N</td>
<td>Natural abundance of stable isotope $^{15}$N</td>
</tr>
<tr>
<td>$\delta^{13}$C</td>
<td>Natural abundance of stable isotope $^{13}$C</td>
</tr>
<tr>
<td>WSOCE</td>
<td>Water-soluble organic carbon</td>
</tr>
<tr>
<td>WSTN</td>
<td>Water-soluble total nitrogen</td>
</tr>
<tr>
<td>TOC</td>
<td>Total organic carbon</td>
</tr>
<tr>
<td>TSN</td>
<td>Total soluble nitrogen</td>
</tr>
<tr>
<td>ANOVA</td>
<td>Analysis of variance</td>
</tr>
<tr>
<td>DMRT</td>
<td>Duncan’s multiple range test</td>
</tr>
<tr>
<td>WFPS</td>
<td>Water-filled pore space</td>
</tr>
<tr>
<td>DNRA</td>
<td>Direct NO$_3^-$ reduction to NH$_4^+$</td>
</tr>
<tr>
<td>NA</td>
<td>Net ammonification</td>
</tr>
<tr>
<td>NN</td>
<td>Net nitrification</td>
</tr>
<tr>
<td>NNM</td>
<td>Net nitrogen mineralization</td>
</tr>
<tr>
<td>GNM</td>
<td>Gross nitrogen mineralization</td>
</tr>
<tr>
<td>GNN</td>
<td>Gross nitrification</td>
</tr>
<tr>
<td>FA</td>
<td>Fulvic acid</td>
</tr>
<tr>
<td>HA</td>
<td>Humic acid</td>
</tr>
<tr>
<td>Abbreviation</td>
<td>Description</td>
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<tr>
<td>--------------</td>
<td>-------------</td>
</tr>
<tr>
<td>NMR</td>
<td>Nuclear magnetic resonance</td>
</tr>
<tr>
<td>Solid-state $^{13}$C CPMASNMR</td>
<td>Solid-state $^{13}$C cross-polarization magic angle spinning NMR</td>
</tr>
<tr>
<td>IHSS</td>
<td>International Humic Substances Society</td>
</tr>
<tr>
<td>HI</td>
<td>Humification index</td>
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</tbody>
</table>
Chapter 1 Introduction

1.1 Riparian zone

1.1.1 Definition of riparian zone

Riparian zone is defined as the interface between terrestrial and aquatic ecosystems, and represents an important ecological component of the landscape. Lowrance et al. (1985) defined riparian ecosystems as “the complex assemblage of organisms and their environment existing adjacent to and near flowing water”. Malanson (1991) described it as “the ecosystems adjacent to the river”. Some other definitions even use riparian to refer to the edges of bodies of water other than streams and rivers, i.e. land adjoining any water body (Davies and Nelson 1994). As a result, the term can also be applied to an area adjacent to the watershed of a dam. The boundaries of a riparian zone extend outward to the limits of flooding and upward into the canopy of streamside vegetation (Gregory et al. 1991).

The riparian zone is a type of ecotones. Although difficult to delineate, the riparian zone encompasses the vegetated strip of land extending along streams and rivers (Gregory et al. 1991). Because of its special location, between the upland and aquatic ecosystems, the riparian zone has the capability of modifying effects, which far exceeds their minor proportion of the location within the landscape and the intricate linkages between terrestrial and aquatic ecosystems (Gregory et al. 1991). Like other ecotones, riparian zones are extremely rich in biodiversity (Gregory et al. 1991; Gregorich and Ellert 1993; Naiman et al. 1993). Naiman et al. (1988) found that ecotones displayed a greater variation than either of the systems they connect. Instead of being averages of the two systems, there are some features to be unique. Interactions between terrestrial and aquatic ecosystems include modification of microclimate (light, temperature, humidity, etc.), alteration of nutrient inputs from slopes, contribution of organic matter to streams and floodplains, and retention of inputs (Gregory et al. 1991).

The functions of the riparian zone can be summarised as follows: (1) storing flood waters; (2) trapping or removing sediments; (3) trapping or removing nitrogen (N) and other nutrients; (4) stabilizing stream banks; (5) trapping or removing other contaminants; (6) maintaining habitat for fish and other aquatic organisms; and (7) providing habitat for terrestrial organisms (Malanson 1991; Salemi et al. 2012). Because of these reasons, riparian buffers are widely considered as tools to protect water quality, maintain wildlife habitat, and provide other benefits to the people and environment (USEPA 1998; Lowrance et al. 2000). Riparian zones are therefore one of the most dynamic portions of the landscape (Swanson et al. 1988)
1.1.2 Factors affecting the riparian zone

Many factors are reported to have an influence on the riparian zone. Water level fluctuations happened in most of rivers, reservoirs and lakes (Zhao et al. 2014). There are two main causes of water fluctuations: climate change and anthropogenic disturbance. Climatic change is expected to cause significant changes in the water regime of watershed, which can result in flooding with seasonal variations related with precipitation, evaporation, and air temperature (Hofmann et al. 2008). Flooding induced by seasonal change is a natural feature of riparian zones. The frequency, duration, timing, and magnitude of floods help to determine both the physical and biological characteristics of the riparian ecosystem (Poff et al. 1997; Brauns et al. 2008). At the same time, the impacts of these water fluctuations by anthropogenic disturbances (dam construction, etc.) are likely enhanced by climatic changes (Brauns et al. 2008).

Research indicates that topography affects the hydrological functioning of riparian zones (Devito et al. 2000). The slope gradient, especially at the riparian zone-upland margin, has an effect on the hydraulic gradient and the volume and velocity of water entering the riparian zone. The slope of the land near the watershed may be the most significant variable in determining effectiveness of the zones in trapping sediments and retaining nutrients. The steeper the slope, the higher the velocity of overland flow and the less time it takes for nutrients and contaminants to traverse the zone (Wenger 1999). A concave riparian profile typically favours interaction between subsurface water and superficial soil horizons where denitrification and root uptake are likely to occur, whereas convex topography favours deeper water tables and subsurface flowpaths. Burt et al. (2002) have shown that when the riparian zone is flat, the stream water level acts as a fixed point around which the water table fluctuates. The upland edges of riparian zones are often characterized with oxic conditions and high nitrate (NO\textsubscript{3}) concentrations in groundwater (Hill 1996); although most of this NO\textsubscript{3} is attenuated within a short distance (Burt 2005) as oxic groundwater flow enters anoxic, organic soils and undergoes denitrification (Hill 1996). At locations deeper into riparian zones, and closer to surface saturated areas or streams, riparian sediments are often organic and anoxic (and thus may be optimal for denitrifying bacteria) but may be limited by N supply.

Dissolved nutrients are transported from terrestrial ecosystems into streams primarily in the form of materials in groundwater. Riparian zones are uniquely situated within watersheds to intercept soil solution as it passes through the riparian rooting zone before entering the stream channel. Nutrient concentrations in riparian soils exhibit patterns related to the composition of streamside plant communities and to the history of fluvial disturbance. As soil solution passes through riparian zones before entering streams, vegetative demand for dissolved nutrients may greatly reduce nutrient loads (Gregory et al. 1991).

Vegetation type can significantly affect labile soil organic carbon (C) pools in subtropical soils (Wang
and Wang 2011), as well as affecting soil microbial processes impacting on production, quality and decomposition rates of litters and root materials, substrate availability, the types of microbial communities and soil microclimates (Hibbard et al. 2005). Substrate quality (C :N) affects the balance between C and N limitation of microbial growth (Chapin et al. 2011). As microbes break down organic matter, they incorporate about 40% of C from their substrates into microbial biomass and return the remaining 60% of C to the atmosphere as CO₂ through respiration. With this 40% growth efficiency, microbes require substrates with a C/N ratio of about 25:1 to meet their N requirement. At higher C/N ratios, microbes import N to meet their growth requirements, and at lower C/N ratios N exceeds microbial growth requirements and is secreted into the litter and soil (Chapin III et al.). Therefore, 25 is often considered to be the critical C: N ratio, above which there is no net N release from decomposing organic matter.

1.1.3 The impacts of dam construction on the riparian zone

Dams serve important functions all over the world, such as seasonal flood regulation, water supply for multiple purposes and irrigation (Lehner et al. 2011; Li et al. 2012). Almost 60% of large river systems are affected by dams (Nilsson et al. 2005), and the impoundments are estimated to represent about one-sixth of the total annual river flow into the oceans (Hanasaki et al. 2006). Dam operations alter the water regime which is the leading force shaping the riparian ecosystems (New and Xie 2008; Egger et al. 2012; Tang et al. 2014). A change in the natural water regime always affects the biogeochemistry of riparian zone as well as its ability to cycle and mitigate nutrients fluxes originating from upslope (Pinay et al. 2002). The construction of dam naturally has a significant environmental influence upon the riparian zone. Dams impacts water regimes by reducing the magnitude and frequency of flood events and by changing their periods of occurrence (Stanford and Gaufin 1974). Some riverine, floodplain and slope habitat processes are changed into lake habitat processes. Inundation areas may be exposed intermittently due to the variation from dam operation and evaporation. Previously existing riparian zone is drowned by the dam pondage, with a new riparian area replacing the previous one from upslope. Also, the variable water level regime in the dam pondage prevents the establishment of permanent vegetation cover (riparian or aquatic) below full supply level (Brizga et al. 2007).

1.2 Soil C cycling in riparian zones

1.2.1 The importance of soil organic C (SOC)

Soil organic C is the key indicator of soil fertility since it has far reaching effects on soil physical, chemical and biological properties (Tiessen et al. 1994; Chen et al. 2004; Schmidt et al. 2011), and is also considered to mitigate global warming by sequestering C from the atmosphere by plants (Bolin and Sukumar 2000). More than two to three times as much organic C is held in the soils worldwide as is in terrestrial biomass (Jacobson et al. 2000; Parry et al. 2007).
Soil has the largest pool of terrestrial organic C in the biosphere, compared with C stored in plants and the atmosphere combined (Schlesinger 1997). The changes in the pool size and turnover rate of SOC can affect the cycling of nutrients in the soil (Blair et al. 1995). In order to predict and ameliorate the consequences of climate and land cover, it is critical to describe SOC distributions and the controls of SOC inputs and outputs (Jobbágy and Jackson 2000). However, in many cases, the changes resulting from the land use are not reflected in SOC (Roscoe and Buurman 2003), due to the high C in the less variable and more stable mineral association (Lal 2006).

1.2.2 SOC pools

The SOC, located in different compartments, has different recycling times and forms of protections (Duxbury et al. 1989; Stockmann et al. 2013). According to different functions, Strosser (2010) classified SOC into three pools: labile SOC, stable SOC, and inert SOC (Table 1.1). Six et al. (2002) divided SOC into labile, slow and recalcitrant organic matter according to the turnover rate. However, some studies also proposed that rather than describing organic matter by decay rate, pool, stability or level of ‘recalcitrance’, they should be described by quantifiable environmental characteristics governing stabilization, such as solubility, molecular size and functionalization (Schmidt et al. 2011).

Table 1.1 Classification of the soil organic carbon (SOC) (Strosser 2010)

<table>
<thead>
<tr>
<th>Classification</th>
<th>Conception</th>
<th>Function</th>
<th>Half-time</th>
</tr>
</thead>
<tbody>
<tr>
<td>Labile SOC</td>
<td>A quickly reactive labile organic matter</td>
<td>Provide energy and nutrients for soil micro-organisms and release part of the nutrients for plant usage</td>
<td>Between days and few years</td>
</tr>
<tr>
<td>Stable SOC</td>
<td>A reservoir of less decomposable organic matter</td>
<td>Cation-exchange capacity</td>
<td>Between years and decades</td>
</tr>
<tr>
<td>Inert SOC</td>
<td>An almost non-reactive organic matter</td>
<td>Affect the physical properties of the soil</td>
<td>Between decades and centuries</td>
</tr>
</tbody>
</table>

Labile organic C pools have been chosen as much more sensitive indicators with which to detect the small changes in total SOC (Ghani et al. 2003; Haynes 2005; Laik et al. 2009; Wang and Wang 2011). The effects of changes in soil management are observed sooner in the labile SOC pool than in the total SOC (Lee et al. 2009; Dodla et al. 2012). Labile fractions of organic C are highly responsive to changes in C inputs to soil and provide a measurable index before changes in total SOC (Gregorich and Janzen 1996; Haynes and Beare 1996; Song et al. 2012). Furthermore, the importance of labile organic C also lies in the fact that it fuels the soil food web as well as influencing the nutrient cycle and many biologically related properties (Weil et al. 2003). However, the large part of soil labile C is susceptible to release due to changes in many factors, such as land-use, management and climate. There are many
methods to analyse labile organic C, such as water-extractable C (Ghani et al. 2003; Tirol-Padre and Ladha 2004; Chen et al. 2016) and microbial biomass carbon (MBC) (Vance et al. 1987; Santos et al. 2012; Shen et al. 2015). A close relationship is also found between hot water-extractable organic C (HWEOC) and soil microbial biomass and activities (Ghani et al. 2003; Wang et al. 2007). The reason may lie in the fact that hot water-extractable methods may extract not only simple organic compounds and carbohydrates, but also parts of the soil microbial components (Rovira and Vallejo 2007).

On the other hand, humic substances (HS) are ubiquitous in the environment (Stocking 2003; Lal et al. 2004). Although investigations of HS can date back to more than 200 years ago, much remains unknown about their structure and properties (Mao et al. 2002). Humic substances are categorised as the chemically resistant component, and may not change markedly over decades (Stevenson 1994; Lal 2004). They have largely remained uncharacterised at the molecular level and have necessarily been defined operationally in terms of the methods used to extract or isolate them. Humic substances can be complex polydisperse polymeric mixtures due to their structural diversity, their state of aggregation, conformation, and surface charge distribution. Thus, their individual molecules may reveal little of the properties that emerge via supramolecular interaction (Cook and Langford 1998). The chemical nature of the litter, composition of the microbial community, and environmental factors play important roles in affecting the chemical and structural components of HS. Humic substances developing from similar geographic locations, but different depositional environments, often have different chemical structures (Rasyid et al. 1992).

1.2.3 Organic matter accumulation and decomposition in the inundation areas

Due to lack of oxygen, the decomposition and accumulation of soil organic matter (SOM) in inundation areas (anaerobic) are totally different from those in their aerobic counterparts. The decomposition of SOM is lessened and incomplete, while the humification of SOM is decreased under flooded condition. Therefore, the overall SOM decomposition rates are slower in submerged soils than those in the aerobic soils, leading to the net accumulation of SOM in soil (Sahrawat 2003).

Studies on the decomposition of SOM in anaerobic environment showed that the decomposition rates are slower in the absence of oxygen than in the aerobic environment. The reduction on oxygen concentration decreased the decomposition rate of SOM (Wershaw 1993). This shows that the rate of SOM decomposition decreases under flooded conditions after peaking at moisture content of 60% of water holding capacity (WHC) (Pal and Broadbent 1975). The optimum water content for aerobic microbial activity is 60% of soil pore space filled with water. This is applied to diverse soils with various textural classes. The increase in soil water content above 60% leads to increased anaerobiosis and decline in aerobic microbial activity which explains slower decomposition and mineralisation of SOM in soils under anaerobic than aerobic conditions (Reddy et al. 1980; Neue and Scharpenseel 1987;
Lack of oxygen has a major effect on microbial physiology and decomposition of SOM.

There are other hypotheses to explain the accumulation of SOM in inundation areas: (1) reduction products such as hydrogen sulfide or volatile fatty acids and toxic concentrations of ammonium, aluminum, iron, and other cations in soil solution have the deleterious effects on microbial activity (Marschner and Kalbitz 2003); (2) the absence of electron acceptors such as iron oxides and hydroxides in submerged soils and sediments slows down SOM oxidation and mineralisation (Lovley 1995; Roden and Wetzel 2002); (3) formation of recalcitrant complex organic molecules with organic matter fractions may render them less available for microbial attack and utilization (Baldock and Skjemstad 2000); and (4) the net primary productivity of wetlands is higher than other ecosystems (Neue et al. 1997).

1.3 Soil N cycling in riparian zones

1.3.1 Soil N cycling

Nitrogen cycling includes the entry of N into the ecosystems, its interval transfers between plants and soils, and its loss from the ecosystems (Fig. 1.1). Nitrogen enters the ecosystems through the biological fixation of atmospheric N, the chemical weathering of rocks, and the deposition of N from the atmosphere. Interval cycling involves the conversion of N from organic to inorganic forms, chemical reactions that change N from one ionic form to another, biological uptake by plants and microorganisms, and exchange of N in surfaces within the soil matrix. Nitrogen is lost through leaching, trace-gas emission, wind and water erosion, and fire (Chapin et al. 2011).
The atmosphere contains 78% N₂. Instead of using N₂ directly from the air, most plants and organisms need to wait for N to be fixed, i.e. the processes transforming N₂ to inorganic compounds (ammonium and nitrate). The two major natural sources of new N entering this cycle are through N₂-fixing organisms and lightening. The N₂ in the atmosphere becomes a part of biological matter mostly through the actions of bacteria and algae in a process known as biological N₂ fixation. Heterotrophic N₂ fixers typically have highest fixation rates in aerobic environments, because aerobic respiration yields much more energy per gram of substrate than anaerobic respiration. Lightening may also indirectly transform N₂ into NO₃⁻, which enters the soil during rain (ESA 1997). However, some studies also indicated that direct utilization of soil native soil organic N (SON) by a large number of forest species, rather than the conversion of SON to NH₄⁺ or NO₃⁻, may present a potentially important new pathway for plant N uptake (Chen and Xu 2008).

Most N absorbed by plants becomes available through the decomposition of organic matter. The N is released as dissolved organic N (DON) through the action of exoenzymes by microbes. Plants and mycorrhizal fungi can absorb some DON, using it to support plant growth. The decomposer microbes also absorb DON for their N and/or C requirement. Immobilization is the removal of inorganic N from the available pool by microbial uptake and chemical fixation. On the other hand, microbial growth is often C limited. Hence the microbes break down the DON, use the C to support their energy, and secrete NH₄⁺ into the soil (Chapin et al. 2011).

Although the pool of insoluble organic N in soil is relatively large, the entire organic N that is eventually mineralized must first becomes DON before it could be absorbed by microbes and
mineralized. Thus the conversion from insoluble organic N to DON is the initial and typically the rate-limiting step in N mineralization (Chapin et al. 2011). Ammonification is the process of the production of ammonia (NH$_3$) from organic compounds. In most soils, the NH$_3$ dissolves in water to form NH$_4^+$. The bacteria that accomplish it are called ammonifying bacteria. The net absorption or release of NH$_4^+$ by the microbes depends on their C status. When microbial growth is C limited, microbes use the C from DON to support growth and respiration and secrete NH$_4^+$ into the soil solution. On the other hand, other N-limited microbes may immobilize some of this NH$_4^+$ and use it for growth. Thus N mineralisation and immobilisation occur simultaneously. Gross N mineralisation is the total amount of N released via N mineralisation, regardless of whether it is subsequently immobilised or not (Nannipieri and Eldor 2009). Net N mineralisation is the net accumulation of inorganic N in the soil solution over a given time interval (Wang et al. 2006). Nitrogen mineralisation rate is controlled by the availability of DON and inorganic N, the activity of soil microbes, and their relative demands for C and N (Hadas et al. 1992; Whitmore and Groot 1997; Moore et al. 1999).

The biological conversion of NH$_4^+$- N to NO$_3^-$-N is called nitrification, and most nitrification is carried out by a restricted group of nitrifying bacteria (autotrophic nitrifiers and heterotrophic nitrifiers). Autotrophic nitrification is a two-step process: bacteria known as Nitrosolobus convert NH$_3$ or NH$_4^+$ to nitrite (NO$_2^-$); then bacteria called Nitrobacter finish the conversion of NO$_2^-$ to NO$_3^-$. The reactions are generally coupled and proceed rapidly to NO$_3^-$; therefore, NO$_2^-$ levels at any given time are usually low. Some NO and N$_2$O are also produced as by-products, typically at a NO to N$_2$O ratio of 10 to 20 (Tortoso and Hutchinson 1990). These nitrifiers are strict “aerobes,” and they must have free dissolved oxygen to perform their work. Nitrification occurs only under aerobic conditions at dissolved oxygen levels of 1.0 mg/L or higher. Although autotrophic nitrification predominates in many ecosystems, heterotrophic nitrification can be important in ecosystems with low N availability or acidic soils. Many heterotrophic fungi and bacteria produce NO$_2^-$ or NO$_3^-$ from NH$_4^+$; while some use organic N in the process (Chapin et al. 2011). Oxygen is also an important factor during nitrification because most nitrifiers require oxygen for oxidation of NH$_4^+$. Oxygen availability is influenced by many factors, including soil moisture, soil texture, soil structure, and respiration by microbes and roots (Chapin et al. 2011).

Denitrifying bacteria can reduce NO$_3^-$ to NO$_2^-$ and free nitrous oxide and N gases (NO$_3^-$ →NO$_2^-$ →NO→N$_2$O→N$_2$) under conditions of high NO$_3^-$ and low oxygen. They use NO$_3^-$ or NO$_2^-$ as an electron acceptor to oxidize organic C for energy when oxygen concentration is low. Most denitrifiers are facultative anaerobes and use oxygen rather than NO$_3^-$ when oxygen is available. Denitrification often happens in waterlogged, anaerobic soils (Knowles 1982). The gases diffuse out the soil and into the atmosphere removing their N from the N exchange pool. Most denitrifiers have the enzymatic potential to carry out the entire reductive sequence but produce variable proportions of N$_2$O and N$_2$, depending in part on the relative availability of NO$_3^-$ versus organic C. When NO$_3^-$ is relatively more abundant than labile organic C, more N$_2$O than N$_2$ is produced (Zaman 2012). Other factors favouring
N$_2$O over N$_2$ production include low pH, low temperature, and high oxygen. Significant denitrification often happens under conditions with low oxygen, high NO$_3^-$, and a supply of organic C. High soil moisture always impedes the diffusion of oxygen. Soil moisture is controlled by other environmental factors such as slope position, soil texture, and the balance between precipitation and evapotranspiration. Therefore, if a wetland receives NO$_3^-$ from outside the system, it has an aerobic zone above an anaerobic zone, and it goes through cycles of flooding and drainage, the wetland should support high denitrification rates (Chapin et al. 2011). However, denitrification does can occur in aerobic condition, such as fungal aerobic denitrification although no N$_2$O reductase has been identified in fungi (Maeda et al. 2015).

### 1.3.2 N transformations in the riparian zone

Riparian zones in any watershed often act as a net sink of NO$_3^-$ depending on the flow path of water draining to the stream. In undisturbed forested watersheds, riparian zones serve as a source of NO$_3^-$ as reported in different studies (Hill 1993; Creed and Band 1998; Coats and Goldman 2001; Bechtold et al. 2003). Similar increases in NO$_3^-$ have also been found in streams draining mixed land use and agricultural watersheds (Correll et al. 1999; David et al. 2004). This behaviour is a called “flushing effect” when a water table rises to the soil surface with subsequent mobilization of nutrients stored near or at the soil surface (Creed et al. 1996; Creed and Band 1998). When the flow is deep below the soil surface, most of the N accumulates in the soil, only small amounts of them export N into adjacent waters. As the flow rises, N is flushed from the soil to the stream with the flow. As the flow below the ground intersects the soil surface, N formed in the highly bioactive surface of the soil is flushed resulting in large N loss into adjacent waters. Riparian zones always act as net sources of NO$_3^-$ during high flows because the rising water table that flushes NO$_3^-$ occurs primarily in the riparian zones (Abdul and Gillham 1989).

Changes to the water regimes, either through alterations in the frequency, duration, period of occurrence, and intensity of water levels, directly affect N cycling in soils by controlling the duration of oxic and anoxic phases (Keeney 1973). Biogeochemistry processes of N are sensitive to the oxido-reduction status of the soil: ammonification of organic N can be realised both under aerobic and anaerobic conditions; nitrification can only occur in aerated soils or sediments; denitrification is strictly anaerobic, requiring saturated soils to operate. Therefore, the riparian zone is ideal for both aerobic and anaerobic N fixation (Fig. 1.2). Sometimes the surface soil is below the partially oxygenated free water in contact with the atmosphere and sometimes above saturated soil devoid of oxygen. The soil below is anaerobic and serves as a source of electron donors to the upper side of the boundary. The riparian zone serves as the interface. Organic products of anaerobic respiration diffuse upward and provide readily
available reduced C energy needed by the N-fixing organisms living on the aerobic side of the interface (Reddy and Patrick 1979). Nitrogen fixation in the anaerobic zone, supplied with cellulose as the energy source, was found to be directly proportional to the interfacial area in soil containers (Magdoff and Bouldin 1970). Short-term periodicity of aerobic-anaerobic conditions through groundwater level movements allow all N-cycling processes to occur simultaneously at the same location in accordance with the level of soil-water saturation. Therefore, the end products of N cycling in the riparian zones are under control of the moisture regime.

Some studies have shown that near-stream saturated zones and riparian zones are active sites of biogeochemical N dynamics (Cooper 1990; Murdoch and Stoddard 1992; Cirmo and McDonnell 1997). Those zones are interfaces between upland slope and watershed dynamics, and they play a critical role in determining the amount and speciation of N entering the stream channel. The N transformation and retention should occur where hydraulic residence time is increased and saturated conditions prevail.

![Diagram](image)

**Fig. 1.2** General model for N cycling in relation to the biogeochemical environment of the riparian zone (Cirmo and McDonnell 1997; Zhu et al. 2013)

### 1.4 Dry-wet (D-W) effects on C and N mineralisation and fluxes in soils

Changes in land-use have strongly affected the global C and N cycles to date, which subsequently affects the global climate (LeQuéré et al. 2009). These changes are associated with increasing frequency and risk for extreme events (Meehl and Tebaldi 2004; Heimann and Reichstein 2008). A globally increasing frequency of severe drought periods will lead to irregular and extreme water stress for plants. Even for the regions with increasing annual amounts of precipitation, increasing evapotranspiration or shifts in precipitation pattern could constrain the availability of soil water during growing seasons. These changes will also exert strong influence on the turnover of SOM, which plays an important role in the global C and N cycles as a major component of the world’s surface C and N reserves (Gruber et al. 2004).
1.4.1 Birch effect

It has been well documented that rewetting dry soil results in a pulse of respiration of a much higher rate than the basal respiration of the moist soil. The phenomenon has been named “the Birch-Effect” in recognition of its first documentation (Birch 1958): cycles of dry-wet of soils stimulated the mineralisation of SOC, leading to the rapid release of mineral N and CO₂. This transient effect was observed in several studies in terrestrial ecosystems (grassland, shrubland, forest, etc.) (Austin et al. 2004; Xu et al. 2004; Tang and Baldocchi 2005; Jarvis et al. 2007; Inglima et al. 2009) and soil scales (Denef et al. 2001; Fierer and Schimel 2002; Fierer and Schimel 2003; Inglima et al. 2009).

The “Birch” pulse could be explained from four aspects: (1) a spontaneous increase in fungal and microbial biomass in response to water availability (Jager and Bruins 1975; Orchard and Cook 1983; Scheu and Parkinson 1994); (2) rewetting of the dry soils shatters soil aggregates exposing previously unavailable organic substrates for decomposition (Denef et al. 2001); (3) drying leads to an increase in dead microbial biomass, which is rapidly decomposed by new microorganisms and fungi after rewetting (Bottner 1985); and (4) nutrient and C pulses are due to a hypo-osmotic stress response of the soil microbial community after sudden changes in soil water status (Fierer and Schimel 2002; Fierer and Schimel 2003; Jarvis et al. 2007). However, a complete understanding of the processes has not yet been achieved (Jarvis et al. 2007).

1.4.2 Mechanisms involved in dry-wet effects

Soil drying is closely related to air humidity, temperature, wind and transpiration and often begins shortly after rain as a result of the water deficit of air (Borken and Matzner 2009). The influence of soil drying on microbial activity is mostly restricted at elevated temperatures, and is a common limitation of microbial activity under extremely dry conditions. Several mechanisms are involved during drying of soils: (1) microorganisms may dehydrate and decrease osmotic potential in the cell by accumulation of compatible solutes such as amino acids, carbohydrates, polyols and inorganic solutes to equilibrate with their environment (Halverson et al. 2000) when drying. Once the threshold is exceeded, microorganisms further dehydrate and die (Sparling and Ross 1988; Van Gestel et al. 1992; Van Gestel et al. 1993) or survive the drought period by forming endospores, cysts or vegetative cells (Chen and Alexander 1973). Hence, the decrease in microbial biomass during drying is likely to be correlated with the duration of drying; (2) drying of soils reduces the mobility of extra-cellular enzymes and the availability of soluble substrates (Stark and Firestone 1995; Ford et al. 2007); (3) drying of soils decreases the reduction in root growth and exudation during drought (Crossman et al. 2004); (4) soil shrinking induced by the drying of soil leads to the exposure of new soil surfaces and of previously protected organic matter (Utomo and Dexter 1982; Appel 1998; Denef et al. 2001), as well as affects the size and stability of pores, being most pronounced in organic soils and forest floors with large portion of macropores; and (5) with the intensity of drying, hydrophobicity of soil surfaces by organic
substances increases and has a large impact on microbial activity during wetting (Doerr et al. 2000; Doerr et al. 2007; Mataix-Solera et al. 2007).

On the other hand, the wetting of the dry soil also involves many processes: (1) the dead microbial cells which accumulate during drying periods, may be hydrated and dissolved during the wetting of dry soils triggers (Halverson et al. 2000); (2) the rewetting of dry soil leads to the mineralisation of nonmicrobial substrates which is much larger than the mineralisation of microbial C and N (Van Gestel et al. 1991); (3) hydrophobicity of SOM considerably slows down the increase in water potential following wetting, which gives microorganisms more time to equilibrate with their environment and to restore their metabolism, including reassimilation of compatible solutes (Halverson et al. 2000; Schimel et al. 2007).

1.4.3 Relationship between dry-wet (D-W) effect and cumulative C and N mineralisation

The relevance of dry-wet (D-W) cycles for cumulative C and N mineralisation and fluxes includes the effects of intensity, duration and frequency of dry-wet on cumulative C and N mineralisation. Few studies focus on the effect of drying or wetting intensity on C and N mineralisation, and a comparison of the study results is difficult given different information on soil moisture (Borken and Matzner 2009). However, the duration of dry-wet is of great importance for cumulative C and N mineralisation. A relatively short drying period and extended wetting period would potentially result in greater cumulative C and N mineralisation relative to a moist control (Mikha et al. 2005; Miller et al. 2005; Hentschel et al. 2007). Moreover, the duration of drought affects the population of soil microorganisms during the dry-wet period. In general, cumulative mineralisation rates theoretically decrease with increasing duration and intensity of desiccation (Borken and Matzner 2009). Rainfall intensity has only a limited effect on C and N mineralisation while the duration of the wetting period will influence the cumulative C and N mineralisation rates. The size of wetting pulses decreases with the frequency of dry-wet cycles, which may be explained by the decrease in labile organic matter over time (Birch 1958), or by a shift in bacterial community composition as reported.

1.5 Research questions and hypotheses

Despite the increasing recognition of riparian zone ecosystem, the spatial and temporal distribution of C and N pools has rarely been documented in the riparian zone of the watershed of a dam (Vidon et al. 2010; Ye et al. 2012; Ye et al. 2015). As envisaged in the literature review, the soil C pools and N availability may be changed after the construction of Wyaralong Dam. However, C and N cycling processes in the riparian zone are complex phenomena influenced by different factors. It is still little known how C and N respond to the water fluctuation in riparian zones. Therefore, there is a need to quantify C and N pools and dynamics in the riparian zone of a dam. This study for the first time evaluated the impact of water fluctuation on soil C and N pools in Southeast Queensland, Australia.
The overall objective of this project was to quantify the key processes of soil C and N dynamics that occur in the riparian zone after the construction of Wyaralong Dam, which are important for the sustainability of the riparian ecosystem and ecosystem services. I sought to provide a comprehensive understanding of soil moisture influences on soil C and N pools via multiple directions of research work, based on a long-term experimental site. Spatial and seasonal variations of soil C and N dynamics were detected in this thesis. The following hypotheses were examined:

(1) Moisture fluctuations would be a driving factor controlling the distribution of soil C and N pools, resulting from water fluctuation by both of seasonal climate change (precipitation and evaporation, etc.) and the Wyaralong Dam operation.

(2) The soil labile organic C pools (stable C isotope, HWEOC, and MBC), and N pools (stable N isotope, HWETN and MBN) would be sensitive indicators on the impacts of water level fluctuation, compared with the soil HS and whole soil.

(3) Riparian zones would be hotspots for biogeochemical C and N dynamics (mineralization and immobilization).

The focal aspects of this study have been shown in the following flowchart (Fig. 1.3).
This study attempted to address different research questions in each chapter. As a general review, a framework was conceptualized including the research questions, hypotheses and thesis chapters as shown in Fig. 1.4.

**Fig. 1.3** Potential effects of soil moisture on soil C and N pools in the riparian zones of Wyaralong Dam
Chapter 1

Fig. 1.4 A flowchart incorporated the research questions, hypotheses and thesis chapters of this research work.
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Chapter 2 Material and Methods

2.1 Materials

2.1.1 Study site

This study was conducted in the riparian zone of Wyaralong Dam. Wyaralong Dam (27°54'28.77"S, 152°52'53.59"E) is located on the Teviot Brook in eastern Beaudesert (Fig. 2.1), Southeast Queensland. The Wyaralong Dam was constructed by the Queensland Government from October 2009 to December 2010. Its main focus is the area to be inundated (ca.1230 ha) with the catchment of 546 km². This area has a subtropical climate with the mean minimum temperature of 13.1 °C (-3.7°C to 25.5 °C), mean maximum temperature of 26.5°C (13.3 °C to 40.1°C), and mean annual rainfall of 965.9 mm (841.0 mm to 1136.0 mm) (since 2007). Climate data were collected from a meteorological station (Beaudesert Drumley Street) about 12.7 km away from the study site. Historical dam storage data were collected from Seqwater.
Fig. 2.1 Soil sampling sites ($S_1$ and $S_2$) in the riparian zone of Wyaralong Dam.

2.1.2 Experimental design

Two sites in the riparian zone of Wyaralong Dam were selected in January 2013 (Fig. 2.1). Both of them were dominated by grassland (including *Cynodon dactylon*, *Heteropogon contortus*, *H. contortus*,
Imperata cylindrical, Chamaecrista rotundifolia and Chrysocephalum apiculatum) (Zheng et al. 2014), but were of different soil types and slope degrees. They represented two main kinds of riparian soils in this area. The soil type of Site 1 (S1) was Dermosols (Isbell 1996), with a relative clayey texture and slope degree of 8-16°; while the Site 2 (S2) soil was Kurosols (Isbell 1996), with a relative sandy texture and slope degree of 4-10° (Table 2.1). Samplings were carried out in February 2013 (summer), May 2013 (autumn), August 2013 (winter), November 2013 (spring), February 2014 (summer) and August 2014 (winter), respectively. Three parallel transects spaced ca. 5 meters apart with 6 to 10 sampling points (depending on the water level) along each transect were selected. All the sampling points were selected according to the 100% of full water level (Fig. 2.2). Three soil cores were collected from each point to be mixed as one composite soil sample within each soil depth (0-10 cm and 10-20 cm) using an auger with 7.5 cm in diameter; while three sediment sample (0-10 cm) from each point along each transect at each site were collected to be mixed as one composite sediment sample with spade (Fig. 2.2). All soil samples were sealed with plastic bags and transferred to laboratory. Although soil samples were collected from the two sites, it is impossible to directly compare soil properties between the sites, since they differed in many aspects such as soil type, slope degree and vegetation type. However, we still can gain some insight into the spatial and seasonal patterns of soil C and N pools in the riparian zones, and expect to observe some underlying mechanisms in common.

Detail information regarding soil sampling design is given in each of the following experimental chapters.
Fig. 2.2 Soil and sediment sampling points of Site 1 ($S_1$) and Site 2 ($S_2$) at Wyaralong Dam.
### Table 2.1 Soil sampling sites (S₁ and S₂) information in the riparian zone of Wyaralong Dam

<table>
<thead>
<tr>
<th>Site</th>
<th>Soil type</th>
<th>Parent material</th>
<th>Slope degree</th>
<th>Coordinates</th>
<th>pH</th>
<th>EC (μS cm⁻¹)</th>
<th>Particle-size distribution (%)*</th>
</tr>
</thead>
<tbody>
<tr>
<td>S₁</td>
<td>Black, Grey, Brown Dermosols</td>
<td>Alluvia</td>
<td>8-16°</td>
<td>27°56'8&quot;S</td>
<td>152°46'9&quot;E</td>
<td>5.22 (0.23)</td>
<td>216.61 (41.02) Sandy 36.6 (6.79) Clay 36.7 (4.38) Silt 36.7 (2.61)</td>
</tr>
<tr>
<td>S₂</td>
<td>Brown, Black Kurosols</td>
<td>Alluvia</td>
<td>4-10°</td>
<td>27°56'19&quot;S</td>
<td>152°51'18&quot;E</td>
<td>4.37 (0.17)</td>
<td>152.98 (22.43) Sandy 57.5 (3.53) Clay 32.6 (3.19) Silt 9.9 (1.21)</td>
</tr>
</tbody>
</table>

* Particle-size distribution (%) (Dong et al., unpublished data)
2.2 Methods

2.2.1 Soil processing

All the soil and sediment samples collected from the field were transferred to the laboratory in plastic bags at field moisture. Soil and sediment samples were mixed thoroughly and sieved through 2 mm screen to remove fine roots and stones. Samples were then separated into two portions: parts of the samples oven dried (40 °C) and the rest stored in the cold room at 4 °C as fresh samples prior to analysis.

2.2.2 Soil analysis

Soil moisture was determined by placing 10 g of fresh soil or sediment samples into the oven at 105 °C until reaching a constant weight (48 h), and all results were expressed on an oven-dry basis. Soil pH and electrical conductivity (EC) were measured with a pH/EC meter using a soil-to-water ratio of 1:5. Approximately 10 g of fresh soil samples were soaked in water for 2 h in water holding capacity (WHC) tubes, and then drained with cling film over the top for 16 h. The soils were then weighed again, and WHC were determined on an oven-dry basis.

Total C (TC), total N (TN), stable C and N isotope composition (δ13C and δ15N respectively) of aired-dried whole soil samples were determined. A fine powder of soil was used to measure TC, TN, δ13C and δ15N using a mass spectrometer (GV Isoprime, Manchester, UK).

Soil hot-water extractable organic C and total N (HWEOC and HWETN) were determined by adding 7 g of air-dried to 35 ml water and incubating in a capped and sealed tube at 70 °C for 18 h. Following incubation, the suspension was shaken by an end-to-end shaker for 5 minutes followed by centrifuging at 10000 rmp for 10 minutes. The suspension was filled through a 33 mm Millex syringe-driven 0.45 μm filter. Soil water-soluble organic C and total N (WSTN) were extracted using only cold water for soil: water ratio (1:5) and followed by the same steps used for hot water extraction. The concentration of total organic C (TOC) and total soluble N (TSN) in the filtered solution was measured using a Shimadzu TOC-VCSH/CSN TOC/N analyser (Chen and Xu 2005).
To measure microbial biomass C and N (MBC and MBN), fumigation-extraction method was used. Two sub-samples of fresh soil (5 g) were weighed (for direct extraction and fumigation). One of the sub-soil samples was fumigated by chloroform for 24 h. Both fumigated and non-fumigated (directly extracted) sub-samples were mixed with 25 ml of 0.5 M K$_2$SO$_4$ and each mixture was shaken with an end-to-end shaker for 30 min, followed by filtering through a Whatman No. 42 filter paper. The TOC and TSN of both extractions were measured using a Shimadzu TOC-VCSH/CSN TOC/N analyser (Chen and Xu 2005). The MBC and MBN were derived from the equations as described by Vance et al. (1987) and Brookes et al. (1985), respectively.

Soil NH$_4^+$ and NO$_3^-$ were extracted with 2 M KCl and their concentrations measured by Westco SmartChem Discrete Wet Chemistry Analyzer (Westco Scientific Instruments, USA). Briefly, 15.0 g of fresh soil samples were weighed into 50-mL plastic centrifuge tubes, extracted with 30 mL of 2 M KCl by shaking for 1 h, centrifuged at 2000 rpm for 10 min and then filtered through Whatman No. 42 filter paper and frozen until analysis.

Reference


Chapter 3 Dynamics of soil labile carbon and nitrogen pools in riparian zone of Wyaralong Dam in Southeast Queensland, Australia

3.1 Introduction

Riparian zone is defined as the interface between terrestrial and aquatic ecosystems, which represents an important ecological component of the landscape, and displays a greater variation in characteristics than either of the systems it connects (Naiman et al. 1988; McClain et al. 2003). Riparian areas play an important role in removing nitrogen (N), from water flowing through riparian soils (Hill et al. 2014). The effectiveness of reducing N concentrations varies, based on the slope of banks, rainfall, soil factors (pH, temperature, redox potential, etc.), soil moisture, land use, vegetation and so on (Wenger 1999). Environmental changes, especially changes in soil aeration condition and flooding, are always considered to affect soil N dynamics in riparian zone (Gergel et al. 2005; Hernandez and Mitsch 2007). Water fluctuation, induced aerobic and anaerobic conditions have a significant influence on soil N mineralization process in the riparian soil (Antheunisse et al. 2007). On the other hand, periodic flooding also leads to the redistribution of soil organic matter (SOM) in riparian sites (Nilsson and Svedmark 2002). Decomposition of SOM is lessened and incomplete, while the humification of SOM is decreased under anaerobic soils (Sahrawat 2003).

River damming is one of the most prominent human impacts on fresh water ecosystems, which has caused global-scale ecological changes in riparian zones (Braatne et al. 2008). Dams affect riparian zones by altering the magnitude, frequencies, and periods of flood events (Nilsson and Berggren 2000). With damming, the previous riparian zones are inundated and drained intermittently, which leads to the alteration of oxic and anoxic periods (Brizga et al. 2007). Therefore, soil C and N dynamics may be influenced by the anthropogenic flood-control measures (Wu et al. 2013; Wu et al. 2015). As inundation can cause sedimentation, and sudden increase of nutrient release in the new reservoirs (Rosa et al. 2004), damming is also related to the climate change problems.

Soil moisture varies not only because of the dry/flooding events, but also due to seasonal precipitation in the riparian zone. It is reported that precipitation is the primary driver for seasonal changes of N cycling in semi-arid and arid areas (Austin et al. 2004). It is well known that rewetting dry soil results
in a pulse of mineral N and CO$_2$, which is called the Birch-Effect (Birch 1958). The flush of N mineralization is thought to occur from enhanced availability of SOM through the increase in fungal and microbial biomass (Meisner et al. 2013; Weise et al. 2016), breakdown and release of occluded SOM from soil aggregates (Denef et al. 2001), decomposition of dead microbial biomass (Bottner 1985), and a hypo-osmotic stress response of soil microbial community (Jarvis et al. 2007). In the riparian areas, soil N cycling would be expected to be closely related to rapid water level changes or a dry-rewetting regime (Venterink et al. 2002). Soil microbial composition may also vary with water contents (Barnard et al. 2013; Barnard et al. 2015). The alteration of soil moisture not only affects microbial activity, but also C and N availability through plant-microbe interaction (Ladwig et al. 2015).

Labile SOM is considered as the more sensitive indicator to be detected even with small change in total SOM, and is highly responsive to changes in C inputs to soil and provides a measurable index before changes in total SOM (Stockmann et al. 2013; Li et al. 2016). This indicates that the influence of changes in soil management can be observed sooner in the labile C and N pools than in the total SOM (Wang and Wang 2011). However, concerns about the impact of flooding on soil C and N pools in riparian areas have mainly been focused on total SOM such as N removal in riparian buffers (Wu et al. 2013; Wu et al. 2015). There has been little work conducted to examine the relationship between soil labile C and N pools, determined by the various techniques, which has important implications for transformation dynamics of SOM. Therefore, the nature of organic C and N determined by these methods has not been fully clarified. To the best of my knowledge, this was the first study investigating soil labile C and N dynamics in the subtropical riparian zone of a dam in the Southern Hemisphere.

In this study, I investigated the spatial and seasonal dynamics of soil labile C and N pools within the riparian zone of Wyaralong Dam in Southeast Queensland, Australia. We aimed to (1) quantify the spatial and seasonal influence of soil moisture change on soil labile C and N pools along the transects, and (2) identify the key factors regulating the spatial and seasonal patterns of soil labile C and N pools in the riparian zone. We hypothesized that labile C (hot-water extractable organic C (HWEOC) and microbial biomass C (MBC)) and N (hot-water extractable total N (HWETN) and microbial biomass N (MBN)), would be influenced by the soil moisture alteration in the riparian zone significantly at both spatial and seasonal levels.
3.2 Materials and methods

3. 2.1 Study site and experimental design

The Wyaralong Dam research site was detailed in Chapter 2. In brief, this area has a subtropical climate with the mean minimum temperature of 13.1 °C, mean maximum temperature of 26.5°C, and mean annual rainfall of 965.9 mm (since 2007). Two sites in the riparian zone of Wyaralong Dam were selected. The soils are classified as Dermosols and Kurosols (Isbell 1996). Three parallel transects spaced ca. 5 meters apart with 6 to 10 sampling points along each transect were selected. Three soil cores were collected from each point to be mixed as one composite soil sample within each soil depth (0-10 cm and 10-20 cm) using an auger with 7.5 cm in diameter; while three sediment sample (0-10 cm) from each point along each transect at each site were collected to be mixed as one composite sediment sample with spade.

3.2.2 Laboratory analyses

All the soil and sediment samples were mixed thoroughly and sieved through 2-mm screen, with parts of the samples oven dried (40 °C) and the rest stored in the cold room at 4 °C as fresh samples prior to analysis. Moisture was determined by placing 10 g of fresh soil or sediment samples into the oven at 105 °C until reaching a constant weight (48h), and all results were expressed on an oven-dry basis.

Soil physiochemical properties (pH, EC, HWEOC, and HWETN) were measured using methods described in Chapter 2. Soil biological parameters (MBC and MBN) were determined using the methods detailed in Chapter 2.

3.2.3 Statistical analyses

Data from the same site (0-10 cm and 10-20 cm) were subjected to three-way analysis of variance (ANOVA) to assess the interaction of slope position, soil depth and sampling season. To quantify the differences in the interaction of slope position and soil depth within each sampling season, two-way ANOVA was used. Changes in labile SOM were analysed using one-way ANOVA with slope position as a factor. All comparisons among the different slope position treatments, soil depths, and seasons were conducted by the Duncan’s Multiple Range Test (DMRT). Significant differences in basic soil properties and labile SOM were reported at $P < 0.05$. The normality of all data was checked before
ANOVA. All above statistical analyses were carried out using SAS for windows version 9.2 (SAS Institute Inc., Cary, NC, USA). Regression analyses were used to assess the spatial distribution of soil moisture, labile SOM, and the correlations among soil moisture, soil labile C and N pools by OriginPro for windows version 8.5 (Analytical Software, MA, USA).

3.3 Results

3.3.1 Monthly rainfall, temperature and dam levels

There was a great reduction in total annual rainfall during 2014 compared with 2013 (Fig. 3.1). A wet summer (174 mm, February) and dry winter (4.4 mm, August) in 2013 occurred; while a dry summer (10.2 mm, February) and relative wet winter (57.4 mm, August) were recorded in 2014. There were marked increases in both of minimum and maximum temperatures for two years, particularly during November to March (Fig. 3.1).

The water levels of Wyaralong Dam fluctuated from 89.5 % to 100.7 % of full water level within 2013 to 2014 (Fig. 3.2). There was a remarkable increase of water level after the construction of Wyaralong Dam. The heavy rain always led to the increase of water level with a time lag between the rainfall episodes and the water level peaks; while the water level stayed relatively stable compared with the variation of monthly total rainfall.
Fig. 3.1 Monthly rainfall, maximum temperature and minimum temperature for the Beaudesert Drumley Street Station from January 2013 to December 2014.

Fig. 3.2 Water level fluctuation of Wyaralong Dam from January 2013 to December 2014.
3.3.2 Soil basic chemical parameters

When all the data from the six sampling times were pooled together for both sites, there were no significant interactions among the soil slope position, soil depth, and sampling time (Table 3.1). However, the interactive influence of soil depth and soil slope position was significant on soil moisture and most of the labile C and N pools at both sites (Table 3.1). Soil moisture, HWEOC, HWETN, MBC, and MBN were significantly higher in the 0-10 cm soil depth than those in the 10-20 cm soil depth except for MBN in summer 2014 (Table S3.1).

Soil moisture

In order to assess the trend for the spatial and seasonal distribution of soil properties, DMRT was used and the relationship between the distance from Sp1 (Slope position 1, the most upper slope position soil) and soil moisture were detected. Generally, soil moisture contents were significantly higher in the lower slope position than in the upper slope position in both depths ($P < 0.01$) at both sites (Fig. 3.3 and Fig. S3.1). Soil moisture contents varied among different seasons, with the lowest observed in summer 2014 (Fig. S3.2).
Table 3.1 Three-way analyses of variation (ANOVA) for soil moisture, hot-water extractable organic C (HWEOC), hot-water extractable total N (HWETN), microbial biomass C (MBC) and microbial biomass N (MBN) in 0-10 and 10-20 cm soil profiles at Site 1 ($S_1$) and Site 2 ($S_2$)

<table>
<thead>
<tr>
<th>Soil Profile (cm)</th>
<th>Moisture (%)</th>
<th>HWEOC (mg kg$^{-1}$)</th>
<th>HWETN (mg kg$^{-1}$)</th>
<th>MBC (mg kg$^{-1}$)</th>
<th>MBN (mg kg$^{-1}$)</th>
</tr>
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<td></td>
<td>df</td>
<td>F</td>
<td>P</td>
<td>df</td>
<td>F</td>
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<tr>
<td>$S_1$ Sd</td>
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<td>290.65</td>
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<td></td>
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<tr>
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</table>

Sd - soil depth; Sp – slope position; and St- sampling time.
Fig. 3.3 The relationship between distance from $Sp_1$ and soil moisture in the 0–10 cm soils $S_1$ (a) and $S_2$ (b).
Chapter 3

**HWEOC and HWETN**

Compared with HWEOC, HWETN was much lower in all corresponding samples. At S1, concentration of HWEOC increased from Sp1 to the peak between Sp2 and Sp4, and then decreased to the minimum values in the sediment ($P < 0.01$) in spring 2013, summer 2014 and winter 2014 in the 0-10 cm soil depth (Fig. 3.4a). Similar pattern could be detected in the 10-20 cm soil depth (Fig. S3.3a). Similar to the pattern of HWEOC, HWETN also decreased along the transects with the peak found between Sp2 and Sp4 in spring 2013, and winter 2014 ($P < 0.01$), respectively in the 0-10 cm soil depth at S1 (Fig. 3.4b). However, HWETN decreased first with the minimum value at Sp7, and then increased in the sediment ($P < 0.05$) in the 10-20 cm soil depth in summer 2014 ($P < 0.05$) (Fig. S3.3b).

More spatial patterns of HWEOC could be found at S2. HWEOC was generally decreased along the transects from upper slope position to the sediment (summer 2013: $P < 0.05$; other seasons: $P < 0.01$) in the 0-10 cm soil depth (Fig. S3.4a). Similar trends were also found in the 10-20 cm soil depth (Fig. S3.4b). The spatial trend of HWETN corresponded well with the HWEOC (Figs. S3.4c and S3.4d).

Pronounced seasonal patterns were found at both sites. Interestingly, among the different seasons, the highest concentration of HWEOC and HWETN were found during autumn 2013 followed by summer 2013 and 2014, and the lowest concentration during winter 2013 and 2014 in both soil layers at both sites (Figs. 3.5, S3.5 and S3.6).
Fig. 3.4 The relationships between distance from Sp\(_1\) and HWEOC (a), and between distance from Sp\(_1\) and HWETN (b) in the 0–10 cm soils at S\(_1\). Sp\(_1\): the most upper slope position; S\(_1\): Site 1; HWEOC: hot-water extractable organic C; and HWETN: hot-water extractable total N.
Fig. 3.5 Seasonal patterns of HWEOC (a) and HWETN (b) in the first six slope positions in 0-10 cm soil depth at S1. Sp: slope position; S1: Site 1; HWEOC: hot-water extractable organic C; and HWETN: hot-water extractable total N. Different letters indicated significant differences among the six sampling times at \( P < 0.05 \).
MBN was low compared with MBC at both sites, and also showed wide spatial variation at both sites (Figs. 3.6, S3.7 and S3.8). Specifically, the highest concentrations of MBC could be found within the first three slope positions, and then decreased to the minimum in the sediment in three seasons at S₁ (Figs. 3.6a and S3.7a). MBN had similar trends. Nevertheless, MBN increased dramatically in autumn 2013 at both soil depths (P < 0.05) (Figs. 3.6b and S3.7b).

On the other hand, much more significant differences were found for MBC and MBN at S₂ (Fig. S3.8). Generally, MBC decreased dramatically in autumn 2013 (0-10 cm depth: P < 0.01; 10-20 cm depth: P < 0.05), winter 2013 (0-10 cm depth: P < 0.01; 10-20 cm depth: P < 0.05), spring 2013 (P < 0.01), and summer 2014 (P < 0.01) for both soil depths. The spatial distribution of MBN was similar to that of MBC.

Large seasonal variations in soil MBC and MBN were also observed at both sites (Figs. 3.7, S3.9 and S3.10). At S₁, maximum concentration of MBC was found in February 2013 and November 2013 at most of slope positions in the 0-10 cm soil depth (Fig. 3.7a). The highest MBN was also detected for both soil depths in summer 2013 and spring 2013 (Figs. 3.7b and S3.9b). Compared with the S₁, seasonal variation at the S₂ was not so obvious and significant (Fig. S3.10). For those at slope positions with significant differences, MBC and MBN were found to be high mainly in summer and spring 2013.
Fig. 3.6 The relationships between distance from Sp₁ and MBC (a), and between distance from Sp₁ and MBN (b) in the 0-10 cm soils at S₁. Sp₁: the most upper slope position; S₁: Site 1; MBC: microbial biomass C; and MBN: microbial biomass N.
**Fig. 3.7** Seasonal patterns of MBC (a) and MBN (b) in the first six slope positions in 0-10 cm soil depth at $S_1$.

Sp: slope position; $S_1$: Site 1; MBC: microbial organic C; and MBN: microbial organic N.
3.3.3 Effects of soil moisture

Soil HWEOC and HWETN displayed different dependencies on soil moisture from both of the soil types during different seasons. At S₁, significant effects of soil moisture on HWEOC and HWETN were observed in most of the seasons for both soil depths (Figs. 3.8 and S3.11). For example, soil HWEOC was significantly related with soil moisture in winter 2013 ($R^2=0.957$); while soil HWETN was also significantly related with soil moisture in winter 2013 ($R^2=0.999$). The relationships between soil moisture and HWEOC, and between soil moisture and HWETN were similar but less sensitive in soil at S₂ (Fig. S3.12). For instance, soil HWEOC and HWETN were significantly related with soil moisture in summer 2014 ($R^2=0.916$ and 0.524, respectively).

The correlations between soil moisture and MBC or MBN in most seasons were also significant for both soil depths at both sites (Figs. 3.9, S3.13 and S3.14). For example, significant relationship were found between soil moisture and MBC, and between soil moisture and MBN in spring 2013 in 0-10 cm soil depth at S₁ ($R^2=0.942$ and 0.989, respectively).
Fig. 3.8 The effects of soil moisture on HWEOC (a) and HWETN (b) in the 0–10 cm soils at S₁, S₂: the most upper slope position; S₁: Site 1; HWEOC: hot-water extractable organic C; and HWETN: hot-water extractable total N.
Fig. 3.9 The effects of soil moisture on MBC (a) and MBN (b) in the 0–10 cm soils at S₁.

Sp₁: the most upper slope position; S₁: Site 1; MBC: microbial biomass C; and MBN: microbial biomass N.
3.3.4 Relationship among the soil C and N pools

Significant and positive relationships existed between HWEOC and MBC at S1, such as in summer 2014 ($R^2 = 0.719$ to 0.846, $P < 0.01$) (Figs. S3.15a and S3.15b). Similar trends were found between MBN and HWETN (for instance, in summer 2014: $R^2 = 0.841$ to 0.960, $P < 0.01$) (Figs. S3.15c and S3.15d). At S2, it was observed that MBC was positively and significantly related to HWEOC at both soil depths in autumn 2013 ($P < 0.05$), winter 2013 ($P < 0.01$), spring 2013 ($P < 0.01$), and summer 2014 ($P < 0.01$) (Figs. S3.16a and S3.16b). The trends of correlation between MBN and HWETN in the 0-10 cm soil depth were similar to those between MBC and HWEOC (Figs. S3.16c and S3.16d).

3.4 Discussion

3.4.1 Spatial variation of soil labile C and N pools

Given the labile nature of HWEOC and HWETN, they could give an early indication of SOM changes (Ghani et al. 2003). Hot-water extractable methods were thought to extract both simple organic compounds and carbohydrates, and some parts of soil microbial components (Curtin et al. 2006; Rovira and Vallejo 2007). Ghani et al. (2003) indicated that about 40-50% of the C in the HWEOC extract was present as carbohydrates. Sparling et al. (1998) reported that MBC took up 45% of HWEOC. Generally, HWEOC and HWETN decreased along the transects at both soil depths for two different soil types with the peak or bottom of values detected mainly in the riparian zone between Sp2 and Sp4 in this study. Similar trend was also found in the spatial distribution of MBC and MBN. Significant and positive correlations between HWEOC and MBC, and between HWETN and MBN could be found in some seasons for both soil depths at the two sites. These results were consistent with previous findings (Zhang et al. 2006), indicating that HWEOC consisted of a labile pool of SOM including MBC (Ghani et al. 2003). Higher soil total C and N, and larger pools of labile C and N in these slope positions of the riparian zone between Sp2 and Sp4, together with their strong relationships, tend to suggest that these areas may have greater quantity and quality of SOM available for decomposition by the microbial community compared with the other slope positions. Microbial dynamics and diversity may also be high in these positions.
Similar trend could also be detected when comparing the spatial distribution of soil labile C and N pools, with the correlation between soil moisture and soil labile C and N pools. Therefore, we assume that soil moisture is an important driving factor for the spatial distribution of labile C and N pools along the transect. The hydrological cycle could lead to the changes in the duration of the oxic and anoxic phases, soil moisture and SOM (De Jager et al. 2012). Studies reported that soil moisture could affect the structure and diversity of the soil community (Tang et al. 2011). The water regime was clearly critical factor in regulating soil microbial diversity in floodplain soils (Rinklebe and Langer 2006). Unfortunately, we did not analyze the fungi and bacterial communities in this study. However, MBC: MBN ratios may shed some light on it. Generally, bacteria have the C: N of 3-6, while fungi have higher C: N ratios of 5-17 (Strickland and Rousk 2010; Liao et al. 2016). In this study, MBC: MBN ratios varied dramatically along the transects, indicating the spatial shift of microbial composition. For example, soil MBC: MBN ratios decreased dramatically from 7.69 in Sp1 to 3.85 in the sediment in the 0-10 cm soil depth at S1 in winter 2013 (P < 0.01), suggesting the microbial communities may shift from fungi to bacteria. It was consistent with the previous findings that bacteria were commonly dominant under flooded conditions while fungi under upland conditions (Bai et al. 2000; Nakamura et al. 2003).

However, the distribution of HWEOC did not always show to be consistent with that of MBC. For example, significant difference was detected for MBC in the 0-10 cm soil depth at S1 in winter 2013 (P < 0.01), but no obvious variation in HWEOC was found. This may be due to the fact that HWEOC would have extracted not only MBC, but also root exudates, soluble carbohydrates and amino acids (Kalbitz et al. 2000). Vegetation can directly contribute a C input to the soil through litterfall and root exudation (Anaya et al. 2007). Additionally, plants could also affect the quantity and activity of microorganism as the energy source (Chen et al. 2004), and then lead to the chain variation of the soil components (Ros et al. 2010).

It is also interesting to note the inconsistency between the spatial distribution of HWEOC and HWETN in this study. For example, HWEOC increased to the peak of value between Sp2 and Sp3 in the 0-10 cm soil depth at S1 in summer 2014; whereas the corresponding HWETN decreased dramatically from Sp1
to $\text{Sp}_4$. It is reported that although HWETN was mainly in organic form (80% on average), significant amounts of $\text{NH}_4^+$-N were also related due to hydrolysis of heat-labile organic N (Curtin et al. 2006). Thus, $\text{NH}_4^+$-N may participate in the soil N dynamics immediately. This assumption was supported by Jiang et al. (unpublished data) who actually measured the spatial variation of $\delta^{15}\text{N}$ in the same experiment, and found the extraordinary low value of $\delta^{15}\text{N}$ in those slope positions.

HWEOC, HWETN, MBC, and MBN decreased significantly with increasing soil depth. It was consistent with previous findings (Zhang et al. 2006; Ros et al. 2009). Because the majority of the organic matter input occurs in the topsoil, the topsoil layer shows the highest SOM and microbial activity (Ros et al. 2009). In addition, the vertical distribution of roots and plant litter might also contribute to the labile C and N distribution (Zhang et al. 2006). On the other hand, it was possible that labile organic C and N in the deeper layers might have been consumed due to the longer residence time of the substrate (Dodla et al. 2012).

The concentrations of labile C and N at $S_1$ were significantly higher than those at $S_2$. The differences in soil texture were considered to affect soil microbial properties significantly (Ausec et al. 2009; Li et al. 2010). Due to the capacity of clay to retain SOM, the positive correlation between HWEOC, or MBC and clay content was reported previously (Poret-Peterson et al. 2007). However, it seems that more significant spatial and seasonal patterns were found at $S_2$, suggesting the influence of slope degree (Wenger 1999).

3.4.2 Seasonal variation of soil labile C and N pools

Factors such as precipitation, temperature, and plant growth, regulate soil C and N cycling, and seasonal patterns of soil labile C and N dynamics can simultaneously reflect the effects of these factors (Parker and Schimel 2011). HWEOC and HWETN concentrations were significantly higher in spring 2013 followed by the summer and winter 2013 and 2014 in this study (Figs. 3.5, S3.5 and S3.6). The dynamics of precipitation and water fluxes were largely responsible for the seasonal changes in the concentration and fluxes of HWEOC and HWETN in the soils in November 2013. The riparian areas subjected to prolonged drying since September 2013 due to low precipitation, and then received the
total rainfall of 60.8 mm within eight days before sample collection on 19 November 2013. The corresponding water level also increased from 96.2 to 97% of full water level. The dry-rewetting cycles can induce large flushes of SOC and nutrients by releasing drought accumulated SOM, inorganic N, and microbial necromass (Schimel et al. 2007; Iovieno and Bååth 2008; Butterly et al. 2009). Rapid water level changes are always tightly coupled with C and N cycling, which may lead to the variation of soil microbial composition (Schimel et al. 2007; Barnard et al. 2013; Barnard et al. 2015). Bacteria are believed to dominate in the wet season while fungi may become active and even predominate in dry systems (Ipsilantis and Sylvia 2007). Bossio and Scow (1998) found winter flooding resulted in a decrease in fungal and aerobic indicators and an increase in bacterial indicators. The intensity, duration and frequency of dry-rewet affect soil C and N cycles differently. A relatively short drying period and extended wetting period would potentially result in greater cumulative C and N mineralisation relative to a moist control (Mikha et al. 2005; Miller et al. 2005; Hentschel et al. 2007). However, the size of wetting pulses decreases with the frequency of dry-rewetting cycles, which may be explained by the decrease in labile organic matter over time (Birch 1958), or by a shift in bacterial community composition as reported for a forest soil (Fierer and Schimel 2003). In our study, the rewetting in November 2013 was featured with high frequency of rainfall. It seemed that dry-rewetting moment appeared at the onset of the wet season.

Seasonal changes lead to differences in temperature and moisture which are important factors controlling soil labile C and N dynamics (Schmidt et al. 2007). Plant litter and root exudates are considered to be the primary source of water-soluble SOM (Qualls et al. 1991). Soil HWEOC and HWETN were high in summer 2013 and 2014 although soil moisture was much lower in summer 2014 (Figs. 3.5, S3.5 and S3.6), suggesting that this increase may cause by other factors such as temperature and plant residues. Higher temperature is known to stimulate microbial activity, plant rhizodeposition, the decomposition of organic C and the release of dissolved organic C from soil organic matter (Clark et al. 2009). In contrast, the lowest values of HWEOC and HWETN were observed in winter although soil moisture in winter 2014 was also high. Therefore, temperature and plant residues may exert stronger influence in the seasonal pattern of HWEOC and HWETN compared with soil moisture in this
study, although the dry-rewetting events were believed to play a pivotal role in the changes of HWEOC and HWETN in November 2013.

However, concentrations of MBC and MBN were not as significantly high as HWEOC and HWETN (Figs. 3.7, S3.9 and S3.10). Soil MBC was also high in November 2013, indicating the influence of dry-rewetting event. However, MBC in November 2013 showed no significant difference with that in summer 2013. We attributed it to the differences of duration, intensity, and frequency of rainfall. In February 2013, soil and sediment samples were collected two weeks after the high intensity rainfall (174.6 mm) on 28 January. Wu et al. (2015) studied the influence of early dry season on soil microbial biomass in Dongting Lake wetland, and found that a shorter flooding duration and a longer period after flooding could lead to a higher soil microbial biomass. They attributed it to two reasons: the recovery of soil aerobic microorganisms from stress in drying period (Anderson and Domsch 1993), and the appearance of plants which could supply litterfall and the exudation of the plant roots to soil microorganisms (Hamilton and Frank 2001). For samples collected on 19 November 2013 in our experiment, there was still time lag effect for microbial biomass to reach the summit of MBC and MBN. In addition, the seasonal patterns of MBC and MBN coincided with the corresponding seasonal patterns of soil moisture. Nevertheless, MBC in summer 2014 was still higher than that in winter 2013 at some lower slope positions with higher soil moisture. It is assumed that temperature turned to be the limiting factor when water was no longer a limitation for MBC in those slope positions. Those findings suggest that soil moisture may exert strong influence on soil MBC and MBN together with temperature and plant residues. Previous studies have also demonstrated that soil temperature and moisture play key roles in controlling soil microbial communities (Fierer et al. 2009; Deng et al. 2010). The hydrological cycle could affect the duration of the oxic and anoxic phases, inducing different soil microbial community structure and diversity (Tang et al. 2011; De Jager et al. 2012). We also found significant and strong correlation between MBC and MBN in this study. However, MBN showed more pronounced seasonal fluctuations than MBC, because microorganisms differ much more in their N content than in their C content (Anderson and Domsch 1989).
3.5 Conclusions

Soil moisture, induced by precipitation and water level fluxes, seems to be the prime driving factor regulating the spatial distributions of labile C and N pools in the riparian areas of Wyaralong Dam in southeast Queensland, Australia. Under the same precipitation regime, soil labile C and N varied with the slope position, soil depth and soil texture. The significant correlation between different labile C and N pools indicates that they may partly represent similar soil C and N pools. However, other factors rather than soil moisture might exert stronger influence in the seasonal pattern of HWEOC and HWETN although dry-rewetting events were shown to affect the seasonal changes of HWEOC and HWETN significantly in November 2013; while soil moisture might play a much more important role in the seasonal variation of soil MBC and MBN together with temperature. Compared with MBC, MBN is a much more sensitive indicator for soil quality changes in the riparian zone. Riparian areas would be the hotspots for microbial activity driven by different environmental factors, especially in the environment at the beginning of the rewetting dry seasons.
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Chapter 3


Fig. S3.1 Relationship between distance from Sp\textsubscript{1} and soil moisture in the 10-20 cm soils at S\textsubscript{1} (a) and S\textsubscript{2} (b).
Fig. S3.2 Seasonal patterns of soil moisture within the first six slope positions in 0-10 cm (a) and 10-20 cm (b) soil depth at S₁, and in 0-10 cm (c) and 10-20 cm (d) soil depth at S₂. Sp: slope position; S₁: Site 1; and S₂: Site 2. Different letters indicated significant differences among the six sampling times at \( P < 0.05 \).
Fig. S3.3 Relationships between distance from Sp$_1$ and HWEOC (a), and between distance from Sp$_1$ and HWETN in the 10-20 cm soil depth at S$_1$ (b). Sp$_1$: the most upper slope position; S$_1$: Site 1; HWEOC: hot-water extractable organic C; and HWETN: hot-water extractable total N.
Fig. S3.4 Relationships between distance from Sp₁ and HWEOC in the 0-10 cm (a) and 10-20 cm (b) soil depth, and between distance from Sp₁ and HWETN in the 0-10 cm (c) and 10-20 cm (d) soil depth at S₂. Sp₁: the most upper slope position; S₂: Site 2; HWEOC: hot-water extractable organic C; and HWETN: hot-water extractable total N.
**Fig. S3.5** Seasonal patterns of HWEOC (a) and HWETN (b) in the first five slope positions in 10-20 cm soil depth at S₁. Sp: slope position; S₁: Site 1; HWEOC: hot-water extractable organic C; and HWETN: hot-water extractable total N. Different letters indicated significant differences among the six sampling times at $P < 0.05$. 
Fig. S3.6 Seasonal patterns of HWEOC in the first six slope positions in 0-10 cm (a) and 10-20 cm (b) soil depth, and seasonal patterns of HWETN in the first six slope positions in 0-10 cm (c) and 10-20 cm (d) soil depth at S2. Sp: slope position; S2: Site 2; HWEOC: hot-water extractable organic C; and HWETN: hot-water extractable total N. Different letters indicated significant differences among the six sampling times at $P < 0.05$. 

70
Fig. S3.7 Relationships between distance from Sp$_1$ and MBC (a), and between distance from Sp$_1$ and MBN (b) in the 0–10 cm soils at S$_1$. Sp$_1$: the most upper slope position; S$_1$: Site 1; MBC: microbial biomass C; and MBN: microbial biomass N.
Fig. S3.8 Relationships between distance from Sp$_1$ and MBC in the 0–10 cm (a) and 10–20 cm (b) soil depth, and between distance from Sp$_1$ and MBN in the 0-10 cm (c) and 10-20 cm (d) soil depth at S$_2$. Sp$_1$: the most upper slope position; S$_2$: Site 2; MBC: microbial biomass C; and MBN: microbial biomass N.
Fig. S3.9 Seasonal patterns of MBC (a) and MBN (b) in the first five slope positions in 10-20 cm soil depth at S1.

Slope position; S1: Site 1; MBC: microbial organic C; and MBN: microbial organic N. Different letters indicated significant differences among the six sampling times at $P < 0.05$. 

Sp: slope position; S1: Site 1; MBC: microbial organic C; and MBN: microbial organic N. Different letters indicated significant differences among the six sampling times at $P < 0.05$. 

73
**Fig. S3.10** Seasonal patterns of MBC in the first six slope positions in 0-10 cm (a) and 10-20 cm (b) soil depth, and seasonal patterns of MBN in the first six slope positions in 0-10 cm (c) and 10-20 cm (d) soil depth at S2. Sp: slope position; S2: Site 2; MBC: microbial organic C; and MBN: microbial organic N. Different letters indicated significant differences among the six sampling times at $P < 0.05$. 

74
Fig. S3.11 The effects of soil moisture on HWEOC (a) and HWETN (b) in the 10-20 cm soils at S1. Sp1: the most upper slope position; S1: Site 1; HWEOC: hot-water extractable organic C; and HWETN: hot-water extractable total N.
Fig. S3.12 The effects of soil moisture on HWEOC in the 0-10 cm (a) and 10-20 cm (b) soils, and the effects of soil moisture on HWETN in the 0-10 cm (c) and 10-20 cm (d) soils at S2. Sp1: the most upper slope position; S2: Site 2; HWEOC: hot-water extractable organic C; and HWETN: hot-water extractable total N.
Fig. S3.13 The effects of soil moisture on MBC (a) and MBN (b) in the 10-20 cm soil depth at Sᵢ. Spᵢ: the most upper slope position; Sᵢ: Site 1; MBC: microbial biomass C; and MBN: microbial biomass N.
**Fig. S3.14** The effects of soil moisture on MBC in the 0-10 cm (a) and 10-20 cm (b) soil depth, and the effects of soil moisture on MBN in the 0-10 cm (c) and 10-20 cm (d) soil depth at S2. Sp1: the most upper slope position; S2: Site 2; MBC: microbial biomass C; and MBN: microbial biomass N.
Fig. S3.15 Relationships between hot-water extractable C (HWEOC) and microbial biomass C (MBC) in 0-10 cm (a) and 10-20 cm (b) soil depth, and between hot-water extractable total N (HWETN) and microbial biomass N (MBN) in 0-10 cm (c) and 10-20 cm (d) soil depth at Site 1 (S1) of Wyaralong Dam. HWEOC: hot-water extractable organic C; and HWETN: hot-water extractable total N. MBC: microbial biomass C; MBN: microbial biomass N; and S1: Site 1.
Fig. S3.16 Relationships between hot-water extractable C (HWEOC) and microbial biomass C (MBC) in 0-10 cm (a) and 10-20 cm (b) soil depth, and between hot-water extractable total N (HWETN) and microbial biomass N (MBN) in 0-10 cm (c) and 10-20 cm (d) soil depth at Site 2 (S₂) of Wyaralong Dam. HWEOC: hot-water extractable organic C; and HWETN: hot-water extractable total N. MBC: microbial biomass C; MBN: microbial biomass N; and S₂: Site 2.
Table S3.1 Two-way analyses of variation (ANOVA) for hot-water extractable organic C (HWEOC), hot-water extractable total N (HWETN), microbial biomass C (MBC) and microbial biomass N (MBN) in 0-10 and 10-20 cm soil profiles at Site 1 ($S_1$) and Site 2 ($S_2$)

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Sd - soil depth; and Sp – slope position
Chapter 4 Soil and sediment $\delta^{15}$N and $\delta^{13}$C provide insights into N and C dynamics of riparian zones in Southeast Queensland, Australia

4.1 Introduction

Damming has one of the most prominent anthropogenic impacts on natural wetlands, which can lead to greenhouse gas emission, anoxia, and sedimentation (Nilsson et al. 2005; Braatne et al. 2008). Impoundments create totally different hydrological regimes compared with free-flowing rivers through changing the timing, intensity, duration, frequency of water level fluctuations (Nilsson and Berggren 2000). As a transitional and interface zone between terrestrial and aquatic ecosystems, riparian areas are particularly sensitive to the variation of hydrological cycle, and can serve as good indicators of the environment changes caused by dam operations (Nilsson and Berggren 2000). Hydrological regimes, especially seasonal variations in flow and alternating wet-dry cycles, greatly impact the characteristics of riparian zones (New and Xie 2008).

The alternation of aerobic and anaerobic conditions caused by flooding has an important influence on soil organic matter (SOM) decomposition and nitrogen (N) cycling (Gergel et al. 2005; Ye et al. 2012). Soil N could be removed from water flowing through riparian soils (Schade et al. 2001), or by absorption of plants in riparian zones (Bardgett et al. 2007; Ye et al. 2012). A number of studies have been carried out to relate soil N dynamics to environmental changes in the riparian zone (Venterink et al. 2002; Gergel et al. 2005; Hernandez and Mitsch 2007). The ability of riparian zone to remove large quantities of N is affected by the type and quantity of plants and by soil conditions (Ashby et al. 1998; Jordan et al. 1998). High water tables in the riparian zones often lead to anoxic conditions, where denitrification occurs (Hill 1996). On the other hand, SOM decomposition is also affected by the soil moisture change in the riparian zones. Riparian areas possess high levels of soil moisture because of the water in the watershed, as well as the movement of groundwater towards the rooting zone (Bilby 1988). Accompanied by the flushing of organic litter from riparian sites by water, sediment periodically deposits to the riparian areas during floods.

Stable isotope compositions serve as a time-integrated measure of ecosystem processes, which is
important in studying the patterns of N cycling (Högberg 1997; Robinson 2001; Sah et al. 2006; Templer et al. 2007; Makarov 2009). Soil δ\(^{15}\)N can be used to identify source, deduce processes, determine rates, estimate inputs and confirm models (Martinelli et al. 1999; Sulzman 2008; Makarov 2009). Soil \(^{15}\)N could be used as an indicator of soil N dynamics and soil N sources in different ecosystems, which could reflect the fractionation of the soil N pools during microbial N transformations (Ibell and Blumfield 2010; Ibell et al. 2013; Bai et al. 2015; Wang et al. 2015). \(\delta^{15}\)N can be affected by numerous of factors, such as uptake of N sources with different isotopic signatures (Michelsen et al. 1996), isotopic discrimination (Högberg 1997; Robinson 2001; Brearley 2013), denitrification and vitalization (Houlton and Bai 2009; Makarov 2009). The enrichment of \(^{15}\)N in the soil is related to the fact that microbial discrimination which prefers the \(^{14}\)N over \(^{15}\)N (Makarov 2009). The products have more light \(^{14}\)N (Högberg 1997), while the residuals contain higher \(^{15}\)N abundance (Shearer and Kohl 1986; Natelhoffer and Fry 1988; Piccolo et al. 1996). There are several possible mechanisms involved. First, high rates of nitrification and N leaching contribute to a gradual enrichment in \(^{15}\)N in the products (Mariotti et al. 1981; Högberg 1997; Wang et al. 2015). Secondly, denitrification is a natural process of N loss with a large isotopic fractionation in soils with high nitrification rates. Lastly, in N-limited environment, plants need greater N deprived from mycorrhizal fungi with high retention of \(^{15}\)N-enriched N; while in N-enriched environment, plants rely on less N from mycorrhizal fungi, which results in higher foliar \(\delta^{15}\)N (Hobbie et al. 2000; Hobbie and Colpaert 2003). It is also found that \(^{15}\)N accumulation in ecology accompanies with more open N cycles (Makarov et al. 2003). A N-enriched ecosystem with an open N cycle, is gradually enriched with \(\delta^{15}\)N; while an ecosystem poor in available N with a slower N turnover, contains less \(^{15}\)N (Högberg et al. 1996; Martinelli et al. 1999; Robinson 2001).

Similarly, SOM dynamics and C turnover can be studied by \(\delta^{13}\)C (Xu et al. 2008; Ibell and Blumfield 2010; Cattaneo et al. 2014). The variations in \(\delta^{13}\)C of SOM have been recognized in several ecosystems, which could shed light on the soil C physical and biological processes (Högberg et al. 1995; Bernoux et al. 1998). Spatial patterns of soil \(\delta^{13}\)C have been reflected by a data set collected from different transects across the world, including several natural gradients, such as climatic, edaphic, and biotic control (Bird et al. 2002a; Bird et al. 2002b; Bird et al. 2004). However, previous studies mostly
focused on the spatial patterns of soil δ\textsuperscript{13}C at large scales, regional soil δ\textsuperscript{13}C data sets are currently incomplete. Studies on theoretical analysis of δ\textsuperscript{13}C dynamics in soil ecosystems are very limited (Ågren et al. 1996). The reasons partly lie in the fact that δ\textsuperscript{13}C of SOM varies both as a result of isotopic fractionation during the decomposition process, and the isotopic heterogeneity among various components in plant materials that decay at different rates (Feng 2002). The most fundamental observation is that soil δ\textsuperscript{13}C increased with soil depth (Ehleringer et al. 2000; Garten et al. 2000; Henn and Chapela 2000; Fernandez et al. 2003). The possible mechanisms are: changing isotopic ratios in atmospheric CO\textsubscript{2} over the past 200 years (the Suess effect) (Balesdent et al. 1993; Ehleringer et al. 2000); the enrichment of δ\textsuperscript{13}C as a result of fractionation during organic matter decomposition (Ehleringer et al. 2000; Accoe et al. 2002); the mixing of new C inputs with older SOM with different δ\textsuperscript{13}C (Balesdent et al. 1993); and preference decomposition by soil microorganisms (Feng 2002; Sulzmann 2008).

However, most of the previous studies on riparian soils were focused on N cycling (Chai et al. 2009; Chen et al. 2009; Iqbal et al. 2010; Ye et al. 2012). Few studies are available about the changes in soil N and C dynamics and how flooding would affect N and C dynamics in the water level fluctuation zone, especially in the subtropical riparian zone of a dam in the Southern Hemisphere (Smith et al. 2012). In this study, we studied the spatial and seasonal dynamics of soil δ\textsuperscript{15}N and δ\textsuperscript{13}C natural abundance within the riparian zones of Wyaralong Dam in southeast Queensland, Australia. We hypothesized that δ\textsuperscript{15}N and δ\textsuperscript{13}C would be affected significantly by the water fluctuation in the riparian areas. Our objectives of this study were to 1) determine whether δ\textsuperscript{15}N and δ\textsuperscript{13}C would vary along the transects in the riparian zone during different seasons; and 2) assess whether δ\textsuperscript{15}N and δ\textsuperscript{13}C could be used as the sensitive indicators for the soil N and C dynamics in the riparian zone.

4.2 Materials and methods

4.2.1 Site selection and soil collection

Because this is the second of two papers presenting results from a common set of experiments, only a brief description of the site selection and soil collection is give here; full details can be found in
Chapter 2. Two soil types commonly found in riparian zone of Wyaralong Dam were selected for study: Dermosols and Kurosols (Isbell 1996).

Composite soil (depths 0-10 and 10-20 cm) and sediment (depths 0-10 cm) samples were collected from six to ten slope positions (depending on the water level) along three parallel transects spaced ca. 5 meters apart in February 2013 (summer), May 2013 (autumn), August 2013 (winter), November 2013 (spring), February 2014 (summer) and August 2014 (winter), respectively. All samples were transported to the laboratory where field moist soils were well mixed and passed through a 2-mm sieve (roots were separated from soil during sieving) and stored at 4 °C until further analysis. Summary of soil properties in riparian areas was previously reported by Jiang et al. (2017).

4.2.2 Laboratory analyses

Soil moisture, soil pH, EC, TC, TN, δ¹³C and δ¹⁵N were analysed, using the methods described in Chapter 2.

4.2.3 Statistical analyses

Repeated measure analysis of variance (ANOVA) was used to determine if there was a significant difference in the interaction of slope position, soil depth and sampling season. The normality of all data was checked before ANOVA. All above statistical analyses were carried out using SAS for windows version 9.2 (SAS Institute Inc., Cary, NC, USA). Regression analyses were used to assess the spatial distribution of soil properties, and the relationships among soil moisture, soil C and N pools using OriginPro for windows version 8.5 (Analytical Software, MA, USA).

4.3 Results

4.3.1 Soil δ¹⁵N and δ¹³C

When all the data were pooled together for each site, repeated measure ANOVA showed that there were significant interactions among the soil slope position, soil depth, and sampling times for soil δ¹⁵N and δ¹³C, especially at S₁ (Table 4.1).
The spatial variation of soil $\delta^{15}$N along the transects at $S_1$ could be predicted by bivariate regression curves (0-10 cm) (Fig. 4.1a), and linear regression (10-20 cm) (Fig. S4.1a) in some seasons. For those in the 0-10 cm soil depth, soil $\delta^{15}$N decreased from the upland soil to the riparian soil, then increased to the sediment in summer 2013 ($P < 0.01$), spring 2013 ($P < 0.01$), summer ($P < 0.01$) and winter 2014 ($P < 0.05$), respectively (Fig. 4.1a). At $S_2$, soil $\delta^{15}$N showed similar trends as those at $S_1$ (Fig. 4.1b and Fig. S4.1b). Significantly seasonal differences in soil $\delta^{15}$N were found at almost all slope positions in both soil depths at both sites (Fig. 4.2 and Fig. S4.2). Generally, the highest soil $\delta^{15}$N was found in winter 2013 at both sites (Fig. 4.2 and Fig. S4.2).

Soil $\delta^{13}$C decreased along the transects within the first three positions, and then increased to the sediment in summer and winter 2014 ($P < 0.01$) in 0-10 cm soil depth at $S_1$ (Fig. 4.3). The seasonal patterns of soil $\delta^{13}$C were shown in Fig. S4.3.

Differences of TC and TN along the transects in both soil depths were only found in some seasons at both sites.
Table 4.1 Repeated measure analysis of variation (ANOVA) for total C (TC), total N (TN), stable C isotope composition (δ\(^{13}\)C) and N isotope composition (δ\(^{15}\)N) in 0-10 and 10-20 cm soil profiles at Site 1 (S\(_1\)) and Site 2 (S\(_2\)).

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Sd: soil depth; Sp: slope position; and St: sampling time.
Fig. 4.1 Relationship between distance from Sp\textsubscript{1} and $\delta^{15}$N in the 0-10 cm soil depth at S\textsubscript{1} (a) and S\textsubscript{2} (b).

Sp\textsubscript{1}: the uppermost slope position; $\delta^{15}$N: soil stable N isotope composition; S\textsubscript{1}: Site 1; and S\textsubscript{2}: Site 2.
Fig. 4.2 Seasonal patterns of $\delta^{15}$N within the first six slope positions in 0-10 cm soil depth at $S_1$ (a) and $S_2$ (b). Sp: slope position; $\delta^{15}$N: soil stable N isotope composition; $S_1$: Site 1; and $S_2$: Site 2. Different letters indicated significant differences among the six sampling times within a slope position at $P < 0.05$. 

Chapter 4
Fig. 4.3 The relationship between distance from Sp$_1$ and $\delta^{13}$C in the 0-10 cm soil depth at S$_1$.

Sp$_1$: the uppermost slope position; $\delta^{13}$C: soil stable C isotope composition; S$_1$: Site 1.
4.3.2 Relationship among soil moisture and soil C and N pools

Significant relationship between soil moisture and soil δ\textsuperscript{15}N was observed at S\textsubscript{1} (Figs. 4.4a and S4.5a). For the 0-10 cm depth soil (Fig. 4.4a), δ\textsuperscript{15}N increased with soil moisture in winter 2013 (\(P < 0.05\)), spring 2014 (\(P < 0.05\)), summer 2014 (\(R^2 = 0.875, P < 0.01\)) except that in summer 2013 (\(P < 0.05\)). The relationship between soil moisture and soil δ\textsuperscript{15}N was similar in the 10-20 cm soil depth as that in the 0-10 cm soil depth (Fig. S4.5a). On the other hand, positive correlations between soil moisture and soil δ\textsuperscript{15}N could be detected in summer 2013 (\(P < 0.05\)) and 2014 (\(P < 0.05\)) in the 0-10 cm soil depth (Fig. 4.4b), and winter 2014 (\(P < 0.01\)) in the 10-20 cm soil depth at S\textsubscript{2} (Fig. S4.5b).

The relationships between soil moisture and soil δ\textsuperscript{13}C at both sites were shown in Fig. 4.5 and Fig. S4.6. At S\textsubscript{1}, soil δ\textsuperscript{13}C was positively related to soil moisture in spring 2013 (\(P < 0.05\)), summer 2014 (\(P < 0.01\)) and winter 2014 (\(P < 0.05\)) in the 0-10 cm soil depth (Fig. 4.5a), as well as in winter 2013 (\(P < 0.05\)), summer 2014 (\(P < 0.01\)) in the 10-20 cm soil depth (Fig. 4.5b). By contrast, soil δ\textsuperscript{13}C was negatively related with soil moisture in the 10-20 cm soil depth at S\textsubscript{2} in some seasons (Fig. S4.6).
Fig 4.4 The effects of soil moisture on $\delta^{15}N$ at $S_1$ (a) and $S_2$ (b) in the 0–10-cm soil depth.

$\delta^{15}N$: soil stable N isotope composition; $S_1$: Site 1; and $S_2$: Site 2.
Fig. 4.5 The effects of soil moisture on $\delta^{13}C$ at $S_1$ in the 0–10-cm (a) and 10–20 cm (b) soil depths.

$\delta^{13}C$: soil stable C isotope composition; and $S_1$: Site 1.
4.3.3 Relationships of soil $\delta^{15}N$ with labile N pools

Because this is the second part of the two papers presenting results from a common set of experiments, the results of soil labile N and C pools were not given here, and full details can be found in Jiang et al. (2017). Negative correlations were found between soil $\delta^{15}N$ and hot-water extractable total N (HWETN), and between soil $\delta^{15}N$ and microbial biomass N (MBN) in some seasons in both soil depths at the two sites (Fig. 4.6). However, soil $\delta^{15}N$ was positively correlated with HWETN in winter and spring 2013 at S$_2$ (Figs. 4.6b and S4.8a). Positive correlations between soil $\delta^{15}N$ and MBN could be also found in winter 2013 (0-10 cm) and spring 2013 (10-20 cm) at S$_2$ (Figs. 4.6d and S4.8b).

4.3.4 Relationships of soil $\delta^{13}C$ with labile C pools

Similarly, negative correlations between soil $\delta^{13}C$ and labile C pools (HWEOC: hot-water extractable organic C; and MBC: microbial biomass C) could be found in some seasons in both soil depths at S$_1$ (Figs. 4.7 and S4.9). But soil $\delta^{13}C$ was positive correlated with HWEOC and MBC in 10-20 cm soil depth at S$_2$ (Fig. S4.10).

4.3.5 Relationship of soil $\delta^{15}N$ with soil C/N ratios and TN

Positive relationship was found between soil $\delta^{15}N$ and soil C/N ratios (0-10 cm) in summer 2013 ($P < 0.05$) where soil C/N was less than 12.2, while it turned to negative relationship after soil C/N ratios > 12.2 in autumn and spring 2013 (0-10 cm) ($P < 0.01$ and $P < 0.05$) respectively except soil collected in winter 2014 (0-10 cm) ($P < 0.05$) (Fig. 4.8a). Similar relationship was found at S$_2$ with the threshold of soil C/N ratios at 14 (Fig. S4.11a).
Fig. 4.6 Relationships between soil $\delta^{15}$N and HWETN at $S_1$ (a) and $S_2$ (b), and between soil $\delta^{15}$N and MBN at $S_1$ (c) and $S_2$ (d) in 0-10 cm soil depth.

$\delta^{15}$N: soil stable N isotope composition, HWETN: hot-water extractable total nitrogen, MBN: microbial biomass nitrogen, $S_1$: Site 1 and $S_2$: Site 2.
Fig. 4.7 Relationships between soil δ\(^{13}\)C and HWEOC (a) and between soil δ\(^{13}\)C and MBC (b) in 0-10 cm soil depth at Site 1 (S\(_1\)). δ\(^{13}\)C: soil stable C isotope composition, HWEOC: hot-water extractable organic C, MBC: microbial biomass carbon, and S\(_1\): Site 1.
Fig. 4.8 Relationships between $\delta^{15}$N and soil total C:N (a), and between $\delta^{15}$N and TN (b) in 0-10 cm soil depth at Site 1 ($S_1$).

$\delta^{15}$N: soil stable N isotope composition; and TN: total nitrogen.
Soil δ\textsuperscript{15}N was also found to be negatively related to soil TN in spring 2013 (0-10 cm, \(P < 0.01\)), summer 2014 (0-10 cm, \(P < 0.01\)) and winter 2014 (0-10 cm, \(P < 0.01\)) at S\textsubscript{1} (Fig. 4.8b), as well as in winter 2013 (0-10 cm, \(P < 0.01\)), summer 2014 (0-10 cm, \(P < 0.01\)) and winter 2014 (0-10 cm, \(P < 0.05\)) at S\textsubscript{2} (Fig. S4.11b).

4.4 Discussion

4.4.1 Spatial and seasonal patterns of δ\textsuperscript{15}N

In this study, soil δ\textsuperscript{15}N was spatially heterogeneous along the transects for the most of seasons at S\textsubscript{1}, but was not evident at S\textsubscript{2}. The changes in soil δ\textsuperscript{15}N were much more pronounced compared with soil δ\textsuperscript{13}C, which was consistent with a previous study by Tiunov (2007). Among those with significant spatial changes, soil δ\textsuperscript{15}N firstly decreased along the transects, and then increased towards to the sediment in the 0-10 cm soil depth at both sites. Most of the lowest levels of soil δ\textsuperscript{15}N were observed between Sp\textsubscript{2} and Sp\textsubscript{4}. The natural abundance of \textsuperscript{15}N (δ\textsuperscript{15}N) in ecosystem is always related to the soil N dynamics (Piccolo et al. 1994; Piccolo et al. 1996; Makarov 2009). Therefore, spatial changes in \textsuperscript{15}N enrichment or δ\textsuperscript{15}N in the riparian zone soil may reflect soil N dynamics, driven by the C availability. In these slope positions between Sp\textsubscript{2} and Sp\textsubscript{4}, the C availability was expected to be high due to the residual inputs from the topsoil (Jiang et al. 2017). Nitrogen assimilation by microorganisms was also high in these hotspots, and δ\textsuperscript{15}N fractionation during N assimilation could be compensating for δ\textsuperscript{15}N fractionation during N dissimilation. Hence, δ\textsuperscript{15}N was relatively low in the riparian zone than the adjacent area. Alternatively, more labile N pools were moved and accumulated in the riparian zone with lower δ\textsuperscript{15}N in these N pools. Previous studies showed that N fluxes in riparian zones may be more complex, since N often moves towards waterways (Likens et al. 1970; Puckett et al. 1999). These explanations could also be verified by the relationships between soil δ\textsuperscript{15}N and labile N pools. Slope positions with low soil δ\textsuperscript{15}N were always accompanied with high HWETN and MBN. Conversely, for the slope positions after Sp\textsubscript{4}, the relative availability of C was low but the activity of microorganisms was still high in the riparian zone. The organic N is mainly used as the source of C and energy, and \textsuperscript{15}N fractionation during the N dissimilation leads to the loss of light \textsuperscript{14}N isotope and high enrichment in \textsuperscript{15}N (Peterson and Fry 1987; Pörtl et al. 2007; Templer et al. 2007). Another possible reason for the enriched δ\textsuperscript{15}N in the riparian
zone soil in lower slope position is that soil microbial processes such as N mineralization, nitrification and denitrification discriminate against $^{15}$N. Lower slope positions often suffer from inundation caused by the water fluctuation. Thus, anoxic condition resulted from high water level always lead to denitrification (Del Grosso et al. 2000; Heinen 2006). This may cause the losses of $^{15}$N-depleted N forms from soils via nitrate ($\text{NO}_3^-$) leaching and N$_2$O or N$_2$ emission, and the remaining soil N enriched in $^{15}$N (Mariotti et al. 1981; Högberg 1997). Similarly, soil $\delta^{15}$N increased along the transects in summer 2014 in the 10-20 cm soil depth at both sites. It could also be explained by denitrification and leaching in the lower soil depth. Although negative relationships between soil $\delta^{15}$N and labile N pools could be detected in most of the seasons at both sites, soil $\delta^{15}$N was found to be positively related with HWETN and MBN in winter and spring 2013 at S$_2$ (Figs. 4.8 and S4.8). This might also be explained as the above. When soil C availability was not high enough for soil microbial activities (in winter or after dry-rewetting effects, etc.), soil organic N was released as the source of C and energy, which led to the enrichment in $^{15}$N especially in the riparian zones with higher microbial activities.

Generally, soil $\delta^{15}$N increased with soil depth in this study. It was consistent with previous findings (Shearer and Kohl 1986; Fry 1991; Högberg 1997). Koopmans et al. (1997) investigated soil $\delta^{15}$N in two forest stands in the Netherlands exposed to elevated N inputs for approximately 40 years. They found that soils at both sites showed characteristic low $\delta^{15}$N values in the organic layers, increasing strongly in the mineral soil. And they attributed it to fractionation during decomposition of SOM followed by losses of $^{15}$N-depleted products, such as uptake by the vegetation or leaching. The profile difference of soil $\delta^{15}$N is controlled by a combination of the N mineralization, humification, and the redistribution of isotopes in the soil (Högberg 1997). The mechanisms involved are: discrimination during decomposition; differential preservation of components enriched in $^{15}$N; and illuviation of $^{15}$N enriched organic matter in deeper soil horizons (Natelhoffer and Fry 1988). Soil microorganisms substantially fractionate $^{15}$N during N assimilation. Tens of ppm changes of $\delta^{15}$N could occur accompanied with nitrification or ammonification (Högberg 1997; Robinson 2001). In addition, SOM in the lower horizons of profile was subjected to more microbial transformation cycles, which led to more enriched $^{15}$N (Högberg 1997). However, we still do not know the exact mechanism responsible for pronounced $^{15}$N depletion with soil depth in this study, and further research is needed to evaluate
the contributions of natural processes.

Clear seasonal variations were also obtained, but not exactly the same for all slope positions and different sites. Generally, the lowest level of soil δ15N was observed in summer and highest in winter 2013. During the warm period, organic N is available in excess and is converted into nitrate. After the vigorous vegetation growth in spring, N release is particularly marked in summer. As a result, soil contains more depleted 15N with more plant litter input. Natelhoffer et al. (1988) studied formation and decay of SOM in surface soils of two oak forests in Wisconsin, and found that low surface soil δ15N resulted from high litter inputs. Another assumption is that more labile N pools were flushed from upper slope position to lower position by flooding on 28 January 2013 (174.6 mm), which led to the relative higher soil δ15N in the upper slope position soil compared with the relative low one in soils at lower slope position. On the contrary, isotopic fractionation exerted more influence in winter, leading to greater 15N abundance during N transformations. With fewer residue input, the light N isotope is lost to atmosphere as gas emission during the N mineralization, nitrification, and denitrification and is leached from the soil as NO3⁻ (Makarov 2009). However, soil δ15N in winter 2014 was not as high as it was in 2013 from Sp3 afterwards at S1. It was consistent with the seasonal variation of soil moisture. But soil δ15N in summer 2014 was almost the same as it was in winter 2014. On the other hand, the patterns at S2 were not so clearly, due to both of the gentle slope degree and coarser sandy sediment associated with low level of organic material (Brunet and Astin 1997).

Soil C and N availability play a pivotal role in microbial metabolism. Thus, C and N isotope fractionation by N loss processes, especially soil δ15N, may be controlled by soil C and N (Dijkstra et al. 2008; Zhou et al. 2014). Previous studies found that the combination of TN, C/N and soil δ15N could provide more information on the ‘N status’ than if only one of these variables were measured (Stevenson et al. 2010; Mudge et al. 2014; Zhou et al. 2014). Enrichment of soil δ15N was related to higher TN, lower C/N ratios and greater N losses were observed in many studies (Högberg 1990; Högberg and Johannisson 1993; Högberg et al. 1996). Our study did not support the previous findings, but also showed soil C/N was an important factor controlling patterns of soil δ15N. In this experiment, negative correlations between soil δ15N and TN and between δ15N and C/N in the 0-10 cm soil depth at
S1 in spring 2013 and 0-10 cm soil depth at S2 in winter 2013 were observed, which may indicate the possibility of lost organic N (Mudge et al. 2014). But in winter 2014 with relative low C availability, negative correlation between soil $\delta^{15}$N and TN but positive correlation with C/N in the 0-10 cm soil depth at S1 were found, which may suggest the N immobilization (Mudge et al. 2014) or N discrimination by gas emission or leaching (Högberg 1997). Similar trend was also found in soil in the 10-20 cm soil depth as S1 in winter 2013, which showed the possibility of $^{15}$N fractionation or N immobilization in the subsoil. These results are consistent with above analyses of the spatial and seasonal patterns of soil $\delta^{15}$N. A critical value of soil C/N ratio determining whether the relationships between $\delta^{15}$N value and soil C/N ratio were positive or negative were detected in many studies (Stevenson et al. 2010; Mudge et al. 2014; Zhou et al. 2014). For example, Stevenson et al. (2010) analysed 210 New Zealand soils from different land-use systems for soil $\delta^{15}$N, TN and C/N. They found a negative correlation between $\delta^{15}$N and C/N excluding sites with C/N > 18. Zhou et al. (2014) investigated the patterns of TN and $\delta^{15}$N values in different ecosystems and soil profiles on the Qinghai-Tibetan Plateau, and observed a threshold of C/N of about 11 dividing the parabolic relationship between soil $\delta^{15}$N values and C/N into positive (C/N < 11) and negative (C/N > 11) parts across all ecosystems and soil profiles. They attributed the trend to C availability. Lower C availability led to low C/N, which constrained soil microbial activity. With the increase of C/N, soil microbial activity was stimulated by the enhancement of SOM, which led to soil $\delta^{15}$N enrichment. When C/N was below 11 and soil became more N saturated, the fractionation of soil $\delta^{15}$N increased when N became a limiting factor instead of C. Results of this study identified a threshold of 12.2 and 14 at S1 and S2 respectively where positive correlation between soil $\delta^{15}$N and soil C/N below the threshold turned to be negative correlation above the value. These trends could also be explained by the variation of microbial activity driven by soil C and N availability. However, a positive correlation between soil $\delta^{15}$N and C/N was also detected when soil C/N > 13 at S1 in the 0-10 cm soil depth in winter 2014 and in the 10-20 cm soil depth in winter 2013. That would probably be because isotope fractionation N-loss process would be lower at higher C/N, due to high rates of N immobilization into SOM (Stevenson et al. 2010). Therefore, soil C/N explained part of the variation in soil $\delta^{15}$N, indicating that relative C availability may be a key factor controlling pattern of soil $\delta^{15}$N in the riparian of Wyaralong Dam for some seasons.
Soil $\delta^{15}\text{N}$ at $S_1$ was generally higher than those at $S_2$. Soil texture could affect soil $\delta^{15}\text{N}$. Previous studies (Tiessen et al. 1984; Liao et al. 2006; Makarov 2009) showed that the different fractions contain different concentrations of $^{15}\text{N}$ with high $^{15}\text{N}$ being noted only in fine fractions.

### 4.4.2 Spatial and seasonal patterns of $\delta^{13}\text{C}$

Little work has been done on the spatial and seasonal changes of $\delta^{13}\text{C}$ in the riparian soils. In this study, spatial changes of soil $\delta^{13}\text{C}$ were observed for some seasons at both sites (Figs. 4.3 and S4.3). Among them, the lowest levels of soil $\delta^{13}\text{C}$ were found in summer and winter 2014 at $S_1$ within the first four slope positions, separately. On the other hand, there was a peak of soil $\delta^{13}\text{C}$ along the transects within the first four slope positions in winter 2013 at $S_1$, and summer 2014 at $S_2$ in the 10-20 cm soil depth. Feng et al. (2002) conducted a theoretical analysis on $\delta^{13}\text{C}$ of SOM and decomposing plant litters. Their model showed that the variation of $\delta^{13}\text{C}$ may result from both of isotopic fractionation during decomposition and the isotopic heterogeneity of different components decaying at different rates. In the present study, the water level was relative low in summer and winter 2014. As a result, the riparian vegetative stripes moved a little bit further towards the watershed, which may trigger the changes in the hotspot of microbial activity along the transects. The relative low level of soil $\delta^{13}\text{C}$ around $S_2$ in summer 2014 at $S_1$ may be attributed to the high decomposition rate in these slope positions. In winter 2014, the rate of microbial activity and litter decomposition decreased due to the soil moisture and low temperature. As a result, the isotopic fractionation decreased, which led to high soil $^{12}\text{C}$. On the other hand, the water level almost reached 100% of full water level in winter 2013. The position of riparian vegetative stripes was relative high along the transects, with low litter decomposition at the lower slope positions. The correlations between soil $\delta^{13}\text{C}$ and labile C pools also proved theses explanations. Soil $\delta^{13}\text{C}$ in the 10-20 cm soil depth increased compared with those in the upper slope position due to the decreased litter input from the topsoil. In spring 2013, soil $\delta^{13}\text{C}$ decreased along the transects in the 10-20 cm soil depth at $S_2$. It showed that this area received high rainfall in November 2013, which might flood more C from the upper slope position leading to high level of soil $\delta^{13}\text{C}$ with higher HWEOC in the lower positions along the transects in deeper soil depth. It might also be attributed to the dry-rewetting effect (Birch 1958; Jarvis et al. 2007; Inglima et al. 2009), leading to more CO$_2$.
release after rewetting dry soil in upland soil. When comparing the spatial distribution of soil $\delta^{13}$C with the relationship between soil moisture and soil $\delta^{13}$C, it was found that they were similar but not exactly the same. It indicated that although soil moisture play a key role in the spatial distributions of soil $\delta^{13}$C, other factors such as litter decomposition might also exert influence on the temporal patterns obtained.

We also found general increase in soil $\delta^{13}$C with depth at both sites. Our result was consistent with previous studies. It is found that $^{13}$C-abundance always increased with soil depth (Ehleringer et al. 2000; Garten et al. 2000; Henn and Chapela 2000; Fernandez et al. 2003). The underlying mechanisms remain unclear. There may be several mechanisms involved at the same time. Accoe et al. (2002) investigated $\delta^{13}$C of SOM with increasing depth in a soil profile under permanent grassland, and suggested that soil $\delta^{13}$C enrichment could be explained by isotopic discrimination associated with SOM decomposition. Balesdent et al. (1993) measured soil $\delta^{13}$C at 14 locations in a temperate forest, and attributed the enrichment with depth to the mixing of new SOM with older ones with different $\delta^{13}$C. Other explanations include changing isotopic ratios in atmospheric CO$_2$ over the past 200 years (Friedli et al. 1984) and preference decomposition by soil microorganisms (Feng 2002; Sulzman 2008).

Clear seasonal patterns of soil $\delta^{13}$C were not observed, except for few slope positions. Generally, among those with significant difference, the lowest levels of soil $\delta^{13}$C were found in summer and highest in winter at S1. The possible explanations may be the same as we explained above in the spatial patterns, mainly attributed to increased plant cover induced by the decrease of water level.

4.5 Conclusion

Soil $\delta^{15}$N and $\delta^{13}$C showed different spatial and seasonal variations in subtropical riparian zones. There may be some hotspots for microbial activities along the transects in the riparian areas of Wyaralong dam where soil microbial activity was higher, driven by soil moisture and soil C availability. Subsoil $\delta^{15}$N and $\delta^{13}$C in general appeared less affected by environmental changes than did surface $\delta^{15}$N and $\delta^{13}$C in the riparian areas. Soil $\delta^{15}$N and $\delta^{13}$C could provide insights into soil N and C dynamics in the riparian zones of Wyaralong Dam. However, soil $\delta^{15}$N appeared to be a much more sensitive indicator of N dynamics in the riparian areas, particularly in combination with more typical measures of N status.
such as labile N pools, TN and the C/N ratio. While $\delta^{15}N$ and $\delta^{13}C$ varied considerably at both sites, these data highlight the importance of riparian zones as the hotspots for N and C dynamics. Our study fills a unique data gap for soil stable N and C isotope fractionation in the subtropical riparian ecosystems. However, further research would be required to test the specific mechanisms regulating soil $\delta^{15}N$ and $\delta^{13}C$ in the riparian zones.
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Fig. S4.1 Relationships between distance from Sp$_1$ and $\delta^{15}$N in the 10-20 cm soil depth at S$_1$ (a) and S$_2$ (b). Sp$_1$: the uppermost slope position; $\delta^{15}$N: soil stable N isotope composition; S$_1$: Site 1; and S$_2$: Site 2.
Fig. S4.2 Seasonal patterns of $\delta^{15}$N within the first five slope positions in 10-20 cm at $S_1$ (a) and $S_2$ (b)

Sp: slope position; $\delta^{15}$N: soil stable N isotope composition; $S_1$: Site 1; and $S_2$: Site 2. Different letters indicated significant differences among the six sampling times within a slope position at $P < 0.05$. 
Fig. S4.3 Relationships between distance from Sp$_1$ and $\delta^{13}$C in the 10-20 cm soil depth at S$_1$ (a) and S$_2$ (b). Sp$_1$: the uppermost slope position; $\delta^{13}$C: soil stable C isotope composition; S$_1$: Site 1; and S$_2$: Site 2.
Fig. S4.4 Seasonal patterns of δ¹³C within the first six slope positions in 0-10 cm (a) and 10-20 cm (b) soil depth at S₁, and in 0-10 cm (c) and 10-20 cm (d) soil depth at S₂. Sp: slope position; and δ¹³C: soil stable C isotope composition; S₁: Site 1; and S₂: Site 2. Different letters indicated significant differences among the six sampling times within a slope position at $P < 0.05$. 

Page 118
Fig. S4.5 The effects of soil moisture on $\delta^{15}$N at $S_1$ (a) and $S_2$ (b) in the 10-20-cm soils depth.

$\delta^{15}$N: soil stable N isotope composition; $S_1$: Site 1; and $S_2$: Site 2.
Fig S4.6 The effects of soil moisture on δ\textsuperscript{13}C at S\textsubscript{2} in the 10-20 cm soil depth.

δ\textsuperscript{13}C: soil stable C isotope composition; and S\textsubscript{2}: Site 2.
Fig. S4.7 Relationships between soil $\delta^{15}$N and HWETN (a), and between soil $\delta^{15}$N and MBN (b) in 10-20 cm soil depth at S$_1$. $\delta^{15}$N: soil stable N isotope composition, HWETN: hot-water extractable total nitrogen, MBN: microbial biomass nitrogen, and S$_1$: Site 1.
Fig. S4.8 Relationships between soil $\delta^{15}$N and HWETN (a), and between soil $\delta^{15}$N and MBN (b) in 10-20 cm soil depth at $S_2$. $\delta^{15}$N: soil stable N isotope composition, HWETN: hot-water extractable total nitrogen, MBN: microbial biomass nitrogen, and $S_2$: Site 2.
Fig. S4.9 Relationships between soil $\delta^{13}$C and HWEOC (a), and between soil $\delta^{13}$C and MBC (b) in 10-20 cm soil depth at $S_1$. $\delta^{13}$C: soil stable C isotope composition, HWEOC: hot-water extractable organic C, MBC: microbial biomass carbon, and $S_1$: Site 1.
Fig. S4.10 Relationships between soil δ^{13}C and HWEOC (a), and between soil δ^{13}C and MBC (b) in 10-20 cm soil depth at Site 2 (S_2). δ^{13}C: soil stable C isotope composition, HWEOC: hot-water extractable organic C, MBC: microbial biomass carbon, and S_2: Site 2.
Fig. S4.11 Relationships between $\delta^{15}$N and soil total C:N (a), and between $\delta^{15}$N and TN (b) in 0-10 cm soil depth at Site 2 ($S_2$).

$\delta^{15}$N: soil stable N isotope composition; and TN: total nitrogen.
Chapter 5 Effects of soil moisture on nitrogen transformations of subtropical riparian soils in Southeast Queensland, Australia

5.1 Introduction

Nitrogen (N) is a major limiting factor for plant growth and net primary productivity in many terrestrial ecosystems. Nitrogen cycling in riparian soils is thought to be highly sensitive to soil moisture variations (Bai et al. 2004; Sleutel et al. 2008). Soil moisture is a key factor affecting the vitality and activity of soil microorganisms by controlling the water and oxygen availability in the soils (Borken and Matzner 2009), thus impacting microbially mediated N transformations (Bengtson et al. 2005; Chen et al. 2011). In other words, the importance of soil moisture on N cycling lies in the fact that it controls soil microbial activity, and thus determines rates of N transformations. Therefore, the influence of soil moisture on soil N transformations should be understood explicitly.

Generally, soil moisture varies with the balance between inputs and outputs of water in the soil profile, such as precipitation, and surface and ground water (Yu and Ehrenfeld 2009). Riparian areas are subject to changes in soil moisture as a result of both seasonal climate variations and anthropogenic water management (e.g. dam water releases), both of which can modify the dynamics of ground and surface water inputs. Depending on the hydrological status, N cycling is likely to be heavily impacted by climate conditions, such as the pattern of precipitation (Chen et al. 2012). More dynamic changes of water table in future is expected due to shifts in the precipitation patterns (IPCC 2014). Therefore, the responses of soil N availability to global environmental changes are critical (Luo et al. 2004).

Nitrogen turnover in riparian soils is sensitive to water fluctuation and O$_2$ supply (Pal et al. 2010). Autotrophic nitrification (the oxidation of ammonium ($\text{NH}_4^+$-N) to nitrate ($\text{NO}_3^-$-N)) rarely occurs under anaerobic conditions; while it is possible for ammonification (the transformation of organic N to $\text{NH}_4^+$-N) (Pinay et al. 2002; Hefting et al. 2004) and immobilization of $\text{NH}_4^+$ (Ambus et al. 1992) under both aerobic and anaerobic conditions. Thus, nitrification decreases as soils become increasingly anoxic; while denitrification increases under the same condition (Pinay et al. 2002). Among them, of
special interest are microbial N transformations resulting in N loss from the soil-water system, such as
denitrification (the transformation of NO$_3^-$ to gaseous N (N$_2$ and N$_2$O)) (Zumft 1997), and the process
of annammox (anaerobic NH$_4^+$ oxidation, the transformation of NH$_4^+$ to N$_2$) (Van de Graaf et al. 1995).
Soil N could be lost as N$_2$O through nitrification under aerobic conditions, as well as through
denitrification under anaerobic conditions (Stevens et al. 1997; Wolf and Russow 2000). Since rates of
N mineralization and nitrification play a key role in N cycling, better knowledge about the variability in
rates of N mineralization and nitrification in the riparian zones is needed to further improve the current
terrestrial ecosystem N cycling models (Asner et al. 2001).

N mineralization is known as the transformation process from organic N to inorganic N, which
primarily determines soil N availability (Wang et al. 2006). The effect of soil moisture on net N
transformation rates has been extensively studied (Owen et al. 2003; Vernimmen et al. 2007; Yu and
Ehrenfeld 2009). Soil moisture can affect net N mineralisation and nitrification rates significantly
(Sierra 1997; Paul et al. 2003). Stanford and Epstein (1974) examined the relationships between soil
moisture and net N mineralization for nine different soil types. Maximum net N mineralization rates
occurred at soil matric potential between 0.3 and 0.1 Mpa depending on soil types, which was close to
field water holding capacity. However, net N transformation is the outcome of competitions between
several N cycling processes, particularly N mineralization and immobilization, that together determine
the net release (Murphy et al. 2003).

To quantify the influence of soil moisture on each N cycling process, gross N transformation rates need
to be determined, which provides a deeper and fundamental understanding of the N cycling processes
(Nannipieri and Eldor 2009). Contradicting results in relation to soil moisture effects on gross N
mineralization have been reported from both field and laboratory experiments. Rates of gross N
mineralization under anaerobic conditions were reported smaller than those under aerobic conditions
(Ambus et al. 1992; Wray and Bayley 2008). Inundation of aerobic peat soil enhanced N losses
compared to the soils under continuously aerobic or anaerobic conditions (Reddy and Patrick Jr 1975).
Generally, gross N mineralization and nitrification rates increase with increasing soil moisture below
field capacity, and then progressively decline as the soils become saturated in arable (Nishio et al. 1985;
Mathieu et al. 2006), pasture (Zaman et al. 1999) and forest lands (Bengtson et al. 2005; Burton et al. 2007; Chen et al. 2011). For example, Linn and Doran (1984) studied different tillage regimes, and pointed out that the highest nitrification activity is usually occurred at water-filled pore space (WFPS) of ~ 60% in surface soils. Similarly, the relationship between soil moisture status and gross nitrification rates could be best described by O’Neill functions which have optimum condition for nitrification at 65% WFPS in both montane and lowland tropical rainforest sites (Kiese et al. 2008). Nevertheless, the optimal soil moisture content for gross nitrification depends on various factors, such as the soil type, pore size distribution, bulk density, and soil organic matter content. The optimum soil moisture content for gross nitrification is around 65-80% water holding capacity (WHC) (Gödde and Conrad 1998; Kiese et al. 2008; Sleutel et al. 2008). Low soil moisture contents usually limit the diffusive transport of solutes or soil drying can partially kill and/or suppress soil microorganisms (Borken and Matzner 2009); while high moisture regime always leads to insufficient oxygen concentration which is not conductive for nitrification (Mathieu et al. 2006; Cheng et al. 2012).

Previous in situ and laboratory incubation studies have demonstrated that net N transformation rates generally differ substantially from the gross rates (Hart et al. 1994a; Verchot et al. 2001; Lang et al. 2010), indicating that the responses of net and gross rates to changes in soil moisture may be different. In fact, net rates of soil N transformation are a poor indicator of actual rates of N cycling in soils. Therefore, gross N transformation rates provide information about each microbial process; while the net N transformation rate may be a better indicator for the actual soil N availability (Zaman and Chang 2004). Therefore, a better understanding of the effect of soil water availability on net and gross N transformations in the riparian soil will facilitate our predictions of soil N dynamics in riparian ecosystems.

Although much progress has been achieved in understanding the influence of soil moisture on gross N transformations in ecosystems such as forests (Kiese et al. 2008; Chen et al. 2011; Cheng et al. 2014), our knowledge about the rates and dynamics of N in the riparian areas is very limited. In subtropical regions, soil moisture varies frequently and widely throughout the year with precipitation. Therefore, studies on the effects of moisture on soil N dynamics are of great importance to predict the N
availability in subtropical riparian zones. The aim of this study was to investigate the effect of soil moisture changes on individual N gross transformation processes in two subtropical riparian soils. By comparing the responses of gross N transformation rates to soil moisture changes in two different soils, we expected to improve our capacity to predict the N availability in relation to soil moisture changes arising from either precipitation or anthropogenic management. We hypothesized that changes in moisture status of different types of riparian soils would affect N dynamics in similar ways but in different magnitudes. In order to test this hypothesis, a laboratory incubation study was carried out to assess net and gross N mineralization and nitrification under different moisture regimes in two soil types by the $^{15}$N dilution method. In addition, to improve our understanding of the N transformations in soil, distributions of $^{15}$N in different forms were also determined. Although short-term laboratory studies do not necessarily reflect field conditions (Booth et al. 2005), our laboratory study aimed to provide a mechanistic understanding of the dynamics of soil gross N transformations in response to soil moisture changes.

5.2 Materials and methods

5.2.1 Sample collection

Soils used in this study were collected from Site 1 (S$_1$) and Site 2 (S$_2$) in the riparian zone of Wyaralong Dam (27°54'28.77"S, 152°52'53.59"E), Southeast Queensland in August 2015 (dry season). Details of the study area were provided in Chapter 2 and by Jiang et al. (2017). The reason to choose soils in August 2015 was that soil moisture was low in dry season and could be readily adjusted to higher soil moisture levels. These soils are representative of the soils in this area in terms of texture, N and C contents, and hydrologic properties.

Soil samples (0-10 cm) used in this study were collected from three slope positions along three transects. They represented the upper slope position, the riparian zone and sediment. We used an auger with 7.5 cm in diameter to collect soil samples. Three soil cores were collected from each position, and the nine cores from the same positions of three transects were mixed thoroughly as one composite sample. Sediment samples were collected with a shovel, and then mixed to form one composite sample.
All samples were transported to the laboratory where the field moist soils were well mixed and passed through a 2-mm sieve and stored at 4 °C until the laboratory incubation could be conducted. The samples with high soil moisture could not be sieved, thus were mixed as thoroughly as possible after removing roots. Subsamples of each soil were air-dried for chemical analysis.

5.2.2 Soil C and N pools

There were two groups with different soil types from S₁ and S₂. For soil samples from the upper slope position, four soil moisture regimes were applied by adding distilled water to fresh soil samples collected in the dry season to simulate typical soil conditions in four typical circumstances: (1) the dry season (50% WHC), (2) the wet season (70% WHC), (3) after transforming into the riparian zone (90% WHC), and (4) under waterlogged conditions (200% WHC). For soil samples collected from the riparian zone during the dry season, there were two soil moisture regimes representing: (1) the riparian soil (90% WHC), and (2) the soil transformed from the riparian zone soil into the sediment (200% WHC). The soil moisture was kept at 200% WHC for the sediment which was waterlogged all the time. Those treatments were used to represent a broad range of possible situations in situ, including (1) different soil moistures to simulate upland and riparian soil under water fluctuations; and (2) the long-term field water regimes for the upland, riparian soils and sediment.

Total C (TC), total N (TN), stable C and N isotope composition (δ¹³C and δ¹⁵N, respectively), soil mineral N (NH₄⁺ and NO₃⁻), soil hot-water extractable organic C (HWEOC) and hot-water extractable total N (HWETN), water-soluble organic C (WSOC) and water-soluble total N (WSTN), microbial biomass C (MBC) and microbial biomass N (MBN) were measured for the 6 pre-incubation samples (3 slope positions × 2 sites). Soil subsamples were analysed in triplicate to determine average initial physicochemical properties. Details of measurements were described in Chapter 2.

5.2.3 Measurement of gross N mineralisation and gross nitrification

This experiment was conducted under different moisture conditions. After conditioning at field moisture for two weeks at 25°C, two sub-samples of soils (10 g, dry weight equivalent) were labelled with either (¹⁵NH₄)₂SO₄ solution (10 atom% ¹⁵N excess), or K¹⁵NO₃ solution (5 atom% ¹⁵N excess).
They were evenly sprinkled to the soil using a syringe. The tubes were then capped and placed into an incubator at 25 °C. After 3 h, as suggested by Murphy et al. (2003), the time zero (T₀) samples were removed from the incubator and extracted with 50 ml of 2 M KCl. Samples were shaken for 1 h, centrifuged at 2000 rpm for 10 min and then filtered through Whatman No. 42 filter paper and frozen until analysis. The remaining samples (T₁) were removed from the incubator after 72 h and extracted as above. The residual soil samples after the KCl extractions (T₀ and T₁) were washed with about 40 ml of 0.01 M CaCl₂ solution by dispersing and centrifuging for 3-4 times to remove the residual ¹⁵N-NH₄⁺ and ¹⁵N-NO₃⁻ (Wang et al. 2001), and then analysed on an isotope ratio mass spectrometer to quantify the N losses via the ¹⁵N mass balance approaches. Soil organic N was assumed to be the N remaining in soil after KCl extraction.

5.2.4 Microdiffusion process for ¹⁵N enrichment of KCl extracts

¹⁵N enrichment was determined by a modified micro-diffusion method according to Stark and Hart (1996). The 30 ml KCl extracts were added into 50 ml plastic bottles. Since both concentrations of the NH₄⁺-N and NO₃⁻-N were low in the extracts, either a known amount of standard (NH₄)₂SO₄ or KNO₃ solution was added as a spike in order to reach the lowest detection limit for the mass spectrometer (total N: 50 μg). After adding 4 μl 2.5 M KHSO₄ solution onto the filter paper discs (~0.5 cm diameter cut from a Whatman No. 42 filter paper), a Teflon tape was used to carefully seal around the disc to form an acid trap. One hundred milligram of MgO was added to each bottle containing the KCl-extract, the acid trap was simultaneously added and the bottle was capped immediately. The bottles were placed on a rotary shaker at 150 to 200 rpm at 25 °C for 5 days. Thereafter, the bottles were opened and the acid traps for NH₄⁺-N were carefully put into 1.5-ml reaction tubes and placed into a desiccator containing a beaker with concentrated sulphuric acid for drying for at least 24 h. Then, 50 mg of Devarda alloy was added to the same bottle containing the KCl-extract for determination of ¹⁵N enrichment of NO₃⁻-N. After addition of another acid trap, the bottle was closed immediately. Thereafter, the bottles and acid traps were treated the same as described above. Inorganic N concentrations in the KCl extracts were determined using a Discrete Chemistry Analyser (Westco Smartchem SC 200, Discrete Wet Chemistry Analyser). ¹⁵N enrichments of NH₄⁺-N and NO₃⁻-N
recovered in the acid traps were measured using the same isotope ratio mass spectrometer as mentioned above (Wang et al. 2015).

5.2.5 Calculations

Net N ammonification, nitrification and mineralization rates were calculated as the difference of NH$_4^+$-N, NO$_3^-$-N and mineral N concentrations between the start and the end of the 3-day incubation. Rates of gross N mineralisation and ammonium consumption and nitrification were calculated using the equations developed by Kirkham and Bartholomew (1954), and presented by Hart et al. (1994b). The gross N mineralization rates were calculated a Whatman No. 42 filter paper for the $(^{15}\text{NH}_4^+)_2\text{SO}_4$-labelled soils using the following equation:

$$\text{Gross N mineralization rate} = \frac{[\text{NH}_4^+]_0 - [\text{NH}_4^+]_t}{t} \times \frac{\log APE_0}{\log APE_t}$$

where $t$=time (day); $APE_0$=atom percent $^{15}$N excess of NH$_4^+$ pool at time $T_0$; $APE_t$=atom percent $^{15}$N excess of NH$_4^+$ pool at time $T_1$; $[\text{NH}_4^+]_0$ and $[\text{NH}_4^+]_t$ were NH$_4^+$ concentration (mg kg$^{-1}$) at time $T_0$ and $T_1$. The gross nitrification rates were calculated using the same equations by substituting NO$_3^-$ concentrations, and atom percent $^{15}$N excesses of NO$_3^-$ pool.

The gross NO$_3^-$ and NH$_4^+$ removal rates were calculated as the difference between the gross nitrification/mineralisation rates derived from the isotope dilution equation and the net nitrification/mineralisation rates. Soil NO$_3^-$ removal included denitrification, direct NO$_3^-$ reduction to NH$_4^+$ (DNRA) and immobilisation, while NH$_4^+$ removal included autotrophic nitrification, anammox-coupled-nitrification-denitrification, and immobilisation. In this study, heterotrophic nitrification refers only to transformation of organic N to NO$_3^-$ (Matheson et al. 2003). The relative contributions of individual processes to the overall production or removal rate were determined by $^{15}$N tracing techniques in conjunction with the isotope dilution approach.
5.2.6 Statistical analyses

Changes in net and gross N transformation were analysed using one-way analysis of variance (ANOVA) with soil moisture as a factor. All comparisons among the different soil moistures were conducted by the Duncan’s Multiple Range Test (DMRT). Significant differences in net ammonification (NA), net nitrification (NN), net mineralization (NNM), gross N mineralization (GNM) and nitrification (GN) rates in the 0-10 cm soils during the 3-day incubation of soils were reported at \( P < 0.05 \). The normality of all data was checked before ANOVA. All above statistical analyses were carried out using SAS for windows version 9.2 (SAS Institute Inc., Cary, NC, USA). Pearson’s correlation analyses were obtained to test the relationships among net and gross N transformation rates for soils from both sites, which were performed with SPSS Statistics 20.0 (SPSS, Chicago, IL).

5.3 Results

5.3.1 Net N transformation rates

Soil basic properties were shown in Table 5.1. When the data were pooled together for each site, significant effect of slope position and soil moisture could be found on soil net N ammonification, nitrification and mineralization rates \( (P < 0.05, \text{Fig.5.1 and Table S5.1}) \). Specifically for \( S_1 \), net ammonification rates under the field moisture conditions were similar for soils from different slope positions, with slight increase from \(-0.28 \pm 0.04\) mg N kg\(^{-1}\) d\(^{-1}\) in upland soil (U50%), \(-0.19 \pm 0.01\) mg N kg\(^{-1}\) d\(^{-1}\) in riparian soil (R90%), to \(0.04 \pm 0.27\) mg N kg\(^{-1}\) d\(^{-1}\) in sediment (S200%). Within each slope position, net ammonification rates were higher at 200% WHC compared to the lower moisture contents. On the contrary, net nitrification and N mineralization rates decreased significantly from unsaturated to saturated soil within the same slope positions, with negative values recorded at \(\geq 90\%\) WHC. Net N mineralization rate for \( S_2 \) first decreased with increasing soil moisture from 50% WHC to 90% WHC, and then increased again at 200% WHC for the upland soil; positive net N mineralization occurred in the riparian soil at both 90% WHC and 200% WHC \((P > 0.05)\). However, negative net N mineralization occurred in the sediment at \( S_2 \).
Table 5.1 Soil properties (0-10 cm) at study sites of S₁ (Site 1) and S₂ (Site 2) in the subtropical riparian areas of Wyaralong Dam

<table>
<thead>
<tr>
<th></th>
<th>SM (%)</th>
<th>WHC (%)</th>
<th>pH</th>
<th>EC (µs cm⁻¹)</th>
<th>TC (%)</th>
<th>TN (%)</th>
<th>δ¹³C ‰</th>
<th>δ¹⁵N ‰</th>
<th>WSOC (mg kg⁻¹)</th>
<th>WSTN (mg kg⁻¹)</th>
<th>HWEOC (mg kg⁻¹)</th>
<th>HWETN (mg kg⁻¹)</th>
<th>MBC (mg kg⁻¹)</th>
<th>MBN (mg kg⁻¹)</th>
</tr>
</thead>
<tbody>
<tr>
<td>S₁ U</td>
<td>13.4±0.1b</td>
<td>56.9±1.9b</td>
<td>4.9±0.1a</td>
<td>192.1±27.1a</td>
<td>3.2±0.0b</td>
<td>0.25±0.01a</td>
<td>-18.1±0.1b</td>
<td>5.0±0.2b</td>
<td>35.5±2.6b</td>
<td>5.2±0.4b</td>
<td>1362.7±5.0b</td>
<td>158.1±2.9a</td>
<td>108.5±2.1a</td>
<td>13.3±2.5a</td>
</tr>
<tr>
<td>R</td>
<td>35.0±0.2b</td>
<td>67.4±2.4a</td>
<td>5.2±0.1a</td>
<td>261.3±59.5a</td>
<td>3.6±0.1a</td>
<td>0.27±0.01a</td>
<td>-18.8±0.0c</td>
<td>4.5±0.1c</td>
<td>30.7±3.8b</td>
<td>3.3±0.1c</td>
<td>1497.3±7.9a</td>
<td>167.2±2.4a</td>
<td>130.0±16.9c</td>
<td>17.9±2.6a</td>
</tr>
<tr>
<td>S</td>
<td>42.9±0.2a</td>
<td>43.6±1.2c</td>
<td>5.6±0.3a</td>
<td>207.3±5.9a</td>
<td>1.8±0.0c</td>
<td>0.13±0.00b</td>
<td>-16.4±0.1a</td>
<td>5.5±0.1a</td>
<td>64.6±3.2a</td>
<td>6.8±0.1c</td>
<td>657.6±14.9c</td>
<td>74.3±2.0b</td>
<td>46.0±6.6b</td>
<td>0.2±0.1b</td>
</tr>
<tr>
<td>S₂ U</td>
<td>4.9±0.1c</td>
<td>38.4±2.2a</td>
<td>4.3±0.2a</td>
<td>174.8±2.9a</td>
<td>1.3±0.1a</td>
<td>0.08±0.01a</td>
<td>-18.3±0.0b</td>
<td>2.4±0.2a</td>
<td>59.7±4.0a</td>
<td>6.7±0.3b</td>
<td>650.5±4.1b</td>
<td>73.7±0.6b</td>
<td>39.3±5.8a</td>
<td>5.7±1.6a</td>
</tr>
<tr>
<td>R</td>
<td>23.6±0.8b</td>
<td>36.4±1.7a</td>
<td>4.3±0.0a</td>
<td>154.1±36.7a</td>
<td>1.2±0.0a</td>
<td>0.08±0.01a</td>
<td>-17.0±0.0a</td>
<td>2.8±0.1a</td>
<td>62.8±2.8a</td>
<td>7.5±0.2a</td>
<td>947.9±18.6a</td>
<td>110.5±2.7a</td>
<td>28.1±5.2a</td>
<td>5.2±0.4a</td>
</tr>
<tr>
<td>S</td>
<td>33.2±0.6a</td>
<td>25.6±2.1a</td>
<td>4.5±0.1a</td>
<td>130.5±41.5a</td>
<td>0.6±0.1b</td>
<td>0.04±0.01b</td>
<td>-18.0±0.1b</td>
<td>3.1±0.2a</td>
<td>41.9±4.1b</td>
<td>5.5±0.2c</td>
<td>336.4±7.7c</td>
<td>43.8±1.1c</td>
<td>24.0±2.4a</td>
<td>1.6±0.2a</td>
</tr>
</tbody>
</table>

Values are the means ± standard errors. Different lowercase letters indicated significant differences among soil slope position treatments at P<0.05.


*aSoil/water =1:5.
Fig. 5.1 Net ammonification (NA), nitrification (NN) and N mineralization (NNM) rates during the 3-day lab incubation of the 0-10 cm soils from Site 1 (a) and Site 2 (c) in a subtropical riparian area. Gross N mineralization (GNM), and gross nitrification (GNN) rates during the 3-day lab incubation of the 0-10 cm soils from Site 1 (b) and Site 2 (d) in a subtropical riparian area. Values are the means ± standard errors (n=3). Different lowercase letters indicated significant differences among soil moisture treatments at $P<0.05$. 

Chapter 5
Negative correlations were found between net ammonification rates and net nitrification rates, and between net ammonization rates and net N mineralization rates; while net nitrification rates was positively correlated with net N mineralization rates at S1 (Table S5.2). At S2, there were also positive correlations between net nitrification and net N mineralization rates (Table S5.3).

5.3.2 Gross N transformation rates

Gross N transformation rates in the soils from both S1 and S2 were summarized in Fig 5.1 and Table S5.1. When the data were pooled together for S1, gross N mineralization was detectable only at 200% WHC for soils from different slope positions; while gross nitrification rates significantly decreased with soil moisture within each slope position. Similar trends were observed for soils from S2 where gross N mineralization rates were also low (< 0.5 mg N kg\(^{-1}\) d\(^{-1}\)) and increased with soil moisture, but no significant gross N mineralization was observed in the sediment at 200% WHC. Significant decrease was also observed for gross nitrification rates with soil moisture for the upland soil; while gross nitrification rates increased with soil moisture for those from the riparian soil and the sediment from S2.

For soils collected at S1, gross N mineralization rates were correlated positively with net ammonification rates, but negatively with net nitrification and net N mineralization rates (Table S5.2). However, the correlation between gross nitrification rates and net ammonification rates was negative; while the correlation between gross nitrification and net nitrification or net N mineralization rates were positive (Table S5.2). For soils from S2, positive correlation between N gross mineralization and net ammonification rates was found. Gross nitrification rates were positively correlated with both net nitrification and net N mineralization rates. Gross N mineralization rates were negatively correlated with gross nitrification rates at both sites (Table S5.3).

5.3.3 Fate of \(^{15}\text{N-NO}_3^-\) and \(^{15}\text{N-NH}_4^+\)

\text{Fate of } ^{15}\text{N-NH}_4^+

For soils from S1, most \(^{15}\text{N-NH}_4^+\) removal was due to fixation/immobilization (T\(_0\): 90.52-111.54 %; T\(_1\):
90.58-109.43%) at both times (Table 5.2). At T₀, the added \( ^{15}\text{N}-\text{NH}_4^+ \) distributed in the following order for the upland and riparian soils: immobilization > other \( ^{15}\text{N} \) (anammox or coupled nitrification–denitrification, only in the upland soil) > autotrophic nitrification > remaining \( ^{15}\text{N}-\text{NH}_4^+ \). However, \( ^{15}\text{N} \) in the form of \( ^{15}\text{N}-\text{NH}_4^+ \) was higher than autotrophic nitrification for the sediment. At T₁, the distribution of added \( ^{15}\text{N}-\text{NH}_4^+ \) still followed the same trend for those unsaturated soils; while for the saturated upland soil there was more \( ^{15}\text{N} \) distributed in the form of \( ^{15}\text{N}-\text{NH}_4^+ \) than \( \text{NO}_3^- \).

For soils from \( S_2 \), most of the added \( ^{15}\text{N}-\text{NH}_4^+ \) was fixed/immobilized (T₀: 89.55 – 99.20 %; T₁: 89.59 – 94.83%) followed by other \( ^{15}\text{N} \) (anammox or coupled nitrification - denitrification, in upland and riparian soils) > \( ^{15}\text{N}-\text{NH}_4^+ \) > autotrophic nitrification (Table 5.2).

**Fate of \( ^{15}\text{N}-\text{NO}_3^- \)**

For soils from \( S_1 \), the distribution of added \( ^{15}\text{N}-\text{NO}_3^- \) was as follows: immobilization > denitrification > \( ^{15}\text{N} \cdot\text{NO}_3^- \) > nitrification and DNRA/remineralisation at T₀, except those in the sediment where there was more \( ^{15}\text{N}-\text{NH}_4^+ \) than \( ^{15}\text{N} \cdot\text{NO}_3^- \) (Table 5.3). At T₁, there were similar trends for the distribution of added \( ^{15}\text{N}-\text{NO}_3^- \) in the unsaturated soils; while for those saturated soils (200% WHC), more \( ^{15}\text{N} \) was in the \( \text{NH}_4^+ \) than the remaining \( ^{15}\text{N} \cdot\text{NO}_3^- \).
Table 5.2 Excess $^{15}$N removed from the added $^{15}$N-$\text{NH}_4^+$ pool and transformed into other $^{15}$N pools during the incubation following addition of $^{15}$N-$\text{NH}_4^+$ solution at different soil moisture levels

<table>
<thead>
<tr>
<th>Site</th>
<th>Time</th>
<th>Slope</th>
<th>%</th>
<th>Excess $^{15}$N removed from the added $^{15}$N-$\text{NH}_4^+$ pool and transformed into other $^{15}$N pools</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>$^{15}$N-$\text{NH}_4^+$ remaining</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>$\mu g \text{ N g}^{-1}$</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>soil</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>S</td>
<td>T₀</td>
<td>U 50</td>
<td>70</td>
<td>0.0104±0.0003$^b$</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>0.0100±0.0001$^b$</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>0.0101±0.0003$^b$</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>0.0101±0.0000$^b$</td>
</tr>
<tr>
<td>R</td>
<td>90</td>
<td>100</td>
<td>200</td>
<td>0.0094±0.0001$^b$</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>0.0105±0.0002$^b$</td>
</tr>
<tr>
<td>S</td>
<td>200</td>
<td>100</td>
<td>200</td>
<td>0.0236±0.0013$^c$</td>
</tr>
<tr>
<td>T₁</td>
<td>U 50</td>
<td>100</td>
<td>70</td>
<td>0.0073±0.0002$^d$</td>
</tr>
<tr>
<td></td>
<td>70</td>
<td>0.0075±0.0002d</td>
<td>0.080±0.002</td>
<td>0.0563±0.0016b</td>
</tr>
<tr>
<td>---</td>
<td>------</td>
<td>----------------</td>
<td>-------------</td>
<td>----------------</td>
</tr>
<tr>
<td></td>
<td>90</td>
<td>0.0072±0.0001d</td>
<td>0.076±0.001</td>
<td>0.0547±0.0021b</td>
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<tr>
<td></td>
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<td>0.0177±0.0004b</td>
<td>0.187±0.004</td>
<td>0.0121±0.0002c</td>
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<tr>
<td>R</td>
<td>90</td>
<td>0.0072±0.0000d</td>
<td>0.071±0.000</td>
<td>0.0613±0.0002a</td>
</tr>
<tr>
<td></td>
<td>200</td>
<td>0.0142±0.0003c</td>
<td>0.140±0.003</td>
<td>0.0144±0.0016c</td>
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<tr>
<td>S</td>
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<td>0.0239±0.0025a</td>
<td>0.479±0.051</td>
<td>0.0057±0.0004d</td>
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Excess $^{15}$N removed from the added $^{15}$N-NH$_4^+$ pool and transformed into other $^{15}$N pools

<table>
<thead>
<tr>
<th>Site</th>
<th>Time</th>
<th>Slope</th>
<th>%</th>
<th>WHC Position</th>
<th>WHC Remaining</th>
<th>WHC Autotrophic Nitrification</th>
<th>WHC Fixation/Immobilisation</th>
<th>Other $^{15}$N Anammox or Coupled Nitrification/Denitrification</th>
</tr>
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<tbody>
<tr>
<td>S$_2$</td>
<td>T$_0$</td>
<td>U</td>
<td>50</td>
<td>$^{15}$N-NH$_4^+$ remaining</td>
<td>$^{15}$N-NO$_3^-$</td>
<td>$^{15}$N-unexchangeable N</td>
<td>Other $^{15}$N</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>$\mu$g N g$^{-1}$ soil</td>
<td>$\mu$g N g$^{-1}$ soil</td>
<td>$\mu$g N g$^{-1}$ soil</td>
<td>$\mu$g N g$^{-1}$ soil</td>
<td></td>
</tr>
<tr>
<td>S$_2$</td>
<td>T$_0$</td>
<td>U</td>
<td>50</td>
<td>0.0188±0.0006$^a$</td>
<td>0.596±0.020</td>
<td>0.0091±0.0001$^a$</td>
<td>0.289±0.003</td>
<td>2.912±0.014$^{bc}$</td>
</tr>
<tr>
<td></td>
<td></td>
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<td></td>
<td>0.0168±0.0003$^d$</td>
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<td>0.0090±0.0001$^a$</td>
<td>0.286±0.002</td>
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<tr>
<td></td>
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<td>0.0159±0.0004$^d$</td>
<td>0.502±0.012</td>
<td>0.0086±0.0001$^b$</td>
<td>0.273±0.004</td>
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<tr>
<td></td>
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<td></td>
<td></td>
<td>0.0163±0.0003$^d$</td>
<td>0.517±0.009</td>
<td>0.0084±0.0001$^b$</td>
<td>0.266±0.004</td>
<td>2.847±0.036c</td>
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<tr>
<td>R</td>
<td>90</td>
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<td></td>
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<td>1.072±0.012</td>
<td>0.0014±0.0001$^c$</td>
<td>0.043±0.004</td>
<td>3.030±0.003$^a$</td>
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<tr>
<td></td>
<td>200</td>
<td></td>
<td></td>
<td>0.0343±0.0002$^a$</td>
<td>1.087±0.008</td>
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<td>S</td>
<td>200</td>
<td></td>
<td></td>
<td>0.0256±0.0013$^b$</td>
<td>1.692±0.088</td>
<td>0.0012±0.0000$^c$</td>
<td>0.081±0.003</td>
<td>1.498±0.035$^d$</td>
</tr>
<tr>
<td>T$_1$</td>
<td>U</td>
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<td></td>
<td>0.0145±0.0001$^e$</td>
<td>0.459±0.004</td>
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<td>0.401±0.010</td>
<td>2.888±0.019$^{ab}$</td>
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<tr>
<td></td>
<td>70</td>
<td></td>
<td></td>
<td>0.0144±0.0005$^e$</td>
<td>0.456±0.016</td>
<td>0.0116±0.0004$^b$</td>
<td>0.369±0.012</td>
<td>2.897±0.015$^{ab}$</td>
</tr>
<tr>
<td>Site</td>
<td>Time</td>
<td>90</td>
<td>200</td>
<td>90</td>
<td>200</td>
<td>90</td>
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<td>Time</td>
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<td>--------</td>
<td>------</td>
</tr>
<tr>
<td>U</td>
<td>90</td>
<td>0.0138±0.0001&lt;sup&gt;c&lt;/sup&gt;</td>
<td>0.436±0.004</td>
<td>0.0110±0.0001&lt;sup&gt;b&lt;/sup&gt;</td>
<td>0.348±0.003</td>
<td>2.843±0.041&lt;sup&gt;b&lt;/sup&gt;</td>
<td>90.04±1.28</td>
<td>0.290±0.040&lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
<tr>
<td>R</td>
<td>90</td>
<td>0.0174±0.0001&lt;sup&gt;c&lt;/sup&gt;</td>
<td>0.551±0.004</td>
<td>0.0043±0.0002&lt;sup&gt;c&lt;/sup&gt;</td>
<td>0.137±0.006</td>
<td>2.899±0.046&lt;sup&gt;ab&lt;/sup&gt;</td>
<td>91.82±1.45</td>
<td>0.237±0.046&lt;sup&gt;ab&lt;/sup&gt;</td>
</tr>
<tr>
<td>S</td>
<td>200</td>
<td>0.0363±0.0002&lt;sup&gt;b&lt;/sup&gt;</td>
<td>1.148±0.007</td>
<td>0.0027±0.0001&lt;sup&gt;d&lt;/sup&gt;</td>
<td>0.085±0.005</td>
<td>2.997±0.020&lt;sup&gt;a&lt;/sup&gt;</td>
<td>94.83±0.64</td>
<td>0.125±0.020&lt;sup&gt;c&lt;/sup&gt;</td>
</tr>
<tr>
<td>S</td>
<td>200</td>
<td>0.0379±0.0007&lt;sup&gt;a&lt;/sup&gt;</td>
<td>1.198±0.021</td>
<td>0.0026±0.0002&lt;sup&gt;d&lt;/sup&gt;</td>
<td>0.081±0.005</td>
<td>2.907±0.026&lt;sup&gt;ab&lt;/sup&gt;</td>
<td>91.98±0.83</td>
<td>0.213±0.025&lt;sup&gt;abc&lt;/sup&gt;</td>
</tr>
</tbody>
</table>

S<sub>1</sub>: Site 1; S<sub>2</sub>: Site 2; U: upland soil; R: riparian zone soil; S: sediment; anammox: anaerobic NH<sub>4</sub><sup>+</sup> oxidation; T<sub>0</sub>: the start of 3-day incubation; and T<sub>1</sub>: the end of 3-day incubation. Different lowercase letters indicated significant differences among soil slope position treatments at each time at P<0.05.
Table 5.3 Excess $^{15}$N removed from the added $^{15}$N-NO$_3^-$ pool and transformed into other $^{15}$N pools during the incubation following addition of $^{15}$N-NO$_3^-$ solution at different soil moisture levels

<table>
<thead>
<tr>
<th>Site</th>
<th>Time</th>
<th>Slope</th>
<th>% WHC</th>
<th>Excess $^{15}$N removed from the added $^{15}$N-NO$_3^-$ pool and transformed into other $^{15}$N pools</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>$^{15}$N-NO$_3^-$</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>$\mu$g N g$^{-1}$</td>
</tr>
<tr>
<td>S</td>
<td>T$_0$</td>
<td>U</td>
<td>50</td>
<td>0.0514±0.0016$^b$</td>
</tr>
<tr>
<td></td>
<td>70</td>
<td></td>
<td>0.0507±0.0005$^b$</td>
<td>0.538±0.006</td>
</tr>
<tr>
<td></td>
<td>90</td>
<td></td>
<td>0.0506±0.0012$^b$</td>
<td>0.536±0.013</td>
</tr>
<tr>
<td></td>
<td>200</td>
<td></td>
<td>0.0494±0.0009$^b$</td>
<td>0.523±0.010</td>
</tr>
<tr>
<td>R</td>
<td>90</td>
<td></td>
<td>0.0568±0.0007$^a$</td>
<td>0.560±0.007</td>
</tr>
<tr>
<td></td>
<td>200</td>
<td></td>
<td>0.0563±0.0005$^a$</td>
<td>0.555±0.005</td>
</tr>
<tr>
<td>S</td>
<td>200</td>
<td></td>
<td>0.0054±0.0002$^c$</td>
<td>0.110±0.003</td>
</tr>
<tr>
<td>T$_1$</td>
<td>U</td>
<td></td>
<td>50</td>
<td>0.0526±0.0006$^a$</td>
</tr>
<tr>
<td></td>
<td>70</td>
<td></td>
<td>0.0502±0.0021$^a$</td>
<td>0.531±0.022</td>
</tr>
<tr>
<td></td>
<td>90</td>
<td></td>
<td>0.0448±0.0059$^a$</td>
<td>0.474±0.063</td>
</tr>
<tr>
<td></td>
<td>200</td>
<td>0.0131±0.0004&lt;sup&gt;b&lt;/sup&gt;</td>
<td>0.138±0.004</td>
<td>0.0185±0.0009&lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
<tr>
<td>----</td>
<td>-------</td>
<td>----------------------------</td>
<td>--------------</td>
<td>----------------------------</td>
</tr>
<tr>
<td>R</td>
<td>90</td>
<td>0.0537±0.0050&lt;sup&gt;a&lt;/sup&gt;</td>
<td>0.529±0.049</td>
<td>0.0069±0.0002&lt;sup&gt;c&lt;/sup&gt;</td>
</tr>
<tr>
<td></td>
<td>200</td>
<td>0.0131±0.0009&lt;sup&gt;b&lt;/sup&gt;</td>
<td>0.129±0.009</td>
<td>0.0138±0.0003&lt;sup&gt;b&lt;/sup&gt;</td>
</tr>
<tr>
<td>S</td>
<td>200</td>
<td>0.0043±0.0002&lt;sup&gt;b&lt;/sup&gt;</td>
<td>0.087±0.003</td>
<td>0.0173±0.0010&lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
</tbody>
</table>
(To continue)

<table>
<thead>
<tr>
<th>Site</th>
<th>Time</th>
<th>Slope</th>
<th>% WHC</th>
<th>Excess $^{15}$N removed from the added $^{15}$N-NO$_3^-$ pool and transformed into other $^{15}$N pools</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>$^{15}$N-NO$_3^-$</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>(DNRA/remineralisation)</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>$\mu g\ N\ g^{-1}$</td>
</tr>
<tr>
<td>S$_2$</td>
<td>$T_0$</td>
<td>U</td>
<td>50</td>
<td>0.0097±0.0002$^a$</td>
</tr>
<tr>
<td></td>
<td>70</td>
<td>0.0096±0.0001$^{ab}$</td>
<td>0.311±0.004</td>
<td>0.0139±0.0002$^c$</td>
</tr>
<tr>
<td></td>
<td>90</td>
<td>0.0093±0.0000$^b$</td>
<td>0.301±0.001</td>
<td>0.0142±0.0005$^c$</td>
</tr>
<tr>
<td></td>
<td>200</td>
<td>0.0094±0.0001$^b$</td>
<td>0.303±0.003</td>
<td>0.0130±0.0005$^c$</td>
</tr>
<tr>
<td>R</td>
<td>90</td>
<td>0.0024±0.0000$^c$</td>
<td>0.077±0.001</td>
<td>0.0317±0.0004$^a$</td>
</tr>
<tr>
<td></td>
<td>200</td>
<td>0.0024±0.0000$^c$</td>
<td>0.078±0.001</td>
<td>0.0316±0.0003$^a$</td>
</tr>
<tr>
<td>S</td>
<td>200</td>
<td>0.0026±0.0001$^c$</td>
<td>0.183±0.009</td>
<td>0.0238±0.0008$^b$</td>
</tr>
<tr>
<td>T$_1$</td>
<td>U</td>
<td>50</td>
<td>0.0118±0.0001$^a$</td>
<td>0.383±0.002</td>
</tr>
<tr>
<td></td>
<td>70</td>
<td>0.0118±0.0001$^a$</td>
<td>0.382±0.003</td>
<td>0.0108±0.0004$^c$</td>
</tr>
<tr>
<td></td>
<td>90</td>
<td>0.0085±0.0013$^b$</td>
<td>0.274±0.042</td>
<td>0.0120±0.0005$^c$</td>
</tr>
<tr>
<td></td>
<td>200</td>
<td>0.0050±0.0002$^c$</td>
<td>0.161±0.005</td>
<td>0.0139±0.0002$^b$</td>
</tr>
<tr>
<td></td>
<td>Slope</td>
<td>pH</td>
<td>Nitrate (mg L⁻¹)</td>
<td>Ammonium (mg L⁻¹)</td>
</tr>
<tr>
<td>----</td>
<td>-------</td>
<td>-------</td>
<td>-----------------</td>
<td>-------------------</td>
</tr>
<tr>
<td>R</td>
<td>90</td>
<td>0.0032±0.0002</td>
<td>0.103±0.006</td>
<td>0.0337±0.0004</td>
</tr>
<tr>
<td></td>
<td>200</td>
<td>0.0031±0.0002</td>
<td>0.101±0.006</td>
<td>0.0343±0.0006</td>
</tr>
<tr>
<td>S</td>
<td>200</td>
<td>0.0038±0.0003</td>
<td>0.262±0.020</td>
<td>0.0144±0.0007</td>
</tr>
</tbody>
</table>

S₁: Site 1; S₂: Site 2; U: upland soil; R: riparian zone soil; S: sediment; DNRA: nitrification and dissimilatory NO₃⁻ reduction to NH₄⁺; T₀: the start of 3-day incubation; and T₁: the end of 3-day incubation. Different lowercase letters indicated significant differences among soil slope position treatments at each time at $P<0.05$. 
All the added $^{15}$N-NO$_3^-$ was distributed as follows in soils from S$_2$: immobilization > denitrification > DNRA/remineralisation > $^{15}$N-NO$_3^-$ ($T_0$). However, the amounts of $^{15}$N distributed in $^{15}$N-NH$_4^+$ and $^{15}$N-NO$_3^-$ were almost the same for U50% and U70% at $T_1$ (Table 5.3).

5.4 Discussion

5.4.1 Effects of soil moisture on soil N mineralization

Compared with net N mineralization rate, gross N mineralization rate is a much better indicator for N mineralization. Gross N mineralization rates were very low (even undetectable for some treatments), and were accompanied by relatively low concentrations of soil NH$_4^+$ contents. Similar results were also reported by Burton et al. (2007) and Grenon et al. (2004). However, gross N mineralization rates were significantly higher in the saturated soils than in the unsaturated ones. Gross N mineralization rates increased with increasing soil moisture at both sites (Fig. 5.1 and Table S5.1), which were consistent with the results found in previous studies (Jamieson et al. 1999; Bengtson et al. 2005; Cheng et al. 2014). For example, Cheng et al. (2014) investigated the influence of soil moisture changes (30-90% WHC) on soil gross N mineralization rates in two contrasting subtropical acidic soils covered by broad-leafed and coniferous forests, and found that the average gross N mineralization rate was significantly higher at 70% and 90% WHC than that at 30% WHC in both soils. Zaman and Chang (2004) studied the effects of soil moisture on gross N mineralization rates under 25°C and 40°C respectively, and reported higher gross N mineralization rates around 100% field capacity than in treatments with less soil moistures at the same temperature. The authors explained that the conditions for microbial activity were best at the higher soil moisture because of sufficient water availability and access of substrates by water movement for microbial metabolism, and of enough soil aeration under the unsaturated conditions.

Furthermore, gross N mineralization rates have been found to be even higher under the saturated soils than the unsaturated conditions in both arable and forest soils (Nishio et al. 1994; Mathieu et al. 2006; Burton et al. 2007). Burton et al. (2007) applied the $^{15}$N isotope dilution method to quantify gross N mineralization rates in adjacent native forest and hoop pine plantation soils under both aerobic and
anaerobic conditions, and found that the gross N mineralization rates in the anaerobic incubation were consistently higher than the rates measured in the aerobic incubation. The authors attributed the results to increased labile organic C and N as a result of lysis of dead microbial biomass under waterlogged conditions (Wu and Brookes 2005). Therefore, the increase in gross N mineralization rates with increasing soil moisture may be attributed to the fact that the diffusive limitations were alleviated, and more labile organic C and N were released as a result of lysis of dead microbial cells at higher soil moistures. Therefore, a fast N mineralization of soil organic N could be maintained under relatively high soil moisture values (Borken and Matzner 2009).

Gross N mineralization rates were positively correlated with net N mineralization rates at both sites, which was consistent with previous study by Zaman and Chang (2004), who attributed it to similar factors regulating the processes of N immobilization and mineralization during the incubation (Burke 1989; Stottlemeyer et al. 1995).

Water availability plays a key role in soil microbial activity, therefore controls the net N mineralization rate (Nicolardot et al. 1994; Stark and Firestone 1996; Nicolardot et al. 2001). It is found that the maximal net N mineralization rates occur when soil moisture is close to field water-holding capacity (Stanford and Epstein 1974; Sleutel et al. 2008). However, a similar trend as previous studies of soil net N mineralization rates was not found in our study. It is still difficult to explain since net N transformation is the outcome of competitions between N mineralization and immobilization (Murphy et al. 2003).

5.4.2 Effects of soil moisture on soil nitrification

Soil moisture had an impact on net and gross nitrification rates of both soils (Fig 5.1). Soil nitrification is thought to be a microbial mediated process. Therefore, soil nitrification is affected by soil moisture through its influence on microbial community (Bai et al. 2004; Borken and Matzner 2009). For soils from S1, gross nitrification rates were similar at soil moistures from 50% to 90% WHC, and then decreased at 200% WHC for the upland soils. Similarly, for the riparian soils and sediment at S1 and the upland soils at S2, gross nitrification rates also decreased significantly at 200% WHC. It was also
found in previous studies that gross nitrification rates increased with increasing soil moisture content to a maximum at a specific soil moisture and decreased thereafter (Breuer et al. 2002; Corre et al. 2003; Kiese et al. 2008). For example, Kiese et al. (2008) studied the seasonal dynamics of gross nitrification rates at two tropical rainforest sites in Queensland of Australia, and found that the relationship between soil moisture and gross nitrification rates could be described by O’Neill functions, with the optimum soil moisture for gross nitrification rates at about 65-80 % WHC (Berg and Rosswall 1989; Gödde and Conrad 1998). Breuer et al. (2002) collected intact soil cores at different seasons (dry season, wet season, and the transition period from dry to wet season) in Australia, and found the lowest value during the dry season, but highest value during the transition period. The possible explanation is that nitrification is water-limited under dry condition. As a result, gross nitrification rate increased with soil moisture in this situation. However, gross nitrification decreases with increasing soil moisture content under wet conditions due to an aeration-limiting phase (Mathieu et al. 2006). The decreased gross nitrification may also be attributed to the low number of nitrifiers (Belser 1979), although we did not detect the microbial biomass in this study. The relative amount of change in gross nitrification rates varied greatly between the two soil types, which may indicate the differences in substrate quality.

On the other hand, it is surprising to note that gross nitrification rates increased with soil moisture for the riparian soils (R70% and R200%) and sediment (S200%) at S2. In fact, Matheson et al. (2003) and Ambus et al. (1992) obtained similar results, and reported high rates of NO₃⁻ production in anaerobic riparian soils. They attributed it to the following two possible reasons: the anaerobic breakdown of soil organics (oximes, nitroso-, nitro compounds etc.) might release NO₃⁻; and there was a possibility that some oxygen was introduced into the soil matrix in the set-up of experiments, which enabled nitrification to occur.

Gross nitrification was also stronger than gross N mineralization for both sites, which was consistent with the previous study by Burton et al. (2007). The authors explained that the high gross nitrification rates were likely due to a high rate of heterotrophic nitrification although both of soil NH₄⁺ contents and gross N mineralization were low at both sites. Heterotrophic nitrification is traditionally not considered to be the dominant process compared with autotrophic nitrification. However, more and
more studies emphasize the importance of heterotrophic nitrification (Schimel et al. 1984; Grenon et al. 2004). For example, heterotrophic nitrification is proved to exist in acidic forest soils or at low oxygen concentrations (Barraclough 1995; De Boer and Kowalchuk 2001). Ingwersen et al. (1999) incubated intact soil cores from a very acidic coniferous forest in an isothermal gas tight system, observed high gross nitrification rates under saturated condition, and exhibited the dominant role of heterotrophic nitrification in this system. Therefore, the higher rates of gross nitrification than gross N mineralization rates would indicate that heterotrophic nitrification might play a significant role in the N transformations in this study. Gross nitrification rates were positively correlated with net nitrification rates, but negatively correlated with gross N mineralization rates at both sites. It may also show the importance of heterotrophic nitrification or the opposite effects of soil moisture on nitrification and mineralisation.

5.4.3 The fate of $^{15}$N addition

The extent of immobilization varied between 35% and 95% in different studies (Schimel and Firestone 1989; Hart and Firestone 1991; Hart et al. 1993). In our study, clay mineral fixation and/or biological immobilization was the principal sink of the $^{15}$N added in the soils regardless of the form of $^{15}$N addition and soil type. In fact, the distributions of $^{15}$NH$_4^+$ or NO$_3^-$ became stabilized even as early as at $T_0$ (3 h), which may indicate that most chemical or microbial reactions occurred in the early stage of the experiment. It probably resulted from the priming effects of soil disturbance or inorganic N addition, which could simulate the soils subjected to natural disturbance and priming effects associated with water table fluctuations, and thus fluxes of N (Matheson et al. 2003). The high N immobilization rates may be due to the effect of added inorganic N on soil microorganisms, enhancing the N-fixing or immobilizing activities in soil (Reddy et al. 1984).

The immobilization and fixation of $^{15}$N-NH$_4^+$ refer to biotic and abiotic processes, respectively. It is found that 10-50% of the NH$_4^+$ immobilized/fixed was by soil microorganisms (Brookes et al. 1985) and only less than 10% of the added NH$_4^+$ was abiotic, although NH$_4^+$ fixation to clay mineral would be fast (< 30 min) (Beauchamp and Drury 1991; Trehan 1996). Nuclear magnetic resonance (NMR) of the organic matter of $^{15}$NH$_4^+$ or $^{15}$NO$_3^-$ treated soils showed that the microbially organic $^{15}$N was mainly
in the form of peptides and proteins (> 80%), nucleic acids, and aliphatic amine groups after several months of in situ incubation (Clinton et al. 1995). Although the amount of immobilization of $^{15}$N-NH$_4^+$ was high, it was difficult to determine the importance of abiotic and biotic processes in our study due to the time limitation. Considering the organic matter and clay contents of the riparian soils, there were still possibilities for abiotic NH$_4^+$ immobilizations (Davidson et al. 1991). Relatively high NO$_3^-$ immobilizations were reported in the riparian soils and sediments (Buresh and Patrick 1981; Ambus et al. 1992; Ragab et al. 1994). However, the amount of NH$_4^+$ immobilization and NO$_3^-$ immobilization were similar, which was opposite to other studies showing higher NH$_4^+$ immobilization than NO$_3^-$ immobilization (Ragab et al. 1994; Matheson et al. 2003). It may be due to assimilation of inorganic N by microorganisms under high biologically available C and low inorganic N contents in the soils regardless of the inorganic N forms added.

For the soils added with $^{15}$N-NH$_4^+$, emissions of gases by anammox or coupled nitrification - denitrification were the second highest pathways of $^{15}$N-NH$_4^+$ for the upland soil at S$_1$ and almost all soils at S$_2$ following the fixation/immobilization (Table 5.2). Nitrification and denitrification processes could produce N$_2$O and NO (Stevens et al. 1997); while denitrification and anammox (the process of anaerobic NH$_4^+$ oxidation) could produce N$_2$ (Van de Graaf et al. 1995). It is believed that nitrification is generally responsible for the N$_2$O formation under aerobic conditions; while denitrification is the dominant processes for N$_2$O formation under anaerobic conditions (Stevens et al. 1997; Wolf and Russow 2000). Studies also showed that denitrification can also happen under aerobic condition, especially in acid soil ecosystems for fungal (Baggs 2011; Maeda et al. 2015). In fact, denitrification may play a key role in N removal for the riparian soils. For example, Hanson et al. (1994) detected that the total denitrification was 5-40 kg N ha$^{-1}$ y$^{-1}$ in riparian wetlands on eastern and western sides of Sandhill Brook, Rhode Island. As the time went on, the amount of autotrophic nitrification decreased in the saturated soils in our study, which would be consistent with the general perception that autotrophic nitrification occur under aerobic condition. On the other hand, similar trends were also found for soils with $^{15}$N-NO$_3^-$ addition from both sites (Table 5.3). It is interesting to have detected more $^{15}$N in the form of DNRA/remineralisation than $^{15}$N-NO$_3^-$ in the saturated treatments for soils at S$_1$, which was consistent with findings in a previous study that DNRA was enhanced by more reducing conditions.
Chapter 5

(Matheson et al. 2003). The nature of microbial activity at T₁ may be representative of the riparian soil response following more prolonged anaerobic conditions during wetter periods of the annual cycle.

5.5 Conclusions

Soil moisture significantly affected net and gross N transformation rates for the soils from both sites. Soil net N ammonification rates and soil moisture were correlated positively for both within and among each slope position treatments for the riparian soils. Gross N mineralization rates were very low, accompanied by relatively low concentrations of soil NH₄⁺ contents, but increased with the soil moisture. Significantly high gross N mineralization rates were found in the saturated soils than in the unsaturated ones. Gross N mineralization rates were positively correlated with net N mineralization rates. Gross nitrification rates generally decreased with the increasing soil moisture. The gross nitrification rates were higher than the gross N mineralization rates may indicate a high rate of heterotrophic nitrification. Most of the ¹⁵N added (>87%) was fixed by clay or immobilized by microbes, but considerable amounts (3-12%) were also lost through N gas emissions. Little added ¹⁵N-NH₄⁺ (<1%) was found as ¹⁵N-NO₃⁻; while more added ¹⁵N-NO₃⁻ was shown in the form of ¹⁵N-NH₄⁺ than the ¹⁵N-NO₃⁻ in the saturated soils at the end of the 3-day incubation.

Further studies on the relationship between gross N transformation rates and N₂O, NO and N₂ production under different soil moisture values using the ¹⁵N tracing technique are needed to investigate the relative contributions of nitrification and denitrification to the gaseous N emissions.
References


Barraclough D (1995) $^{15}$N isotope dilution techniques to study soil nitrogen transformations and plant uptake. Fertilizer research 42:185-192


Chapter 5


Table S5.1 Net ammonification (NA), net nitrification (NN), net mineralization (NNM), gross N mineralization (GNM) and nitrification (GN) rates in the 0-10 cm soils during the 3-day incubation of soils collected at S\textsubscript{1} (Site1) and S\textsubscript{2} (Site 2) in a riparian areas of Wyaralong Dam

<table>
<thead>
<tr>
<th>Site</th>
<th>Slope</th>
<th>% WHC</th>
<th>NA</th>
<th>NN</th>
<th>NNM</th>
<th>GNM</th>
<th>GNN</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>position</td>
<td>(mg N kg\textsuperscript{-1}.d\textsuperscript{-1})</td>
<td>(mg N kg\textsuperscript{-1}.d\textsuperscript{-1})</td>
<td>(mg N kg\textsuperscript{-1}.d\textsuperscript{-1})</td>
<td>(mg N kg\textsuperscript{-1}.d\textsuperscript{-1})</td>
<td>(mg N kg\textsuperscript{-1}.d\textsuperscript{-1})</td>
</tr>
<tr>
<td>S\textsubscript{1}</td>
<td>U</td>
<td>50</td>
<td>-0.28 ± 0.04\textsuperscript{c}</td>
<td>0.62 ± 0.40\textsuperscript{a}</td>
<td>0.35 ± 0.40\textsuperscript{a}</td>
<td>0.00 ± 0.00\textsuperscript{b}</td>
<td>9.17 ± 1.90\textsuperscript{b}</td>
</tr>
<tr>
<td></td>
<td></td>
<td>70</td>
<td>-0.21 ± 0.03\textsuperscript{c}</td>
<td>0.01 ± 0.78\textsuperscript{a}</td>
<td>-0.20 ± 0.08\textsuperscript{a}</td>
<td>0.00 ± 0.00\textsuperscript{b}</td>
<td>10.66 ± 1.01\textsuperscript{b}</td>
</tr>
<tr>
<td></td>
<td></td>
<td>90</td>
<td>-0.25 ± 0.03\textsuperscript{c}</td>
<td>-1.86 ± 2.31\textsuperscript{a}</td>
<td>-2.11 ± 2.29\textsuperscript{a}</td>
<td>0.00 ± 0.00\textsuperscript{b}</td>
<td>8.43 ± 2.07\textsuperscript{b}</td>
</tr>
<tr>
<td></td>
<td></td>
<td>200</td>
<td>0.67 ± 0.02\textsuperscript{a}</td>
<td>-12.70 ± 0.17\textsuperscript{b}</td>
<td>-12.0 ± 30.20\textsuperscript{b}</td>
<td>0.44 ± 0.19\textsuperscript{a}</td>
<td>1.16 ± 0.28\textsuperscript{c}</td>
</tr>
<tr>
<td>R</td>
<td>90</td>
<td>0.19 ± 0.01\textsuperscript{c}</td>
<td>-0.74 ± 2.03\textsuperscript{a}</td>
<td>-0.93 ± 2.02\textsuperscript{a}</td>
<td>0.00 ± 0.00\textsuperscript{b}</td>
<td>16.00 ± 1.51\textsuperscript{a}</td>
<td></td>
</tr>
<tr>
<td></td>
<td>200</td>
<td>0.34 ± 0.03\textsuperscript{ab}</td>
<td>-15.19 ± 0.51\textsuperscript{b}</td>
<td>-14.84 ± 0.49\textsuperscript{b}</td>
<td>0.41 ± 0.06\textsuperscript{a}</td>
<td>1.89 ± 0.97\textsuperscript{c}</td>
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</tr>
<tr>
<td>S\textsubscript{2}</td>
<td>200</td>
<td>0.04 ± 0.27\textsuperscript{bc}</td>
<td>-0.38 ± 0.04\textsuperscript{a}</td>
<td>-0.34 ± 0.24\textsuperscript{a}</td>
<td>0.21 ± 0.16\textsuperscript{b}</td>
<td>0.24 ± 0.24\textsuperscript{c}</td>
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<td>70</td>
<td>-0.21 ± 0.06\textsuperscript{c}</td>
<td>0.85 ± 0.04\textsuperscript{a}</td>
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<td>0.01 ± 0.01\textsuperscript{c}</td>
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<td>90</td>
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<td>-0.28 ± 0.47\textsuperscript{b}</td>
<td>-1.72 ± 0.04\textsuperscript{c}</td>
<td>0.06 ± 0.01\textsuperscript{c}</td>
<td>0.27 ± 0.24\textsuperscript{bc}</td>
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<tr>
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<td>200</td>
<td>0.10 ± 0.02\textsuperscript{b}</td>
<td>-1.54 ± 0.04\textsuperscript{c}</td>
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<td>0.42 ± 0.09\textsuperscript{a}</td>
<td>0.70 ± 0.07\textsuperscript{bc}</td>
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<tr>
<td>S</td>
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<td>-0.86 ± 0.11&lt;sup&gt;a&lt;/sup&gt;</td>
<td>0.45 ± 0.11&lt;sup&gt;a&lt;/sup&gt;</td>
<td>-0.86 ± 0.11&lt;sup&gt;b&lt;/sup&gt;</td>
<td>0.00 ± 0.00&lt;sup&gt;c&lt;/sup&gt;</td>
<td>0.95 ± 0.11&lt;sup&gt;b&lt;/sup&gt;</td>
<td></td>
</tr>
</tbody>
</table>

Values are the means ± standard errors (n=3). Different lowercase letters indicated significant differences between soil moisture treatments at \( P < 0.05 \).

S<sub>1</sub>: Site 1; S<sub>2</sub>: Site 2; U: upland soil; R: riparian soil; S: sediment; WHC: water holding capacity; NA: net ammonification; NN: net nitrification; NNM: net mineralization; GNM: gross N mineralization rates; and GNN: gross nitrification rates.
**Table S5.2** Pearson’s correlation analyses of nitrogen (N) transformation rates and other soil properties at the S$_1$ site (n=21)

<table>
<thead>
<tr>
<th></th>
<th>NA</th>
<th>NN</th>
<th>NNM</th>
<th>GNM</th>
<th>GNN</th>
</tr>
</thead>
<tbody>
<tr>
<td>NA</td>
<td>1</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>NN</td>
<td>-0.783**</td>
<td>1</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>NNM</td>
<td>-0.759**</td>
<td>0.999**</td>
<td>1</td>
<td></td>
<td></td>
</tr>
<tr>
<td>GNM</td>
<td>0.850**</td>
<td>-0.711**</td>
<td>-0.693**</td>
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<td></td>
</tr>
<tr>
<td>GNN</td>
<td>-0.623**</td>
<td>0.563**</td>
<td>0.551**</td>
<td>-0.635**</td>
<td>1</td>
</tr>
</tbody>
</table>

NA: net ammonification; NN: net nitrification; NNM: net mineralization; GNM: gross N mineralization rates; and GNN: gross nitrification rates.

**Table S5.3** Pearson’s correlation analyses of nitrogen (N) transformation rates and other soil properties at the S$_2$ site (n=21)

<table>
<thead>
<tr>
<th></th>
<th>NA</th>
<th>NN</th>
<th>NNM</th>
<th>GNM</th>
<th>GNN</th>
</tr>
</thead>
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<tr>
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<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>NN</td>
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<td>1</td>
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<td></td>
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<td>NNM</td>
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</tr>
<tr>
<td>GNM</td>
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</tr>
<tr>
<td>GNN</td>
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<td>0.720**</td>
<td>0.552**</td>
<td>-0.484*</td>
<td>1</td>
</tr>
</tbody>
</table>

NA: net ammonification; NN: net nitrification; NNM: net mineralization; GNM: gross N mineralization rates; and GNN: gross nitrification rates.
Chapter 6 Characterization of soil organic matter in whole soils and humic acids by solid-state $^{13}$C CPMAS NMR spectroscopy within the riparian zone of Wyaralong Dam in Southeast Queensland, Australia

6.1 Introduction

Riparian zones play a key role in ecosystem services, such as soil stabilization, and are important sites of carbon (C) sequestration (Burger et al. 2010; Smukler et al. 2010). The global soil C pool is more than three times the size of the atmospheric C pool and more than four times that of the biotic pool (Lal 2004). Soil organic C (SOC) represents approximately 62% of global soil C. Therefore, soil organic matter (SOM) is considered to be a vital part of soil because of its high contribution to soil productivity; SOM represents an active environmental component in biogeochemical processes that have a direct impact on primary production and biodiversity (Knicker et al. 2006).

Humic substances (HS) constitute at least 50% of soil organic C pool, and form an important pool of C in the global C budget (Fig. 6.1). Humic substances are categorized as the chemically resistant component; HS are not markedly changing over decades of soil use (Stevenson 1994; Lal 2004). The fraction linked to minerals in aggregates shows a high proportion of recalcitrant compounds with varied aromatic C (aliphatic and alkyl-C compositions) (Golchin et al. 1994). Highly transformed, HS are ubiquitous in the environment (Stocking 2003; Lal et al. 2004). In addition to their role in C cycle, HS also affect soil fertility, soil development, and various soil chemical properties (e.g. cation exchange capacity, buffer capacity, pH, acid–base chemistry, and metal transport) (Petersen 1980; Buol et al. 2003), and serves as a source of energy for soil macro- and microorganisms (Paul and Clark 2006). Much remains unknown about HS structure and properties, except it consists of a wide range of structures and functional groups (Stevenson 1994). Previous studies showed that HS consists of small heterogeneous molecules combined mainly by hydrophobic and H bonding forces in supramolecular association (Piccolo 2002; Nebbioso et al. 2014). Therefore, HS has largely remained uncharacterized at the molecular level. There are two theories about the formation of HS: the decay of plant materials rich in lignin, particularly by condensed aromatic molecules (black C) (Stevenson 1994; DiDonato et al. 2016); and the residues of cutin and suberin which are resistant to biodegradation (Tao et al. 1999).
Among them, the first theory is considered to be the primary source of humification (DiDonato et al. 2016).

![Diagram of soil organic matter fractionation](image)

**Fig. 6.1** Fractionation of soil organic matter (SOM) by classical methods (Strosser 2010).

Humic substances can be described as complex polydisperse polymeric mixtures, whose properties result from its structural diversity, as well as its status of aggregation, conformation, and surface charge distribution. Thus, it is difficult to reveal properties emerging via interaction by individual molecular components alone (Cook and Langford 1998). However, the molecular moieties in HS could reflect the sources of SOM inputs and are related to degradation/decomposition state and depositional environment (Golding et al. 2004). Humic substances have been shown to contain both amorphous and crystalline domain characteristics, which are expected to have different resistivity to environmental attack (Hu et al. 1999). There are many factors affecting HS. The chemical nature of the litters, composition of the microbial community, and environmental factors (e.g. temperature and moisture) play important roles in influencing the chemical and structural components of HS. Humic substances, developing from similar geographic locations but different depositional environments, often has different chemical structures (Rasyid et al. 1992). Therefore, the alteration of HS compositions could indicate flooding effects in the riparian zone (Kimura et al. 2017).

Humic substances can be divided into fulvic acid (FA), humic acid (HA), and humin. As one component of the HS, HA enhances soil C sequestration through hydrophobic protection (Spaccini et al. 2002) and by stabilizing soil aggregates (Hayes and Clapp 2001). The stabilized soil aggregates can, in turn, protect readily degradable C such as polysaccharides (Borken et al. 2006). HA is well known as an active soil fraction, interacting to various extents, although its activity will vary considerably in...
accordance with site condition (Zech et al. 1997). During the process of humification, HA is considered to be more sensitive to environmental changes than other components of whole soils (Arshad and Schnitzer 1989; Zech et al. 1997). Therefore, the characteristics of HA is commonly studied to evaluate the effects of environmental changes. Flooding has been highlighted as a key factor affecting HA formation (Kimura et al. 2017), through modifying soil aggregation, soil microbial communities, and decomposition processes (Yoo et al. 2011).

Soil C sequestration is affected by the amount and forms of C presented in the soil. The use of spectroscopic techniques such as nuclear magnetic resonance (NMR) provides an opportunity to identify functional groups and molecular structures, providing a better understanding of decomposition pathways of organic matter and qualitative alterations induced by management practices (Wilson 1987; Preston 1996; Kögel-Knabner 1997; Smernik and Oades 2001). $^{13}$C NMR is one of the most useful techniques to study the chemical structure of the whole soils (Mathers and Xu 2003) and HS (Preston 1996). Among numerous solid-state $^{13}$C NMR approaches, the most popular one to study the whole soils and HS is solid-state $^{13}$C cross-polarization magic angle spinning (CPMAS) NMR (Wilson 1987). Solid-state $^{13}$C CPMAS NMR can detect important C functional groups of intact soil, allowing for the direct assessment of SOM composition and quality without destruction (Preston 1996; Kögel-Knabner 1997; Mathers et al. 2000). Cross-polarisation between the concentrated $^1$H and dilute $^{13}$C spins leads to considerable signal enhancement that allows for the investigation of solid samples at natural $^{13}$C abundance (Wilson 1987; Kögel-Knabner 1997). Routinely, the generalised chemical shift regions for the C functional groups are integrated, across the $^{13}$C chemical shift domain, to obtain some measure of the relative representation of these functional groups, and hence the biomolecular composition, in samples of interest. While the line widths of resonances in the solid state spectra of homogeneous and heterogeneous samples are typically broad, because chemical shift anisotropy and/or dipolar broadening contribute to their line widths, soil and plant samples demonstrate additional “apparent broadening”. This “apparent broadening” is not attributable to actual NMR line width, but simply reflects the degree of chemical shift diversity within these inherently heterogeneous mixtures of materials. The resonances of similar, but not identical, functionality appear within expected ranges, but there is generally low absolute coincidence within any given shift region and hence resonances are only...
loosely clustered within each region. The summed resonant intensities of the different functional group
types (e.g. alkyl, O-alkyl, aromatic and carbonyl carbons), can be obtained by integration of their
respective chemical shift regions. Changes in the proportions of C types during decomposition and
humification can then be identified (Zech et al. 1997).

Few studies, however, have investigated changes in the chemical nature of soil C in riparian soils using
solid-state $^{13}$C CPMAS NMR spectra, especially on whole soils and HAs in the Southeast Queensland
(Alcântara et al. 2004; Smith et al. 2012). Therefore, information on the influence of water fluctuation
on the chemical composition of SOM and HA in the riparian zones is scarce. The soil water gradient
across the riparian zone reflects the integrated impact of vegetation, and the microbial community, as
well as the physicochemical condition of the humification process. This has not been fully investigated.
Since the residence time of C in the soil is linked to its chemical nature (Smernik and Oades 2001), this
represents a significant knowledge gap. Understanding the changing nature of SOM under different soil
moisture regimes is critical in managing the ecological health of the riparian zone. To test the
hypothesis that SOM humification is associated with change in soil moisture along the transects, we
used solid-state $^{13}$C CPMAS NMR spectra to investigate the chemical compositions of SOM in whole
soils and HAs in the riparian areas of Wyaralong Dam, in Southeast Queensland, Australia. The
objective of this research was to examine the spatial and temporal variations in the structural
characteristics of the whole soils and HAs in the riparian zone of Wyaralong Dam in Southeast
Queensland.

6.2 Materials and methods

6.2.1 Sample collection

Because this is part of the four papers presenting results from a common set of experiments, only a
brief description of the site selection and soil collection is given here; full details can be found in
Chapter 2 and by Jiang et al. (2017). Two soil types commonly found in the riparian zone of Wyaralong
Dam were selected for study: Dermosols and Kurosols (Ishell 1996). Soils (0-10 cm) from the upper
slope position (U) and the riparian zone (R), and sediment samples (S) (0-10 cm) were collected at
each site in August 2013 and 2015 respectively. Samples were selected according to the results from the samplings in the previous field work, which could represent the characteristics of SOM in this area. All samples were transported to the laboratory where field moist soils were well mixed and passed through a 2-mm sieve (roots were separated from soil during sieving) and air-dried until further analysis.

6.2.2 Soil analyses

The methods to measure total C (TC), total N (TN), stable C and N isotope composition ($\delta^{13}$C and $\delta^{15}$N respectively), soil mineral N ($\text{NH}_4^+$ and $\text{NO}_3^-$), soil hot-water extractable organic C (HWEOC) and hot-water extractable total N (HWETN), soil microbial biomass C (MBC) and microbial biomass N (MBN) were described in Chapter 2.

6.2.3 Procedure for extraction, isolation, and purification of HS

The procedures used for the extraction, fractionation, and purification of HS were summarized in Fig. 6.2. Soil HS was attracted with sodium hydroxide (NaOH) using the International Humic Substances Society (IHSS) method. There were four steps in total to extract, isolate and purify the HS.
Fig. 6.2 Procedure for extraction, isolation, and purification of humic substances (HS).

6.2.4 Solid-state $^{13}$C CPMAS NMR spectroscopy

Before $^{13}$C CPMAS NMR spectra were obtained, all compositied soil samples were pre-treated with 10% hydrofluoric acid (HF) as recommended by Rumpel et al. (2006) and He et al. (2009), to reduce the amount of paramagnetic material and concentrate the organic matter. Since obtaining a single spectrum was laborious and expensive and the yield of fraction samples after hydrofluoric acid treatment was not enough to warrant further analysis, NMR analysis was carried out on the combined samples bulked from all the five replications.

Solid-state $^{13}$C CPMAS NMR spectra were obtained on a Varian Unity Inova 400 spectrometer (Varian Inc., Palo Alto, CA), operating at a frequency of 100.6 MHz, or on a Varian VNMRS 300 (Varian Inc., Palo Alto, CA), operating at a frequency of 75.4 MHz. In each case, a suitably processed sample was packed into a silicon nitride rotor (Outer Diameter = 7 mm) and spun at 5 kHz at the magic angle.
unless otherwise noted. The NMR software processing package MestReNova 11.0 (Mestrelab Research S.L.) was used to process and integrate the spectra. A Lorentzian line broadening function of 20 Hz was applied to all spectra. Chemical shift values were referenced externally to hexamethylbenzene at 132.1 ppm, equivalent to tetramethylsilane at 0 ppm.

Spectra acquired at 100 MHz used the $xpol1$ cross-polarization pulse sequence. A contact time of 2 ms, an acquisition time of 14 ms, and a recycle delay of 2.5 s were used in all cases. 8000 or 20000 transients were collected for each sample, depending on sample type. Spectra acquired at 75 MHz used the $tanp$ cross-polarization pulse sequence. A contact time of 1.2 ms, an acquisition time of 20 ms, and a recycle delay of 2.5 s were used in all cases. 4000 or 20000 transients were collected for each sample, depending on sample type. Both 75 MHz and 100 MHz $^{13}$C CPMAS NMR spectra of the whole soil samples were divided into eight major chemical shift regions: alkyl C (0 to 45 ppm), N-alkyl/methoxy C (45 to 60 ppm), O-alkyl C (60-90 ppm), di-O-alkyl C (90-110 ppm), aromatic C (110-145 ppm), O-aromatic C (145-160 ppm), carboxyl C (160-180 ppm) and carbonyl C (180-210 ppm). Additional regions -50 to 0 ppm, 210 to 230 ppm and 230 to 245 ppm were integrated to permit spinning side band compensation where required.

Carbon distribution was summarized based on spectral peak area for all C groups. Amount of each functional group C per unit soil was calculated based on their relative distribution and the total C in soil. The humification index (HI) was used to evaluate the extent of SOM decomposition in the whole soil samples (Knicker et al. 2006). This was based on the previous studies that demonstrated that alkyl C increases concomitantly with a decrease in the O-alkyl C (Baldock and Preston 1995; Mathers et al. 2003).

$$HI = \frac{\text{alkyl } C \ (0 - 45 \text{ ppm})}{\ O - \text{alkyl } C \ (45 - 110 \text{ ppm})}$$

6.3 Results and discussions

6.3.1 $^{13}$C solid state NMR studies on the whole soils

Tables 6.1 and 6.2 provided the general physical and chemical characteristics of SOM in the two
sampling years. Exemplar spectra of the whole soils are presented in Fig. 6.3. In general, the major signals of organic C were found at regions clustered around δ 28, 50, 67, 100, 125, and 168 ppm for bulk soil collected from S1 in 2013 (Fig. 6.3a); while much weaker signals were detected around δ 28, 67, 100, and 168 ppm for those collected from S2 in 2013 (Fig. 6.3b).

The results for integration of $^{13}$C-NMR spectra for whole soils were present in Table 6.3. During the two years, the O-alkyl C (carbohydrate) and alkyl C peaks appears to dominate over the aromatic and carboxyl C peaks in the whole soils at both sites in both years (Table 6.3).

The HI ratio (the proportion of alkyl C/O-alkyl C) is provided in Table 6.3. Despite the small difference of the HI ratio between the upland soil to the sediment, the HI was smaller in the upland soil than in the sediment.

Comparing soils collected from different slope positions at both sites in two years, there is little detectable variation in the structural characteristics of the upland soil, riparian soil or sediment (Fig. 6.3 and Table 6.4).

Fig. 6.3 showed that soil and sediment samples collected from S1 contained considerable more signals of aromatic C than those from S2, indicating some loss of identity of original biochemical components at S2. Other notable differences were the decrease and eventual disappearance of methoxy and aromatic peaks at chemical shifts around δ 50 and 125 ppm at S2, respectively.
Table 6.1 Physical and chemical characteristics of soil organic matter (SOM) at Site 1 (S₁) and Site 2 (S₂) in 2013

<table>
<thead>
<tr>
<th>Site</th>
<th>Position</th>
<th>TC (%)</th>
<th>TN (%)</th>
<th>¹³C ‰</th>
<th>¹⁵N ‰</th>
<th>HWEOC (mg kg⁻¹)</th>
<th>HWETN (mg kg⁻¹)</th>
<th>MBC (mg kg⁻¹)</th>
<th>MBN (mg kg⁻¹)</th>
</tr>
</thead>
<tbody>
<tr>
<td>S₁</td>
<td>U</td>
<td>3.12 ± 0.24</td>
<td>0.245 ± 0.022</td>
<td>-17.5 ± 0.6</td>
<td>6.0 ± 0.6</td>
<td>879.1 ± 123.8</td>
<td>94.0 ± 11.1</td>
<td>113.7 ± 10.7</td>
<td>14.8 ± 1.4</td>
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<tr>
<td></td>
<td>R</td>
<td>2.67 ± 0.04</td>
<td>0.203 ± 0.005</td>
<td>-17.5 ± 0.2</td>
<td>6.5 ± 0.3</td>
<td>640.5 ± 19.4</td>
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<td>112.9 ± 4.4</td>
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<td>S</td>
<td>2.63 ± 0.28</td>
<td>0.210 ± 0.021</td>
<td>-17.3 ± 0.3</td>
<td>7.5 ± 0.5</td>
<td>622.6 ± 113.0</td>
<td>67.4 ± 13.7</td>
<td>48.5 ± 9.2</td>
<td>12.6 ± 5.9</td>
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<tr>
<td>S₂</td>
<td>U</td>
<td>1.00 ± 0.13</td>
<td>0.070 ± 0.005</td>
<td>-17.0 ± 0.5</td>
<td>8.1 ± 2.4</td>
<td>464.5 ± 7.8</td>
<td>45.3 ± 3.7</td>
<td>35.9 ± 3.7</td>
<td>6.3 ± 0.2</td>
</tr>
<tr>
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<td>R</td>
<td>0.87 ± 0.03</td>
<td>0.056 ± 0.002</td>
<td>-17.0 ± 0.7</td>
<td>5.8 ± 0.3</td>
<td>307.3 ± 8.9</td>
<td>24.2 ± 2.7</td>
<td>21.4 ± 4.0</td>
<td>2.9 ± 1.0</td>
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<td></td>
<td>S</td>
<td>0.78 ± 0.07</td>
<td>0.057 ± 0.006</td>
<td>-16.4 ± 0.2</td>
<td>5.7 ± 0.4</td>
<td>231.8 ± 23.5</td>
<td>22.5 ± 3.9</td>
<td>11.3 ± 3.2</td>
<td>1.5 ± 1.0</td>
</tr>
</tbody>
</table>

S₁; Site 1; S₂; Site 2; U: upper slope position soil; R: riparian zone soil; S: sediment; TC: total carbon; TN: total nitrogen; δ¹³C: stable C isotope composition; δ¹⁵N: stable N isotope composition; HWEOC: hot-water extractable organic C; HWETN: hot-water extractable total N; MBC: microbial biomass C; and MBN: microbial biomass N.

Mean ± SE is shown in the figure.
Table 6.2 Physical and chemical characteristics of soil organic matter (SOM) at Site 1 (S1) and Site 2 (S2) in 2015

<table>
<thead>
<tr>
<th>Site</th>
<th>Position</th>
<th>TC (%)</th>
<th>TN (%)</th>
<th>13C (%)</th>
<th>NH4-N (mg kg⁻¹)</th>
<th>NO3-N (mg kg⁻¹)</th>
<th>NH3-15N (atm%)</th>
<th>NO3-15N (atm%)</th>
<th>HWEOC (mg kg⁻¹)</th>
<th>HWETN (mg kg⁻¹)</th>
<th>WSOC (mg kg⁻¹)</th>
<th>WSTN (mg kg⁻¹)</th>
<th>MBC (mg kg⁻¹)</th>
<th>MBN (mg kg⁻¹)</th>
</tr>
</thead>
<tbody>
<tr>
<td>S1</td>
<td>U</td>
<td>3.10 ± 0.19</td>
<td>0.250 ± 0.015</td>
<td>-18.2 ± 0.6</td>
<td>5.0 ± 0.2</td>
<td>11.6 ± 0.1</td>
<td>0.3677 ± 0.1</td>
<td>0.3688 ± 0.0</td>
<td>1439.7 ± 96.5</td>
<td>164.5 ± 13.7</td>
<td>29.8 ± 5.8</td>
<td>4.2 ± 0.5</td>
<td>111.8 ± 6.6</td>
<td>17.0 ± 2.0</td>
</tr>
<tr>
<td></td>
<td>R</td>
<td>3.46 ± 0.11</td>
<td>0.265 ± 0.011</td>
<td>-18.7 ± 0.4</td>
<td>4.5 ± 0.2</td>
<td>12.5 ± 1.4</td>
<td>0.3678 ± 0.0</td>
<td>0.3690 ± 0.1</td>
<td>1584.0 ± 23.1</td>
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S1: Site 1; S2: Site 2; U: upper slope position soil; R: riparian zone soil; S: sediment; TC: total carbon; TN: total nitrogen; δ13C: stable C isotope composition; δ15N: stable N isotope composition; HWEOC: hot-water extractable organic C; HWETN: hot-water extractable total N; WSOC: water soluble organic C; WSTN water soluble total N; MBC: microbial biomass C; and MBN: microbial biomass N.

Mean ± SE is shown in the figure.
Table 6.3 Relative intensities (%) of each chemical shift region and humification index (HI) in the 0-10 cm soil and sediment determined after integration and correlation for spinning side bands (SSB) of solid-state $^{13}$C CPMAS NMR spectra

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S₁: Site 1; S₂: Site 2; U: upland soil; R: riparian soil; S: sediment; T: transect; and HI: humification index.
Fig. 6.3 Solid-state $^{13}$C cross-polarization magic-angle nuclear magnetic resonance spectra for the whole soils at $S_1T_2$ at $S_1$ (a) and $S_2$ (b) in 2013, and $S_1$ (c) and $S_2$ (d) in 2015. $S_1$: Site 1; $S_2$: Site 2; and $T_2$: Transect 2.
6.3.2 $^{13}$C solid state NMR studies on the isolated humic acids (HA)

On initial inspection of the $^{13}$C CPMAS spectra of the HA isolated and examined in this work, it appears that there is an “ordinary” distribution of functional groups within the materials; i.e. it appears that the sample comprises functionality that can be assigned as alkyl, O-alkyl, di-O-alkyl and so on (Fig. 6.4a).

However, in other studies, our collaborators (Zhang/Chen/Boyd/Gray: Personal Communication) have examined the $^{13}$C CPMAS spectra obtained for manufactured biochars (Zhang 2016). Those biochars were derived from four different feedstocks and manufactured by anaerobic pyrolysis over a temperature range of 300-900°C. Although acquisition of data of many of the resultant biochars was hampered due to the presence of persistent free radicals in some samples (dramatically reducing the signal noise ratio obtained in spectra of those samples with significant concentrations of PFRs), the samples manufactured in the 550-650°C range provided excellent spectra. The spectrum of one such biochar (obtained from a sugarcane feedstock at 600°C) is provided in Fig. 6.4b. This spectrum can be compared to the spectrum obtained for the HA sample obtained from $S_1T_3S$ in this work (Fig. 6.4a).

**Figure 6.4** Exemplar $^{13}$C CPMAS spectra obtained for (a) humic acid sample $S_1T_3S$ obtained from the Wyaralong Dam site and (b) a biochar manufactured from sugar cane feedstock by pyrolysis under $N_2$ gas at 600°C.
The similarities in the appearance of the spectra of the HAs obtained from $S_1T_3S$ and spectra obtained for the manufactured biochars are striking.

6.4 Results and discussions

6.4.1 $^{13}$C solid state NMR studies on the whole soils

For the whole soils in 2013, the signals around $\delta$ 28 ppm are attributed to methylene C in long-chain aliphatic compounds (such as fatty acids, lipids, and cutin acids), which is relatively stable towards microbial attack (Schnitzer and Preston 1986; Kögel-Knabner 1997; Ussiri and Johnson 2003; Alcântara et al. 2004). The shoulders appearing around $\delta$ 25 ppm in some spectra indicated of shorter branched-chain polymethylene groups (Mathers et al. 2003). The signals at the region around $\delta$ 50 ppm were assigned to methoxyl C groups; these are normally associated with lignin (Hatcher 1987; Kögel-Knabner 1997; Ussiri and Johnson 2003). The high relative intensity of the signal around $\delta$ 67 ppm indicated that the presence of carbohydrate-derived structures, particularly hexoses, the $C_\alpha$ of some amino acids, and higher alcohols (Kögel-Knabner 1997; Ussiri and Johnson 2003). The signal at around $\delta$100 ppm was assigned to anomeric C of carbohydrates and $C_2$, $C_6$ of syringyl units of lignin; while the signals around $\delta$ 125 ppm indicate the presence of aromatic units, contained in lignin, and olefinic C (Hatcher 1987; Kögel-Knabner et al. 1988; Baldock and Preston 1995; Ussiri and Johnson 2003). The signals around $\delta$ 168 ppm are attributable to carboxyl C; amides and esters contribute to this peak (Kögel-Knabner 1997; Ussiri and Johnson 2003). The highest signal, $\sim$ $\delta$ 67 ppm, together with the signals $\sim$ $\delta$100 ppm and the shoulder $\sim$ $\delta$ 50 and 65 ppm (Fig. 6.3), were characteristics of O-alkyl-C content, specifically a polysaccharide component (Kögel-Knabner 1997). These results were similar to previous studies (Preston et al. 1994; Jien et al. 2011; Chung et al. 2012). The input of fresh organic matter in the riparian areas was easily seen in the spectra of the samples, with a pronounced carbohydrate signature (at around $\delta$ 67 ppm) and a typical anomeric signal (at around $\delta$ 100 ppm) (Larsson et al. 1999; Schmidt et al. 2000). The signal characteristics of lignin (substituted aromatic regions at around $\delta$ 125 ppm and the methoxy peak at around $\delta$ 50 ppm) were also seen in the NMR spectra of the whole soils. Signals originating from waxes typically found in leaves were also detected (methylene groups in long aliphatic chains at around $\delta$ 28 ppm) (Smith et al. 2012).
From the results for integration of $^{13}$C-NMR spectra for whole soils, the intensity of alkyl C and carboxyl C increased in abundance relative to other fractions; while aromatic C signal appeared to decrease at both sites comparing samples collected in 2013 and 2015 (Table 6.3). The lability of SOM was found to be dependent on both chemical recalcitrance and physical protection from microbial degradation (McLauchlan and Hobbie 2004; Rovira and Vallejo 2007; Silveira et al. 2008). Organic compounds with simple structures, such as polysaccharides, followed by proteins and lipids, were rapidly utilized by microbes (Rodger Harvey et al. 1995); whereas complex compounds with high molecular weight and irregular structure were more recalcitrant (Krull et al. 2003; Dodla et al. 2008). Because of the utilization of the easily decomposable carbohydrates by microorganisms and selective preservation of the recalcitrant alkyl-C content in the original plant materials, alkyl-C was considered to increase with decomposition (Wilson et al. 1983; Nordén and Berg 1990; Hayes 1998; Dai et al. 2001; Ussiri and Johnson 2003). Microorganisms are also known to synthesize alkyl C as metabolic products of decomposition (Harvey et al. 1989). In addition, there were no significant differences found except much stronger signals appeared at around δ 20 ppm when we compared whole soil samples collected at S_1 between 2013 and 2015. This indicated more terminal methyl groups at S_1, particularly for the hemicelluloses (Kögel-Knabner 1997; Mathers et al. 2003). However, the signals of samples at S_2 were still weak after two years (Fig. 6.3).

The HI can also provide an index of the extent of decomposition (Baldock and Preston 1995; Knicker et al. 2006), as well as the resource quality of soil C as a substrate for microbes (Webster et al. 2000). This result of HI is in contrast to those of the previous studies, which showed decomposition of SOM was lessened and incomplete, while the humification of SOM was decreased under anaerobic soils (Wershaw 1993; Sahrawat 2003). Such an increase of HI in the sediment might be due to the transportation of the recalcitrant components (alkyl C) to the lake by leaching from the upper sites. In general, the degree of humification in 2015 was larger than that in 2013 at both sites. It reflected that the humification process increased during the two years’ time. However, the HI seemed to be a limited index to compare the humification degree of SOM between the two sites, because it can be affected by the nature of the original C input into the soils (Baldock et al. 1997; Huang et al. 2008).
The little difference in the structural characteristics of the upland soil, riparian soil or sediment suggests that soil moisture and slope position resulted in few or no significant effects on the C structural characterizations. It is possible that the treatment effects were too small to be detected in the NMR spectra, or that would take longer time to observe the effects of soil moisture on SOM.

Soil texture and location effects also exerted a significant control on the characterization of soil organic C (Kögel-Knabner 1997). Clay fraction and fine silt are considered to be associated with refractory organic matter; while sand-size fractions have a single pattern characteristics for plant litter (Baldock et al. 1992). More signals of aromatic C in whole soils from S1 than S2 may indicate the degradation of lignin in clay soil was slower compared with that of sandy soil. Since aromatic C was preferentially preserved relative to other organic C, the abundance of aromatic C was reported to increase with the extent of decomposition in many studies (Wilson et al. 1983; Nordén and Berg 1990; Dai et al. 2001; Ussiri and Johnson 2003). Therefore, our study may indicate that the extent of humification of soil and sediment samples at S1 was stronger than that at S2.

6.3.2 $^{13}$C solid state NMR studies on the isolated humic acids (HA)

As stated, ordinarily, the generalised chemical shift regions for the C functional groups are integrated, across the $^{13}$C chemical shift domain (as discussed above), to obtain some measure of the relative representation of these functional groups, and hence the biomolecular composition, in samples of interest.

In their previous studies on the biochar systems, a series of variable spin speed experiments and spectral fitting experiments were undertaken by our collaborators’ team. In contrast to familiar $^{13}$C CPMAS spectral profiles obtained in SOM and plant residue studies, the spectra obtained for the biochars showed minimal functional group diversity. Consequently, the resonances displayed low chemical shift diversity and relatively tight clustering. The resonances did, however, also evidence intense resonance broadening. In contrast to the line widths typically observed for SOM,
where linewidths are typically <10 kHz (as evidenced by the lack of spinning side bands typically seen across the $^{13}$C NMR spectral domain in CPMAS experiments conducted at the common 5 kHz spinning speed), the linewidths of the species identified in the $^{13}$C CPMAS spectra of the biochars were >30 kHz (Zhang 2016). The fitting of the major isotropic chemical shifts for the series of spectra obtained from the variable spin speed experiments (with spin rate dependent fitting of the intense spinning side bands), showed reasonable matches for the experimental spectral intensity, when a resonance model based on four principal resonance frequencies was employed. The fitted resonances were consistent with the chemical shift regions expected for small condensed aromatic systems, such as pyrenes and coronenes, with minor peripheral substitution (Fig. 6.5). Chemically, these types of species could reasonably be expected to be generated in the anaerobic pyrolysis of organic feedstocks, such as sugarcane and peanut shell. Furthermore, these species would be expected to exhibit a very high degree of resonance broadening, as planar aromatic systems would be expected to exhibit extensive chemical shift anisotropy (McBeath et al. 2014).

Given the apparent similarity of the $^{13}$C CPMAS spectra previously obtained in the biochar experiments and the spectra observed here for the HAs obtained from $S_1T_3S$, we conducted equivalent variable spin speed experiments on the HA samples. Our intent was to confirm whether the various “resonance” regions observed in the $^{13}$C CPMAS spectra of the HAs, reflected actual $^{13}$C functional group populations, or whether they were in fact a potentially misleading series of intense spinning side bands, arising from a high concentration of small condensed, planar aromatic functionality in the isolated HAs. The resultant $^{13}$C CPMAS spectra are compared and contrasted with the spectra obtained from the sugarcane biochar (manufactured at 600 °C) at equivalent spin speeds in the exemplar spectra provided in Figs. 6.6, 6.7 and 6.8. A comparison of the $^{13}$C CPMAS spectra obtained for $S_1T_3S$ at three different spin speeds (3480Hz, 4410Hz and 5000Hz) is provided in Fig. 6.9. The striking similarities evident in the $^{13}$C CPMAS spectra of the $S_1T_3S$ and the sugarcane derived biochar (600 °C) obtained at 5000Hz spin speeds (Fig. 6.4) are still apparent in the spectra of the same samples recorded at 4410Hz (Fig. 6.6). There is considerable peak to peak correspondence throughout the $^{13}$C chemical shift range at both spin speeds.
Figs. 6.7 and 6.8 highlight the changes in the spectra observed for the sugarcane derived biochar and S₁T₃S for the two different spin speeds. Peaks assignable to the resonance frequencies of functional groups will not alter their positions (i.e. alter their isotropic chemical shifts) as a result of a change in the rate of the CPMAS spin speed. The peaks that are seen to alter their positions in the sets of spectra are assignable to spinning side bands, not unique resonances.

While there is evidence of significant concentrations of other functionality being present in the HAs of S₁T₃S spectra, the bulk of the C content in S₁T₃S would still reasonably be attributed to a high concentration of smaller condensed aromatic systems in these samples, presumably similar, but not identical in formulation, to the materials identified in the manufactured biochars. It is also reasonable that the additional signals observed in the S₁T₃S spectra are indicative of functionality that either links aromatic units or occupies terminating positions on the periphery of the aromatic ring systems.

Given the increased relative complexity of the spectra obtained for the HAs in comparison to the less chemically diverse biochar, the simulation of the resonance regions observed in the spectra of the HAs is consequently more complicated, as there is an increased number of variables to fit, relative to the biochar case. Work, attempting to quantify the relative concentrations of aromatic functionality versus nonaromatic functionality in the HAs isolated here, is still ongoing in our research group. Consequently, the quantification of the functional group proportions of all HAs isolated is not available at this time. However, $^{13}$C CPMAS NMR spectra obtained for the HA samples isolated in this work, acquired at 5 kHz spin rates, are provided in Fig. 6.10 for illustration.
Figure 6.5 Exemplar simulation of the spectrum obtained for $^{13}$C CPMAS spectra of the biochar obtained from sugarcane at 600 °C, at 5300 Hz. The spectrum was fitted to a model of four principal chemical shifts ($\delta_{124}$, $\delta_{134}$, $\delta_{116}$ and $\delta_{145}$ ppm) with spinning side bands spaced at 5300 Hz intervals. Magenta line: the calculated spectra; Black line: the experimental spectra; Red line: residuals. Spectra obtained at 3480, 4410, 5000 and 5300Hz spin speeds were fit using this model (Zhang/Chen/Boyd/Gray personal communication).

Figure 6.6 Exemplar $^{13}$C CPMAS spectra obtained for (a) a humic acid sample obtained from the Wyaralong Dam site ($S_1T_3S$) and (b) a biochar manufactured from sugar cane feedstock by pyrolysis under $N_2$ gas at 600°C at a 4410Hz CPMAS spin rate.
**Figure 6.7** Exemplar $^{13}$C CPMAS spectra obtained for a biochar manufactured from sugar cane feedstock by pyrolysis under N$_2$ gas at 600°C at (a) a 5000Hz CPMAS spin rate and (b) a 4410Hz CPMAS spin rate. The shift in the spacing of the spinning side bands with spin speed is in evidence.

**Figure 6.8** a 5000Hz CPMAS spin rate and (b) a 4410Hz CPMAS spin rate. The shift in the spacing of the spinning side bands with spin speed is in evidence.
Figure 6.9 Exemplar $^{13}$C CPMAS spectra obtained for $S_1T_3S$ at CPMAS spin rates of (a) 3480Hz, (b) 4410Hz, and (c) 5000Hz, respectively.
Fig. 6.10 Solid-state $^{13}$C cross-polarization magic-angle nuclear magnetic resonance spectra for humic acids (HA) at $S_1$ (a) and $S_2$ (b). $S_1$: Site 1; $S_2$: Site 2; and $T_2$: Transect 2.
6.5 Conclusions

In general, solid-state $^{13}$C CPMAS NMR spectra of the whole soils indicated that there was little influence of soil moisture or slope positions on the structural composition of SOM along the transects in the riparian zone. However, most of the intensity of HAs was attributed to the primary aromatic spinning side band and not to authentic carboxylate in this study. In addition, the process of humification also increased at both sites during the two years. Nevertheless, HI may not be an ideal index when comparing the humification process at the two sites. Therefore, solid-state $^{13}$C CPMAS NMR spectra gave us the C framework of the whole soils, but not the extracted HAs by semi-quantification of C types. However, further studies still need to be carried out to detect the influence of soil moisture on C functional groups in SOM and HA in the long run.
Chapter 6

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Chapter 6

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Chapter 7 Summary, conclusions and future perspectives

7.1 General discussion and conclusions

As an important part of the soil-plant-water interface ecosystem, riparian zones were considered to be the dynamic boundaries between terrestrial and aquatic systems. They displayed a greater variation in characteristics than either of the systems it connects. Hydrological regimes, especially water fluctuation caused by seasonal climate variations (precipitation, surface and ground water, etc.) and anthropogenic water management (damming, etc.), significantly influenced the characteristics of the riparian zones.

Construction of the dam affected water regimes by reducing the magnitude, timing and frequency of flood events and by changing their periods of occurrence. With damming, the previous riparian zones were inundated and drained intermittently, which led to the alteration of oxic and anoxic periods. Therefore, soil C and N dynamics could be influenced by the anthropogenic flood-control measures.

Soil C and N cycling processes in the riparian zone are complex phenomena influenced by different factors. It is still unknown how C and N would respond to the water fluctuation in the riparian zones. To fill these research gaps, my PhD work was proposed to test the following hypotheses: (1) soil moisture was a driving factor controlling the spatial and seasonal distribution of soil C and N pools, resulting from water fluctuation by both of seasonal climate change and the Wyaralong Dam operation; (2) the soil labile organic C pools ($\delta^{13}$C, HWEOC, and MBC), and N pools ($\delta^{15}$N, HWETN and MBN) were sensitive indicators on the impacts of water level fluctuation, compared with the soil HS and whole soil; and (3) riparian zones were hotspots for biogeochemical C and N dynamics (mineralization and immobilization). We aimed to investigate the key processes of soil C pools and N dynamics that occurred in the riparian zone after the construction of Wyaralong Dam, which would be important for the sustainability of the riparian ecosystem and ecosystem services.

Despite of the increasing recognition of the riparian ecosystem, previous studies on the impact of soil moisture have rarely looked into the spatial and seasonal distributions of soil C and N pools in the riparian areas, particularly over the watershed of a dam in the Southern Hemisphere. To the best of our knowledge, this thesis is the first study examining impacts of moisture regime on soil C and N
dynamics at different spatial and seasonal scales. Specifically, each of the above three hypotheses was tested in an individual experiment and the results were presented in each of the four chapters.

Firstly, in Chapter 3, the spatial and seasonal dynamics of soil labile C (HWEOC and MBC) and N (HWETN and MBN) pools were determined within the riparian zone of Wyaralong Dam. Concerns about the impact of flooding on soil C and N pools in the riparian areas were mainly focused on total SOM such as N removal in the riparian buffers. There has been little work conducted to examine the relationship between soil moisture and labile C and N pools. The results suggested that soil labile C and N pools decreased along the transects in both soil depths of the two soil types, with the peak or bottom of values detected between upland slope and the riparian zone. Other factors rather than soil moisture were more important in regulating seasonal changes of soil HWEOC and HWETN except the dry-rewetting influence in November 2013. Soil moisture played a significant role in the seasonal variation of MBC and MBN. Soil labile C (HWEOC and MBC) and N (HWETN and MBN) pools at Site 1 (S1, heavy texture), were significantly higher than those at Site 2 (S2, light texture).

Secondly, we examined whether soil $\delta^{15}$N and $\delta^{13}$C could be used as sensitive indicators for soil N and C dynamics in the subtropical riparian areas (Chapter 4). Most of the previous studies on soil $\delta^{15}$N and $\delta^{13}$C have focused at large scales. The second experiment evaluated the spatial and seasonal dynamics of soil $\delta^{15}$N and $\delta^{13}$C at regional scale. Results showed that significant spatial and seasonal variations of soil $\delta^{15}$N could be explained by microbial activity driven by soil moisture. The highest and lowest rates of soil $\delta^{13}$C along the transects were separately found in the riparian zones for different seasons of two soil types. Clear seasonal patterns of soil $\delta^{13}$C were not observed, except few slope positions with the lowest level in summer and highest in winter. Soil total C (TC) and total nitrogen (TN) were less variable and more uniform at both sites.

Thirdly, this study determined the influence of soil moisture on N transformations based on laboratory incubation (Chapter 5). Soils (0-10 cm) were collected from two sites with different soil textures in the riparian zone of Wyaralong Dam in August 2015 (dry season). The results showed that significant influences of soil moisture could be found on soil net and gross N transformation rates for the riparian soils. Net ammonification rates increased with soil moisture for both within and among each slope
position treatments at both sites. On the contrary, soil net nitrification rates declined significantly within each slope position treatment. Soil net ammonification rate was negatively correlated with net nitrification rate for soils at S1. Gross N mineralization rates increased with soil moisture for both among and within each slope position treatment; while gross nitrification rates significantly decreased with the soil moisture within each slope position treatment. The gross nitrification rates were higher compared with the gross N mineralization rates, which was likely due to possible heterotrophic nitrification. Most of the $^{15}$N addition was immobilized and followed by possible gaseous N emissions. As the time went on, less $^{15}$N-NH$_4^+$ was autotrophically nitrified for the saturated soils; while more $^{15}$N-NO$_3^-$ existed as dissimilatory NO$_3^-$ reduction to NH$_4^+$ (DNRA) under anaerobic condition.

Chapter 6 investigated the structural characterization of soil organic matter (SOM) in the whole soils and humic acids (HAs) by solid-state $^{13}$C CPMAS NMR spectroscopy within the riparian zone. The result showed that O-alkyl C and alkyl C peaks were dominated over the aromatic and carboxyl C peaks in the whole soil at both sites in both years. The intensity of alkyl C and carboxyl C increased in abundance relative to other fractions; while aromatic C signal decreased at both sites comparing samples collected between 2013 and 2015. Little detectable variations of structural characteristics of whole soils among the upland soil, riparian soil, and sediment were detected within each year. Soil and sediment samples collected from Site 1 (S$_1$) contained considerable more signals of aromatic C than those from Site 2 (S$_2$). Peaks assignable to the resonance frequencies of functional groups in HAs did not alter their positions as a result of a change in the rate of the CPMAS spin speed. The degree of humification in 2015 was greater than that in 2013 at both sites, as indicated by humification index (HI).

In conclusion, this PhD work clearly demonstrated that soil moisture would be an important driving factor affecting the spatial and seasonal distribution of soil labile C and N pools. Soil $\delta^{15}$N and $\delta^{13}$C could provide insights into soil N and C dynamics in the riparian areas spatially and seasonally. However, soil $\delta^{15}$N appeared to be a much more sensitive indicator when combined with labile N pools, TN and soil C/N ratio. Laboratory incubation showed that soil moisture significantly affected net and gross N transformation rates in the two riparian soils. Small differences in the structural compositions
of the whole soils were found among different slope positions along the transects in the riparian zone. Nevertheless, the peaks of HAs that were seen to alter their positions in the sets of spectra were assignable to spinning side bands, not unique resonances. Additionally, HI indicated that the process of humification also increased during the two years. Our study highlighted the importance of riparian zones as the hotspot of soil C and N dynamics where microbial activity might be higher than other positions.

7.2 Future work

This body of work has provided valuable information on the dynamics of soil C and N in the riparian zone of Wyaralong Dam. This information would have great ecological implications for global climate change and help ecologists to diversify strategies that promote sustainable development of riparian zones. This study has identified a few knowledge gaps and future work is required in some aspects as follows:

(1) Since surface soils of the riparian zones often undergo rapid wetting followed by drying periods. Understanding how dry-rewetting cycles affect C and N transformations is important in predicting SOM dynamics. However, we did not study the dry-rewetting effect on soil C and N in this study. Future studied should incorporate long-term field experiments and laboratory incubations to investigate the influence of dry-rewetting effect on soil gross N transformation and greenhouse gas emissions (principally CO₂, CH₄ and N₂O).

(2) This study mainly focused on soil C and N dynamics in the riparian zone soil after the construction of Wyaralong Dam. Future studies should focus on the comparison of soil C and N changed before and after the construction of Wyaralong Dam, in order to detect the influence of the dam construction.

(3) More laboratory experiments should be carried out to investigate the effects of different C substrates on soil C mineralization and N dynamics in the riparian zone, in order to better understand the contribution of residue incorporation to soil C and N pools.

(4) Given the simulation of the resonance regions observed in the spectra of the HAs is consequently more complicated, relative to the biochar case, more works should be done to quantify the relative
concentrations of aromatic functionality versus non-aromatic functionality in the HAs isolated.