ABSTRACT

Title of Dissertation: UREA HYDROLYSIS IN SOIL PROFILE TOPOSEQUENCES: MECHANISMS RELEVANT TO NITROGEN TRANSPORT AND WATER QUALITY

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ABSTRACT

Urea has been linked to harmful algal blooms in surface waters, but numerous studies of its hydrolysis in agricultural soils have concluded that urea does not persist long enough to be transported to surface waters. This paradox in the published literature may be explained by our lack of knowledge regarding the soil chemical conditions that affect microbial urease activity in surface and subsurface horizons of soil profiles that lie between agricultural fields and surface waters, particularly in sandy Coastal Plain regions. Laboratory studies were conducted to determine the most influential soil chemical characteristics predicting rates of urea hydrolysis in six Maryland soils. Soils were sampled from both the A and B horizons of toposequences consisting of an agricultural field, a grassed field border, and a transitional zone adjacent to surface waters. A pH-adjustment experiment identified soil C and N as important predictors of urea hydrolysis. Analysis of microbial community composition and *ureC* genes across a toposequence found the greatest abundance of bacteria, fungi, and *ureC* genes in riparian

A horizon soils, despite inhibitory conditions of low pH, low field-sampled moisture content, and high extractable metal concentrations. The high carbon content of A horizon riparian soils likely mediated these toxic characteristics. Of particular note was the significant correlation between *ureC* genes and rate of urea hydrolysis ($r^2 = 0.82$), indicating that the presence of this gene may be useful as a biomarker for predicting rates of urea hydrolysis in other soils. An investigation into the effects of added C revealed that diverse soil C compounds influenced urea hydrolysis differently. In a 24 hr incubation, ascorbic and gallic acid acted as pro- and antioxidants with both enhancement and inhibition of hydrolysis, depending upon concentration, whereas benzoic and cinnamic acids likely enhanced hydrolysis as a result of being metabolized by soil microorganisms. A better understanding of the mechanisms controlling urea hydrolysis in diverse soils will help researchers and policymakers formulate defensible recommendations related to urea fertilizer and animal waste application so that urea-N can be efficiently used by crops and urea movement across the landscape and into surface waters can be minimized.

UREA HYDROLYSIS IN SOIL PROFILE TOPOSEQUENCES: MECHANISMS RELEVANT TO NITROGEN TRANSPORT AND WATER QUALITY

by

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Dissertation submitted to the faculty of the graduate school of the
University of Maryland, College Park in partial fulfillment
of the requirements for the degree of
Doctor of Philosophy
2014

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Acknowledgements

I would like to thank the members of my committee for their thorough and thought-provoking review of my research proposal and dissertation, and their commitment to challenging me throughout the completion of this PhD program. I would especially like to thank Dr. Bruce James, who served as my PhD advisor and inspired me to enjoy the journey of finding the story behind my data, even when that path was long and meandering. I would also like to thank Dr. Stephanie Yarwood for taking me under her wing in the study of microbial genetics, an area of research that was wholly new to me and very exciting to learn. I would like to acknowledge my fellow graduate students, and especially Natalie Lounsbury, Christine Prasse, and Glade Dlott, for their help with questions along the way. In addition, many thanks to the undergraduate students who worked with me in the Soil Chemistry lab and assisted me in the gathering of the data presented in this dissertation: Sara Mack, Shulamit Shroder, and Meg Ryan.

A final thank-you to my family for being interested in learning about soil science and remembering to refer to it as “soil”, rather than “dirt”. I especially thank my husband, Eric Stavale, who experienced this entire PhD program by my side. From our statistical discussions over breakfast, to providing encouragement during my comprehensive exams, and helping me sample soils and complete lab work on a few special weekend days, thank you for your support, enthusiasm, encouragement, and love.

TABLE OF CONTENTS

List of Tables v

List of Figures v

List of Abbreviations xii

Chapter 1: Urea in Soils and Surface Waters 1
- Urea in Surface Waters: Natural Cycles, Eutrophication, and Harmful Algal Blooms 1
- Urea in Animal Wastes and Soils: Transformations and Pathways from Land to Surface Waters 5
- Urease: Enzyme Structure, Variability, and Activity in Soils and Cells 10
- Uncertainties in Understanding and Predicting Urea Persistence in Soils: Justification for My Research 17
- Research Chapters 23

Chapter 2: Soil C, N, and metal effects on urea hydrolysis in soil profile toposequences from the Coastal Plain and Piedmont regions 27
- Introduction 27
- Materials & Methods 31
- Results & Discussion 36
- Conclusions 56

Chapter 3: Urease gene number correlation with urea hydrolysis in soils across an agricultural – riparian landscape and pH gradient 58
- Introduction 58
- Materials & Methods 62
- Results & Discussion 67
- Conclusions 82

Chapter 4: Urea hydrolysis in soils: enhancement and inhibition by ascorbic, gallic, benzoic, and cinnamic acids. 86
- Introduction 86
- Materials & Methods 91
- Results & Discussion 93
- Conclusions 110

Chapter 5: Urea hydrolysis in soils: pedological, chemical, and microbial controls 112

Appendix A: Soil sampling locations 121

Appendix B: Additional data and information related to findings in Chapter 2 123

Appendix C: Additional data and information related to findings in Chapter 3 136

Appendix D: Additional data and information related to findings in Chapter 4 143

Appendix E: Additional data and information related to discussion in Chapter 5. 147
Appendix F: Studies of effects of added P on soil urea hydrolysis 148
References 150

List of Tables

Table 2.1: Selected physical and chemical properties of soils sampled in 2012 along two transects consisting of an agricultural field (AG), a grassed field border (GB), and a riparian zone (RZ) in the Coastal Plain (CP) and a wetland edge (WE) in the Piedmont (PM). 39

Table 2.2: Rate of urea hydrolysis in native Coastal Plain and Piedmont transect soils (AG = agricultural field, GB = grassed field border, RZ = riparian zone, WE = wetland edge) based on zero-order kinetics. 42

Table 2.3: Soil chemical characteristics of Coastal Plain A horizon transect soils treated with diluted HCl or $CaCO_3$ to achieve a range of pH values. 46

Table 2.4: Soil chemical characteristics of Coastal Plain B horizon transect soils treated with diluted HCl or $CaCO_3$ to achieve a range of pH values. 47

Table 2.5: Soil chemical characteristics of Piedmont A horizon transect soils treated with diluted HCl or $CaCO_3$ to achieve a range of pH values. 48

Table 2.6: Soil chemical characteristics of Piedmont B horizon transect soils treated with diluted HCl or $CaCO_3$ to achieve a range of pH values. 49

Table 2.7: Multiple regression model components for pH-adjusted soils from the Coastal Plain (CP) and Piedmont (PM) soil transects. 52

Table 2.8: Linear regression models for rate of urea hydrolysis (Y) vs. pH (X) in the pH-adjusted soils from the A and B horizons of the Coastal Plain and Piedmont soil transects consisting of an agricultural field (AG), a grassed field border (GB), and a riparian zone (RZ) in the Coastal Plain and a wetland edge (WE) in the Piedmont..... 53

Table 3.1: Gene abundances in native Coastal Plain transect soils. AG (agricultural field), GB (grassed field border), RZ (riparian zone), n = 3. 69

Table 3.2: Rates of urea hydrolysis in native Coastal Plain transect soils. AG (agricultural field), GB (grassed field border), RZ (riparian zone). 70

Table 3.3: Fungal-to-bacterial gene copy ratios in native transect soils. AG (agricultural field), GB (grassed field border), RZ (riparian zone). 72

Table 4.1: Selected physical and chemical properties of soils sampled along a transect consisting of an agricultural field (AG) and a riparian zone (RZ) in the Coastal Plain region of Maryland's Eastern Shore. 94

Table B.1: Selected physical and chemical properties of soils sampled in 2013 along three transects consisting of an agricultural field (AG), a grassed field border (GB), and a riparian zone (RZ) in the Coastal Plain..... 126

Table B.2: Date for rates of urea hydrolysis in native Coastal Plain A and B horizon soils plotted in Figure 2.1. Three replicates per soil for each time are listed from the AG (agricultural field), GB (grassed field border), and RZ (riparian zone) transect points sampled in 2013. Data for the 2012 transect is included in Tables B.2 and B.3. 127

Table B.3: Data for rates of urea hydrolysis in native Coastal Plain A horizon transect soils sampled in 2012 and plotted in Fig. 2.2. Three replicates per soil at each time are listed from the AG (agricultural field), GB (grassed border), and RZ (riparian zone) A horizon soils. 128

Table B.4: Data for rates of urea hydrolysis in native Coastal Plain B horizon transect soils sampled in 2012 and plotted in Fig. 2.3. Three replicates per soil at each time are listed from the AG (agricultural field), GB (grassed border), and RZ (riparian zone) B horizon soils. 129

Table B.5: Data for rates of urea hydrolysis in native Piedmont A horizon transect soils, plotted in Fig. 2.2. Three replicates per soil at each time are listed from the AG (agricultural field), GB (grassed border), and WE (wetland edge) A horizon soils. 130

Table B.6: Data for rates of urea hydrolysis in native Piedmont B horizon transect soils, plotted in Fig. 2.3. Three replicates per soil at each time are listed from the AG (agricultural field), GB (grassed border), and WE (wetland edge) B horizon soils. 131

Table B.7: Data for rate of urea hydrolysis in pH-manipulated Coastal Plain A horizon transect soils plotted in Fig. 2.4a. AG (agricultural field), GB (grassed border), and RZ (riparian zone) soils listed. Each mean and SEM derived from three replicates. 132

Table B.8: Data for rate of urea hydrolysis in pH-manipulated Coastal Plain B horizon transect soils plotted in Fig. 2.4c. AG (agricultural field), GB (grassed border), and RZ (riparian zone) soils listed. Each mean and SEM derived from three replicates. 133

Table B.9: Data for rate of urea hydrolysis in pH-manipulated Piedmont A horizon transect soils plotted in Fig. 2.4b. AG (agricultural field), GB (grassed border), and WE (wetland edge) soils listed. Each mean and SEM derived from three replicates. 134

Table B.10: Data for rate of urea hydrolysis in pH-manipulated Piedmont B horizon transect soils plotted in Fig. 2.4d. AG (agricultural field), GB (grassed border), and WE (wetland edge) soils listed. Each mean and SEM derived from three replicates. 135

Table C.1: Data for numbers of Eub 16S genes in pH-manipulated soils presented in Fig. 3.2. AG (agricultural field), GB (grassed border), and RZ (riparian zone) A horizon soils listed. 136

Table C.2: Data for numbers of ITS genes in pH-manipulated soils presented in Fig. 3.3. AG (agricultural field), GB (grassed border), and RZ (riparian zone) A and B horizon soils listed..... 137

Table C.3: Data for numbers of Eub 16S genes in pH-manipulated soils presented in Fig. 3.4. AG (agricultural field), GB (grassed border), and RZ (riparian zone) A horizon soils listed. 138

Table C.4: Data for numbers of ureC genes and rate of urea hydrolysis in pH-manipulated soils presented in Fig. 3.5. AG (agricultural field), GB (grassed border), and RZ (riparian zone) A and B horizon soils listed. 139

Table C.5: Data for the correlation between numbers of ureC and Eub 16S genes in pH-manipulated soils before urea was added. Presented in Fig. 3.6 a and c. AG (agricultural field), GB (grassed border), and RZ (riparian zone) A and B horizon soils listed. 140

Table C.6: Data for the correlation between numbers of ureC and Eub 16S genes in pH-manipulated soils after urea was added. Presented in Fig. 3.6 b and d. AG (agricultural field), GB (grassed border), and RZ (riparian zone) A and B horizon soils listed. 141

Table C.7: ITS gene copy numbers in freshly-sampled vs. stored soils from the agricultural field (AG), grassed field border (GB), and riparian zone (RZ) A and B horizons. Measurements for triplicate samples presented. 142

Table D.1: Chemical characteristics of AG Ap soil treated with different types and concentrations of added C..... 143

Table D.2: Chemical characteristics of AG Bt soil treated with different types and concentrations of added C..... 144

Table D.3: Chemical characteristics of RZ A1/A2 soil treated with different types and concentrations of added C..... 145

Table D. 4: Chemical characteristics of RZ BC soil treated with different types and concentrations of added C..... 146

List of Figures

Figure 1.1: The multi-discipline approach used in this research to investigate urea hydrolysis in soils. Pedalogical techniques were used for sampling soil in the field, soil chemical and microbiological methods were employed in experimental designs, and results relevant to soil fertility and water quality have been emphasized..... 21

Figure 2.1: Changes in the concentration of urea with time in native soil transects sampled in 2012 and 2013 from the Coastal Plain site. Transects consisted of AG (agricultural field), GB (grassed field border), and RZ (forested riparian zone) A and B horizon soils. SEM plotted, but some error bars fall within the symbol areas (n=3). Soil physical and chemical characteristics for the 2013 soil transects are summarized in Table B.1 in Appendix B. 37

Figure 2.2: Urea hydrolysis, ammonium generation, and nitrification in soils sampled from the A horizons of Coastal Plain (CP) and Piedmont (PM) transects consisting of an agricultural field (AG), a grassed field border (GB), and a riparian zone (RZ) in the CP and a wetland edge (WE) in the PM. SEM plotted (some error bars are within the symbols), n=3..... 40

Figure 2.3: Urea hydrolysis, ammonium generation, and nitrification in soils sampled from the B horizons of Coastal Plain (CP) and Piedmont (PM) transects consisting of an agricultural field (AG), a grassed field border (GB), and a riparian zone (RZ) in the CP and a wetland edge (WE) in the PM. SEM plotted (some error bars are within the symbols), n=3..... 41

Figure 2.4: Linear regression of rate of urea hydrolysis vs. pH in both the A (panels a and b) and B (panels c and d) horizons of the Coastal Plain (CP) and Piedmont (PM) soil transects consisting of an agricultural field (AG), a grassed field border (GB), and a riparian zone (RZ) in the CP and a wetland edge (WE) in the PM. Hydrolysis rates at the lowest pH values in the PM GB and WE Ap Horizon soils (symbols not connected to linear regression lines) were removed from the linear regression analysis because these data points were not linear. SEM plotted (some error bars are within the symbols), n=3.50

Figure 3.1: Genes extracted from the native agricultural field (AG), grassed field border (GB), and riparian zone (RZ) soils in both the a) A and b) B horizons. Error bars represent the SEM among triplicate analytical replicates, and in some cases are within the margins of the bar graph. 74

Figure 3.2: Eub 16S genes in pH-adjusted A horizon soils following the addition of urea during the urea hydrolysis rate experiment. Hollow symbols denote native soils, and error bars indicate ± SEM (n=3). 78

Figure 3.3: ITS genes in pH-adjusted A and B horizon soils following the addition of urea during the urea hydrolysis rate experiment. Hollow symbols denote native soils, and error bars indicate ± SEM (n=3). 79

Figure 3.4: An increase in the rate of urea hydrolysis was significantly correlated with Eub 16S gene copies in the pH-adjusted A horizon soils both a) before and b) after incubation with urea ($p < 0.05$). AG Ap (circles), GB Ap (squares), and RZ A1/A2 (triangles) plotted. Hollow symbols denote native soils (n=3). 81

Figure 3.5: Significant correlations between urea hydrolysis rate and ureC genes in both A (a and b) and B (c and d) horizons, before (a and c) and after (b and d) urea added ($p < 0.05$). AG Ap (circles), GB Ap (squares), and RZ A1/A2 (triangles) plotted. Hollow symbols denote native soils (n=3). 83

Figure 3.6: Significant correlations between Eub 16S and *ureC* genes in both A (a and b) and B (c and d) horizons, before (a and c) and after (b and d) urea added ($p < 0.05$). Addition of urea improved r^2 in A horizon (a and b). AG Ap (circles), GB Ap (squares), and RZ A1/A2 (triangles) plotted. Hollow symbols denote native soils (n=3). 84

Figure 4.1: Structural formulas for the four organic acids tested in this study. Gallic and ascorbic acids contain resonance-stabilized –OH groups, which makes them effective reducing agents and anti-oxidants. Benzoic and cinnamic acids do not donate electrons and therefore do not act as reducing agents (Alamed et al., 2009; Brewer, 2011). 96

Figure 4.2: Effects of added ascorbic acid-C on urea hydrolysis and soluble metal concentration, relative to the control treatment, in each soil and horizon. Letters that are not different within the same soil horizon are not significantly different in the amount of urea-N hydrolyzed ($p < 0.05$). †Indicates treatment is not significantly different from the control treatment for that soil horizon. AG: agricultural field, RZ: riparian zone. SEM plotted (n = 3; n = 2 in urea-N in RZ BC 4,000 mg C kg^{-1} soil)..... 98

Figure 4.3: pe-pH diagrams for each organic acid showing FeOOH-Fe^{2+} ((red)/(ox)=10^{-4}; dashed line) and MnOOH-Mn^{2+} ((red)/(ox) = 10^{-4}; solid line) equilibria. AG: agricultural field, RZ: riparian zone. Treatments corresponding to those below the FeOOH-Fe^{2+} equilibrium line are listed in Appendix D..... 101

Figure 4.4: Effects of added gallic acid-C on urea hydrolysis and soluble metal concentration, relative to the control treatment, in each soil and horizon. Letters that are not different within the same soil horizon are not significantly different in the amount of urea-N hydrolyzed ($p < 0.05$). †Indicates treatment is not significantly different from the control treatment for that soil horizon. AG: agricultural field, RZ: riparian zone. SEM plotted (n = 3). 103

Figure 4.5: Effects of added benzoic acid-C on urea hydrolysis and soluble metal concentration, relative to the control treatment, in each soil and horizon. Letters that are

not different within the same soil horizon are not significantly different in the amount of urea-N hydrolyzed ($p < 0.05$). †Indicates treatment is not significantly different from the control treatment for that soil horizon. AG: agricultural field, RZ: riparian zone. SEM plotted (n = 3). 106

Figure 4.6: Effects of added cinnamic acid-C on urea hydrolysis and soluble metal concentration, relative to the control treatment, in each soil and horizon. Letters that are not different within the same soil horizon are not significantly different in the amount of urea-N hydrolyzed ($p < 0.05$). †Indicates treatment is not significantly different from the control treatment for that soil horizon. AG: agricultural field, RZ: riparian zone. SEM plotted (n = 3). 107

Figure A.1: Transect sampling points at the Wye Island Natural Resource Management Area within the Coastal Plain physiographic province, consisting of an agricultural field (AG), a grassed field border (GB), and a riparian zone (RZ). The red transect was sampled in October, 2012 and the blue transects were sampled in October, 2013. AG soil sample was from the Ingleside mapping unit (IgB) (38°54'11.97"N, 76° 8'12.20"W), the GB soil was from the boundary of Ingleside and Longmarsh-Zekiah (LZ) mapping units (38°54'10.37"N, 76° 8'13.79"W), and the RZ soil was from the Longmarsh- Zekiah mapping unit (38°54'9.98"N, 76° 8'14.70"W); all of which were similar to the Ingleside series (coarse-loamy, siliceous, mesic Typic Hapludult). Photo obtained using Google Earth (Version 7.1.2.2041)..... 121

Figure A.2: Transect sampling points at the Central Maryland Research and Education Center, Clarksville Facility, in the Piedmont physiographic province consisting of an agricultural field (AG), a grassed field border (GB), and a wetland edge (WE). The AG soil sample was from the Glenelg unit (GgB) and the GB soil was from the Glenville-Baile unit (GnB); both similar to the Glenelg series (fine-loamy, mixed, semiactive, mesic Typic Hapludults). The WE soil was from the Hatboro-Codorus unit, similar to the Codorus series (fine-loamy, mixed, active, mesic Fluvaquentic Dystrudept). Photo obtained using Google Earth (Version 7.1.2.2041). 122

Figure B.1: First-order kinetics in Coastal Plain (CP or Wye) pH-manipulated transect soils, consisting of an agricultural field (AG), a grassed field border (GB), and a riparian zone (RZ) sampled from both A and B horizons. SEM plotted (some error bars are within symbols), n=3..... 123

Figure B.2: First-order kinetics in Piedmont (PM or Clarksville) pH-manipulated transect soils, consisting of an agricultural field (AG), a grassed field border (GB), and a wetland edge (WE) sampled from both A and B horizons. SEM plotted (some error bars are within symbols), n=3. 124

Figure B.3: Soils sampled in July 2012 from the Ap and Bt horizons of grassy areas near agricultural fields at both the Piedmont (PM) and Coastal Plain (CP) sites were used to

monitor the stability of urea hydrolysis rate in soils stored field-moist in the laboratory (†Due to differences in field sampled moisture content, final concentrations of added N varied; CP Ap received 113 mg urea-N kg^{-1} soil, CP Bt received 110 mg urea-N kg^{-1} soil, PM Ap received 114 mg urea-N kg^{-1} soil, and CP Bt received 121 mg urea-N kg^{-1} soil125

Figure E.1: Temperature effects on urea hydrolysis in Coastal Plain (CP) and Piedmont (PM) A horizon soils. AG (agricultural field), GB (grassed field border), RZ (riparian zone), WE (wetland edge) soils plotted. SEM plotted, error bars may be within symbol area (n=3). 147

Figure F.1: Urea hydrolysis in soils sampled from the Wye Research and Education Center, which have a history of different levels of P_2O_5 application. Lack of evidence of inhibition of urea hydrolysis by soil P directed research toward soil C (Chap. 4). 148

Figure F.2: Urea hydrolysis in soils treated with varying concentrations of NaCl and Na_2HPO_4. Wye (or Coastal Plain) agricultural field, transect 1 (WA1), Wye grassed field border, transect 1 (WEOF1), and Wye riparian zone, transect 1 (WR1) values plotted. Results of this work and that presented in Figure E.2 directed future research toward soluble C effects on soil urea hydrolysis. 149

List of Abbreviations

CP: Coastal plain

PM: Piedmont

AG: Agricultural field

GB: Grassed field border

RZ: Riparian zone

HAB: Harmful algal bloom

PSP: Paralytic shellfish poisoning

qPCR: Quantitative polymerase chain reaction

ITS: Internal transcribed spacer

AOA: Ammonia-oxidizing archaea

Chapter 1: Urea in Soils and Surface Waters

Urea in Surface Waters: Natural Cycles, Eutrophication, and Harmful Algal Blooms

Urea ($CO(NH_2)_2$) is a naturally-occurring form of nitrogen (N) that is present in both aquatic and terrestrial environments. In the 1990s, researchers began to suspect that natural urea concentrations in surface waters were being increased by anthropogenic sources (Glibert and Terlizzi, 1999), and that higher levels of urea in coastal zones might be contributing to an increase in the frequency of harmful algal blooms (HABs) (Glibert et al., 2006). Historically, however, this form of N was not routinely measured in water samples; therefore, pinpointing the natural background concentration of urea was difficult in surface waters such as Chesapeake Bay (but see Lomas et al., 2002). Fluctuations in seasonal rainfall, mixing currents, and a salinity gradient from fresh to saltwater along the axes of many coastal bays makes these ecosystems dynamic and variable over space and time in both their physical and biological characteristics. Nevertheless, in recent years, researchers studying urea in Chesapeake Bay have identified annual mean concentrations in surface waters from 7 to 21 μg urea-N L^{-1} (Lomas et al., 2002; Glibert et al., 2005), with individual measurements as high as 336 μg urea-N L^{-1} (Glibert et al., 2005). A study in an isolated system of striped bass aquaculture ponds found that dinoflagellate blooms were associated with urea-N levels in excess of 21 μg L^{-1}, whereas no dinoflagellate blooms occurred when urea concentrations were below this concentration (Glibert and Terlizzi, 1999). This same threshold has been exceeded immediately preceding harmful algal blooms events in Chesapeake Bay tributaries in the wake of heavy spring rains draining agricultural watersheds. During these events, urea-N levels have reached

between 112-196 μg urea-N L^{-1} (Glibert et al., 2001). Lower concentrations of urea (3.5 μg urea-N L^{-1}) were present during harmful algae blooms in Monterey Bay, California (Kudela et al., 2008). In the East China Sea and off the west coast of Florida, urea is believed to contribute to certain stages and types of harmful algae blooms, perhaps selecting for or being preferentially utilized by specific phytoplankton and cyanobacteria (Heil et al., 2007; Li et al., 2010). Understanding the sources of urea to coastal waters, and whether it is natural or anthropogenic, has become an important research goal.

In aquatic environments, urea is part of the dissolved organic N pool where it is produced by bacteria, fish, zooplankton, and numerous other organisms as a byproduct of metabolism. It can also be released from sediments (Therkildsen and Lomstein, 1994; Tyler et al., 2003) and arise from the microbial decomposition of organic substrates (Berman and Bronk, 2003). Its release from sediments is likely the reason for elevated urea concentrations in bottom waters (1 m above the sediment) relative to surface waters. Average annual surface water concentrations of urea in Chesapeake Bay have ranged from 6.9-13 μg urea-N L^{-1}, whereas urea concentrations in samples taken in bottom waters were higher and more variable, with values ranging from 7-21 μg urea-N L^{-1} (Lomas et al., 2002). The higher concentrations of urea found in bottom waters possibly results from the breakdown of recently settled particulate organic matter. Urea is consumed by bacteria and phytoplankton and is a valuable source of N for these organisms (Altman and Paerl, 2012). Researchers (e.g. Lomas et al., 2002) have observed a close relationship between the levels of urea in the Chesapeake Bay and the rate of urea uptake by the planktonic community, suggesting that urea is an important component of the N cycle in primary producers, averaging 18% of measured N uptake (among nitrate,

ammonium, and urea). In North Carolina's Neuse River Estuary, urea accounted for 16-45% of the total N taken up by the microbial community, despite its concentration remaining consistently below that of dissolved inorganic N (NH_4^+ and NO_3^-) (Twomey et al., 2005). Some of the species that have the ability to use urea as an N source can cause HABs, and sometimes toxins. For example, the HAB dinoflagellate *Alexandrium catenella* is capable of using urea as an N source and can produce a saxitoxin that causes paralytic shellfish poisoning (PSP) (Camargo and Alonso, 2006; Solomon et al., 2010). The preferential use of urea by different types of phytoplankton and under different environmental conditions has been documented (e.g. Li et al., 2012). However, urea is not the only source of N that can contribute to algal blooms and eutrophication; some toxin-producing phytoplankton are capable of growing on urea as well as other forms on N (NH_4^+ and NO_3^-) (Auro and Cochlan, 2013), and increased production of toxins has been measured in some phytoplankton grown on urea, NH_4^+, or NO_3^- as an N source (Leong et al., 2004; Howard et al., 2007; Adolf et al., 2009; Thessen et al., 2009). A simultaneous wordwide increase in both the use of urea fertilizer and the incidence of HABs and PSP in coastal areas has focused some attention on the effects of urea-N in aquatic systems (Glibert et al., 2006). Researchers suspect that urea, which is applied to agricultural fields as both a fertilizer and as a decomposition product of the uric acid in poultry litter, may be leaching from farmland and contributing to organic N enrichment and HABs in these waterways (Glibert et al., 2006).

In some coastal areas, variations in the seasonal concentrations of urea in surface waters have helped researchers separate what is believed to be allochthonous from autochthonous urea. A study in the Neuse River Estuary of North Carolina did not detect

a seasonal pattern of urea concentration in that water body, and attributed the presence of urea to natural microbial mineralization of dissolved organic N (Twomey et al., 2005). In Chesapeake Bay, seasonal variability in urea concentration has been reported, with some years showing a peak in surface urea concentrations in the late winter/spring (Dec/April), and others showing a distinct peak in the summer due to differences in seasonal rainfall in the different years (Lomas et al., 2002). The winter/spring peak may not be solely a result of background urea regeneration resulting from bacterial processes, as this requires organic matter that is often produced during or after the spring bloom. In fact, the highest rates of urea regeneration are found in the fall (Lomas et al., 2002). Therefore, there is support for the assertions made (Glibert et al., 2006) that external and anthropogenic sources of unhydrolyzed urea are entering Chesapeake Bay.

In the absence of the enzyme urease that catalyzes its hydrolysis, urea is stable in aqueous solutions between pH 2 and 12, with a half-life of 3.6 years at 38°C (Zerner, 1991). Under these conditions, urea can slowly break down by an elimination reaction to produce NH_3 and isocyanate (HNCO), by the mechanism shown in Eqn. 1.1 (Krajewska, 2009):

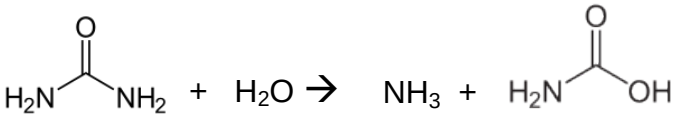
$$CO(N_{H_2})_2 \rightarrow HNCO + NH_3 \xrightarrow{2H_20} H_2CO_3 + 2NH_3 \qquad \text{(Eqn. 1.1)}$$

Urea's stability under these conditions may result from resonance, which donates electrons to the carbonyl carbon and causes it to be less subject to nucleophilic attack (Zerner, 1991). Therefore, in the absence of urease, urea may remain unhydrolyzed, soluble, and bioavailable in surface waters until environmental conditions for bacterial or algal growth are favorable for its hydrolysis.

Urea in Animal Wastes and Soils: Transformations and Pathways from Land to Surface Waters

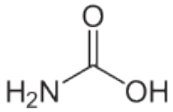
In terrestrial environments, urea is present as a result of both natural and anthropogenic processes. Urea is an intermediate of microbial metabolism, a component of mammalian urine, and a decomposition product of the uric acid excreted by birds, some amphibians, and most insects (Wright, 1995; Hasan, 2000). It is present in the effluent of some sewage treatment plants (Cozzi et al., 2014), animal agriculture excrement, and is the dominant form of N fertilizer used in agriculture worldwide (Glibert et al., 2014). It is also used in aquaculture to stimulate algal growth for fish or shrimp grazing and is spread on oil spills to stimulate the growth of bacteria that can break down the oil. Urea is sometimes used as a feed additive for ruminants, is present in herbicides and pesticides, is used as a de-icer, and is used in the manufacture of plastics, paints, tobacco products, wine, and cosmetics (Glibert et al., 2006). The many sources of both anthropogenic and natural urea make this N source an important component of the N cycle in both terrestrial and downstream aquatic environments.

In soils, urease hydrolyses urea into ammonia and carbamate (H_2N-COOH), the latter of which spontaneously degrades into carbonic acid (H_2CO_3) and a second molecule of ammonia in the presence of water (Krajewska, 2009), by the pathways shown in Equations 1.2 and 1.3:



(Eqn. 1.2)

Urea *Ammonia and carbamate*



$+ H_2O \rightarrow H_2CO_3 + NH_3$ (Eqn. 1.3)

Carbamate *Carbonic acid and ammonia*

Plants produce urea during the catabolism of arginine by the enzyme arginase, when N is remobilized from old leaves to provide necessary N for new growth and flower production (Witte, 2011). The N in urea can also be absorbed from the soil following microbial transformation to ammonium (NH_4^+), or it can be absorbed by active or passive transport of unhydrolyzed urea by root cells, followed by hydrolysis within the plant cells (Witte, 2011). The expression of the active urea transporter in some plant roots can be increased by N-deficiency and decreased by application of NH_4^+ or NO_3^- (Kraiser et al., 2011). However, some plants grown on urea, rather than nitrate or ammonium nitrate, had reduced growth and symptoms of N deficiency (Witte, 2011). Since little work has been done to assess the importance of the direct uptake and assimilation of urea by agricultural plants, the conditions under which unhydrolyzed urea contributes to the N nutrition of agricultural crops is not well understood (Witte, 2011; Andrews et al., 2013).

Urea is capable of moving through soils because it is a soluble, nonionic compound, although it is often quickly hydrolyzed to ammonium and then converted to nitrate by soil microorganisms, and may leach in this form (Gould et al., 1986). In soils, urea is often hydrolyzed within a period of 1-14 days (Singh and Yadev, 1981; Yadev et al., 1987; Khakurai and Alva, 1995; Wali et al., 2003; Antil et al., 2006). Some field studies have shown that this process can take longer, although the distance that urea

migrated through the soil was not measured (Singh and Yadev, 1981; Khakurai and Alva, 1995). Most studies have focused on measuring soil ammonium or nitrate concentrations that result from urea application rather that urea leaching itself (Moe et al., 1968; Alva, 2006; Aparicio et al., 2008), or have only measured trace amounts of urea leaching several centimeters in column and field studies using controlled release fertilizer or urea plus a urease inhibitor (Agrotain) (Singh and Yadev, 1981; Khakurai and Alva, 1995; Sato and Morgan, 2008; R.J. Kratochvil, personal communication, 2011; Dawar et al., 2011) . Since information in the literature has focused on the rate of ammonia loss or the amount of nitrate leaching following urea fertilization, detailed information on the persistence of unhydrolyzed urea in soils is not complete.

Agricultural urea fertilizer is available in prilled or granular form, or as liquid urea ammonium-nitrate (UAN) solution (28-32% N). The different forms of urea behave slightly differently after application to soils; solutions of urea hydrolyze more quickly than pelleted or prilled urea fertilizer, but in the laboratory, all forms completely hydrolyzed in 6-8 days (Gould et al., 1986). Soil column studies have shown that the rate of urea hydrolysis depends on application method, with urea incorporated into the soil hydrolyzing faster than banded urea. In addition, urea incorporated in moist soil hydrolyzed faster than the urea banded in dry soil (Gould et al., 1986). The ease of use, availability, and high N content (46% by weight) of urea have contributed to its popularity; urea is now estimated to make up over 50% of the world's N fertilizer consumption (Glibert et al., 2006; Glibert et al., 2014).

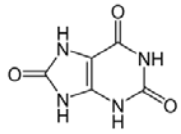
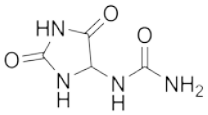
The hydrolysis of urea to NH_3 can be problematic for agricultural producers under certain conditions. Losses in excess of 50% of applied N can result from NH_3

volatilization if this fertilizer is surface-applied or incorporated into sandy soils under warm, dry conditions (Overrein and Moe, 1967; Terman, 1979). Ammonia accumulation in the soil can result in seed, seedling, and young plant damage (Bremner and Krogmeier, 1989). Increases in soil pH that can occur as a result of hydrolysis and the accumulation of NH_4^+ (Bremner, 1995) can also reduce the activity of *Nitrobacter* bacteria, the genus responsible for oxidation of nitrite to nitrate (Aleem and Alexander, 1960). The resulting build-up of nitrite in the soil can be toxic to plants and can pollute ground waters. Foliar application of urea is not an option as it often results in leaf burn and decreased yield (Bremner, 1995). To counter these problems with urea fertilizer use, urease inhibitors are sometimes added to fertilizers to reduce the rate at which urea hydrolysis occurs. A recent study reported the effect of one of these compounds, N-(n-butyl) thiophosphoric triamide, or 'Agrotain', on surface-applied urea. It delayed urea hydrolysis by about seven days and increased the distance that urea traveled in the soil profile, both vertically (down to 5 cm) and laterally (up to 6 cm) (Dawar et al., 2011). In contrast to chemical additives, Dong et al. (2009) found that soils amended with lignite-derived humic acids at the time of urea fertilization decreased urease activity and delayed the conversion of urea to NH_4^+, also delaying nitrification. While the development and use of urease inhibitors have reduced some of the problems associated with the use of urea fertilizer, there is some concern that other problems, namely increased runoff of unhydrolyzed urea, may be more likely (Glibert et al., 2006).

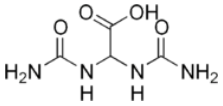
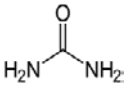
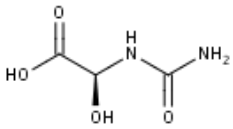
In addition to the use of urea fertilizers, this form of N is also available as a decomposition product of uric acid, which is produced by birds, some reptiles and amphibians, and most insects as the major nitrogenous waste product (Wright, 1995). In

agricultural settings, uric acid represents as much as 70% of the total nitrogen in poultry feces depending on diet (Nahm, 2003), although when mixed with bedding, uric acid represents closer to 19% of the total N in the litter (Gordillo and Cabrera, 1997). About 25% of the bacteria in poultry litter decompose uric acid to urea, and only a few of these are capable of decomposing uric acid directly to NH_3. The majority of these microbes are aerobic (Schefferle, 1965). The pathway for the decomposition of uric acid, and the enzymes involved, is illustrated in Eqn. 1.4 (Nahm, 2003). Simple by-products including CO_2 and H_2O are not shown, to simplify the equation.

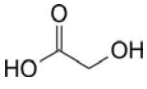
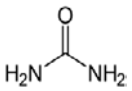
(Eqn. 1.4)

$$C_5H_4N_4O_3\ (uric\ acid) + O_2 + 2H_2O \xrightarrow{Uricase} C_4H_6N_4O_3\ (allantoin) + H_2O \xrightarrow{Allantoinase}$$

$$(allantoic\ acid) + 2H_2O \xrightarrow{Allantoicase} urea\ or\ NH_3 + S\text{-}ureidoglycolic\ acid \rightarrow$$

$$\xrightarrow{r\text{-}and\ s\text{-}ureidoglycosase} (glycolic\ acid\ and\ urea) + H_2O \xrightarrow{urease} 2NH_3\ and\ CO_2$$

Some aerobic microorganisms including protozoa, algae, fungi, and bacteria produce uricase, an enzyme which decomposes uric acid into s(+)-allantoin (Vogels and Vanderdrift, 1976). An anaerobic pathway also exists, but it is much slower (Nahm, 2003). Water is required for several steps in the aerobic decomposition pathway, so not surprisingly, broiler litter with the lowest water content of the samples tested was found to contain the most uric acid N, and the wettest had the least (Gordillo and Cabrera,

1997). Rothrock et al. (2010) made reference to urease activity being a limiting factor in the mineralization reaction of uric acid to ammonia. However, the paper they cited for that only refers to the second step of this decomposition reaction depending on urease activity, pH, and temperature (Nahm, 2003). Therefore, it is not clear whether the hydrolysis of urea is in fact a slower process than the hydrolysis of uric acid, and the implications for this potential bottleneck and build-up of urea in poultry-amended agricultural soils are unknown.

Urease: Enzyme Structure, Variability, and Activity in Soils and Cells

The urease enzyme exists both within living cells and as an extracellular enzyme released into the soil environment upon cell death (Krajewska, 2009). In most bacteria and plants, it is present in the cytoplasm. One exception to this occurs in the case of the bacteria *Helicobacter pylori,* a bacterium causing ulcers in humans, which is believed to contain some urease at the cell surface. However, the functionality of this extracellular urease has been the subject of much debate (Krajewska, 2009). Extracellular soil urease is stabilized by adsorption on soil colloids, particularly clays and organic matter, where it is resistant to microbial attack and continues to function (Krajewska, 2009). However, differentiating between intracellular and extracellular soil urease is challenging (Ciardi, 1998). Chloroform fumigation of soil samples, which lyses microbial cells, results in a total measurement of both intra- and extracellular urease activity, whereas measurement of urease activity without chloroform fumigation is assumed to represent extracellular urease activity. The difference between the two provides a measurement of urease

activity associated with the microbial community (Klose and Tabatabai, 1999; Qin et al., 2010). However, this method assumes that jack bean urease activity interacts with chloroform fumigation the same way soil urease does, which may not be the case. One study looked at jack bean urease that was added to unsterilized soil and found that the jack bean urease did not persist for more than a few days (Zantua and Bremner, 1977). In the soils tested, the urease activity returned to its initial level within a maximum of 14 days. Researchers concluded that soils have a natural saturation level of native extracellular urease that can be protected by associations with soil colloids (Zantua and Bremner, 1977). Using linear regression between urease activity and ATP content to identify intra- and extracellular components of urease activity has been attempted, but is not recommended because increases in ATP content are not always correlated with increases in enzyme activity (Ciardi, 1998). While differentiating between the two types of soil urease can be difficult, it is the combined activity of both intracellular and extracellular enzyme that is responsible for the hydrolysis of applied urea.

Besides plants (Witte, 2011), soil organisms that produce urease include fungi (including ectomycorrhizal fungi), bacteria, actinomycetes, some yeasts, cyanobacteria (Hasan, 2000), and archaea (Tourna et al., 2011; Lu and Jia, 2013). The proportion of the soil community that is ureolytic is not well understood, although one study found that 17-30% of the cultivable bacterial population sampled from six different soils was ureolytic (Lloyd and Sheaffe, 1973). Microbes regulate urease synthesis by three major pathways: 1) synthesis of urease is repressed in the presence of NH_3 and then turned on under N-limiting conditions, 2) urease synthesis is induced by the presence of urea as a substrate, and 3) the production of urease occurs constantly and is not affected by levels of N

compounds in the environment (Mobley and Hausinger, 1989). These mechanisms are species-specific, although only a few dozen soil organisms have been investigated in detail. For example, urease production is repressible in the soil fungus *Aspergillus nidulans*, the soil bacteria *Bacillus megaterium*, and the denitrifying bacteria *Paracoccus denitrificans*. Inducible urease production is found in the soil bacteria *Proteus vulgaris*, which is also a human pathogen, and in the ruminal bacteria *Selenomonas ruminantium*. Constitutive urease production is found in the agriculturally relevant and pathogenic *Agrobacterium tumefaciens* that causes crown gall disease in fruit trees. It is also found in the N_2-fixing bacteria *Rhizobium leguminosarum* and the soil bacterium *Sporosarcina ureae* (Mobley and Hausinger, 1989). Multiple pathways have been found in some species. For example, *Selenomonas ruminatium* is reported to have both inducible and repressible urease. Variations in urease production have also been seen at different stages of microbial cellular differentiation. In particular, increased constitutive urease activity has been noted in swarm cells (Mobley and Hausinger, 1989; Mobley et al., 1995). However, since this knowledge is focused on individual species, further research is necessary to understand ureolytic microbial community dynamics in soils.

The urease enzyme has subunits that are the same in plants and fungi. Each of the subunits has a molecular mass of approximately 90 kDa. The subunits are usually arranged into trimers or a homohexameric structure, with two Ni^{2+} per subunit. The total molar mass, including nickel ions, of a typical hexamer is approximately 550 kDa. In contrast, bacterial ureases are usually composed of three subunits, one of which is between 60-76 kDa, and the other two are smaller and between 6-21 kDa. Since these are usually found in trimers, the resultant molar masses of bacterial ureases are between 190-

300 kDa (Krajewska, 2009). In spite of these differences, ureases from different organisms maintain highly conserved sequences of amino acids and are believed to all be derived from one ancestral enzyme. Following resolution of the crystal structure of two bacterial ureases, researchers found the active sites to be nearly superimposable, which provides further evidence for similarities among ureases from different sources (Krajewska, 2009). Urease is composed of 51.6% C, 7.1% H, 16% N, and 1.2% S. The S is in the form of sulfhydryl groups (H-S-R), one of which is essential to urease activity while the other is not (Hasan, 2000). The two nickel ions that are present in the active site are essential to the enzyme's proper functioning, and they can be released from the enzyme under acid conditions, leading to irreversible loss of activity (Mobley et al., 1995).

The mechanism of urea hydrolysis at the active site has not been entirely elucidated, but it is believed that one of the two nickel ions is more electrophilic than the other. During hydrolysis, the more electrophilic nickel ion may bind to the carbonyl oxygen of urea with the less electrophilic nickel ion binding to an amino N on urea, creating a carbonyl C that is more susceptible to nucleophilic attack by water. The tetrahedral intermediate structure that results from this reaction then decomposes to release NH_3 and carbamate. It is further believed that the proton-donor to the leaving NH_3 molecules is either a bridging hydroxide or a histidine located in the active site. A second possible mechanism suggests that urea is only bound to the first nickel ion and the second nickel ion delivers a nucleophilic water molecule to the bound urea (Karplus et al., 1997; Benini et al., 2001; Krajewska, 2009).

While the urease enzyme activity and active site characteristics have been found to be similar among different organisms, many different types of urease are recognized and isolated from different organisms, and only some of these have been investigated in enough detail to understand variations in the size, structure, and optimum environmental conditions for each (Mobley and Hausinger, 1989; Mobley et al., 1995). Purified enzymes such as jack bean urease have been studied in the laboratory but behave differently from the urease enzyme present in the soil environment. As mentioned earlier, jack bean urease that was added to unsterilized soil did not persist for more than a few days (Zantua and Bremner, 1977). Even when the jack bean urease was added to autoclaved soil that exhibited no native urease activity, the added jack bean urease activity declined to zero within two weeks (Zantua and Bremner, 1977). In addition, jack bean urease has been estimated to have a narrower pH optimum (6-7) than some soil urease (5-8), and is less stable than soil urease at temperatures of 4°C, 25°C, 37°C, 45°C and 70°C measured over a period of two weeks (Pettit et al., 1976). These factors warrant consideration when attempting to apply laboratory-based research to field situations.

Soil urease inhibitors have been developed to reduce the rate of loss of NH_3 from agricultural fields following urea fertilization. These inhibitors fall into two categories: class competitive inhibitors that compete for the same active site on the enzyme and block it from reacting with urea, and non-competitive inhibitors that modify the structure of the enzyme and destroy its ability to react with urea. One type of non-competitive inhibitor interferes with the sulfhydryl group that is essential for the proper function of the enzyme, and another reacts with the nickel in the active site (Hasan, 2000). In

addition, urea may be coated with acidic materials to delay its hydrolysis and retain NH_4^+ in the soil (Ahmed et al., 2008; Junejo et al., 2011).

In contrast to urease inhibitors that were developed for the purpose, Todd and Hausinger (1989) looked at the inhibition of purified urease from *Klebsiella aerogenes* by phosphate. In laboratory studies, they found that phosphate competitively inhibits this urease between pH 5.0 and 7.0 and that the inhibition increases with an increase in pH in this range. There are likely three groups that are protonated or deprotonated that are the reason for this pH effect: one at the active site with a pKa of 6.5, the protonation of which would allow for the entry of the phosphate monoanion ($H_2PO_4^-$); one associated with the deprotonation of $H_2PO_4^-$ at pH 7.2 that would decrease the inhibition effect of phosphate; and the third associated with either the first deprotonation of H_3PO_4 or to the deprotonation of an active site residue (Todd and Hausinger, 1989; Krajewska and Zaborska, 1999; Benini et al., 2001). Jack bean urease has also been shown to be inhibited by H_2PO4^- between the pH of 5.8 and 7.49 (Krajewska and Zaborska, 1999). Whether phosphate inhibits urease activity in soil and field settings is unknown.

The natural factors that affect the activity of urease in soil are the same as those that affect aerobic microbial communities. Urease activity follows Michaelis-Menten kinetics (Gould et al., 1986), increases with plant residue age and degree of decomposition (Hasan, 2000), the addition of N in the form of organic matter, temperature up to 35° C, and water potential up to field capacity (5-10 kPa) (Kumar and Wagenet, 1984; Yadev et al., 1987). Soil urease activity was higher under no-till and reduced tillage agriculture, compared to conventional moldboard plowing (Qin et al., 2010). It also increased after manure application (Zaman et al., 2002) and as a result of

crop rotation (Klose and Tabatabai, 2000). Soil urease activity has been positively correlated with clay content in soils, although this may have been due to higher organic matter content or cation exchange capacity associated with the clay (Yadev et al., 1987). Wali et al. (2003) reported that hydrolysis of urea increased with increasing soil organic matter and clay content. In a comparison of urea hydrolysis of different organic manures, they found that urea hydrolysis was highest with poultry litter containing 40.1% organic C, the highest among the manures tested. However, Kumar and Wagenet (1984) warned against attempting to correlate urease activity with natural soil physical or chemical properties such as cation exchange capacity (CEC), clay or sand content, or pH, as the soils in their study showed wide variability in the correlations among these factors and urease activity. Soil urease activity can be depressed in soils with elevated levels of heavy metals (Hasan, 2000; Antil et al., 2006), under alkaline and saline conditions, in some mineral horizons below 30 cm (Gould et al., 1986; Khakurai and Alva, 1995), under flooded conditions (Wali et al., 2003), at low soil temperatures (Gould et al., 1986; Yadev et al., 1987), after additions of $CaCO_3$ (Kumar and Wagenet, 1984), when Ni levels are low (Mobley and Hausinger, 1989), following long-term N fertilization with NH_4NO_3 (Ajwa et al., 1999), and when soil temperatures exceed 60-70°C (Gould et al., 1986; Hasan, 2000). Although there are some conflicting reports in the literature about optimum conditions for soil urease activity (Gould et al., 1986; Hasan, 2000), in general, warm, moist soils with near-neutral pH result in high urease activity.

Uncertainties in Understanding and Predicting Urea Persistence in Soils: Justification for My Research

A wealth of information on urease activity in soils has come from researchers at universities in the U.S. such as Iowa State (Zantua and Bremner, 1975; Zantua and Bremner, 1976; Zantua and Bremner, 1977; McCarty and Bremner, 1991) and Purdue University (Overrein and Moe, 1967), but they used local soils in their experiments that may not be comparable to Mid-Atlantic soils from the Coastal Plain and Piedmont physiographic provinces of Maryland, which are in the watershed of the Chesapeake Bay and its freshwater tributaries In 2006, Maryland consumed more than 7 thousand tonnes of urea, and more than 86 thousand tonnes of N solutions, approximately one third of which is urea (USDA-NASS, 2006). In addition, there are over 800,000 ha of farmland in Maryland, and almost half of it is located on the poultry-producing Coastal Plain region of Maryland's Eastern Shore (USDA-NASS, 2012). Despite this, research on urea hydrolysis in Maryland soils is lacking. Those studies that have researched urea hydrolysis in soils outside of Maryland have usually only provided the textural class of the soil, although some studies specified using Mollisols (Kumar and Wagenet, 1984), Entisols (Kumar and Wagenet, 1984; Khakurai and Alva, 1995; Wali et al., 2003; Alva, 2006), Spodosols (Khakurai and Alva, 1995; Sato and Morgan, 2008), Inceptisols (Kumar and Wagenet, 1984; Yadev et al., 1987; Wali et al., 2003; Antil et al., 2006; Dawar et al., 2011), Aridisols (Yadev et al., 1987; Wali et al., 2003), or Alfisols (Dunigan et al., 1976). The soils of the Coastal Plain and Piedmont regions of Maryland are dominated by Ultisols. These soils have highly weathered, 1:1 layer phyllosilicate clay minerals, principally kaolinite, and they have argillic horizons with higher clay contents than their surface horizons. They often have low base saturation and tend to be

naturally acidic. Subsurface horizons may have accumulations of iron oxides (Brady and Weil, 2008). No studies were found that looked at urea or uric acid hydrolysis in Ultisols. Ultisols may behave very differently from other soils due to differences in their texture, mineralogy, horizonation, low pH, hydrology and phosphate content, especially those that have been amended with poultry litter over many years on the Delmarva Peninsula of Maryland, Delaware, and Virginia (Codling et al., 2008). Some research suggests that urea can be weakly adsorbed to soil, mainly in complexes with soil organic acids (Chin and Kroontje, 1962). If this is the case, the low organic matter content found in many Coastal Plain Ultisols would not retain urea in the soil, which may result in unhydrolyzed urea leaching vertically through the profile. Agricultural fields that receive lime present an interesting conundrum, in that increasing the soil pH will likely improve urease activity, but the addition of 2, 4 or 8% $CaCO_3$ by weight has been shown to result in significant reductions in urease activity (Kumar and Wagenet, 1984). However the authors of this study did not report changes in pH associated with these $CaCO_3$ additions, and only speculated as to whether the decreased urease activity was due to elevated pH or effects of the $CaCO_3$ itself. The sandy soils of the Coastal Plain may have reduced urea hydrolysis due to the presence of phosphate (Benini et al., 2001), low organic C content, low surface area for adsorbing extracellular enzyme, smaller microbial populations to hydrolyze urea, lower pH, Fe(III)(hydr)oxides which may result in inhibition of urease by Fe(III) (Zaborska et al., 2004), or combinations of these characteristics and others yet to be explored.

The finer-textured Ultisols of the Piedmont region provide a contrast to those of the Coastal Plain, while providing a regional comparison pertinent to the Chesapeake

Bay. Maryland's Piedmont soils tend to be buffered to a greater extent against changes in pH due to higher organic C and clay content, and they also tend to have more micaceous rather than kaolinitic clays. However, they are subject to similar climatic weathering conditions and are also in close proximity to Chesapeake Bay tributaries. Since no studies have been found on urea and uric acid dynamics in Ultisols, research in this area is needed, and a contrast between Piedmont and Coastal Plain soils could determine key differences in soil chemical conditions or other factors that influence the fate and transport of urea in these landscapes.

Some of the contrasting information in the literature about the optimum parameters for urease activity may be a result of testing soils with inherently different microbial communities. Krajewska (2009) listed the activity of ureases derived from some plants, fungi, and bacteria that range from 14.5-180,000 μmol urea/min per mg of enzyme. Most papers do not take these variations into account. One paper by Rothrock et al. (2010) did track the microbiology in acidified poultry litter and found that hydrolysis of urea was delayed as the community shifted from bacterial- to fungal-dominated ureolytic organisms. However, since urease activities can vary by several orders of magnitude among bacteria alone, further work on the influence of specific community characteristics and urea hydrolysis in soils would be valuable. In addition, traditional urease enzyme assays take place in a pH and temperature-buffered solution with unlimited substrate concentration and provide a measure of potential enzyme activity rather than *in situ* activity under normal soil temperature, moisture, pH, and substrate concentration. The use of current technologies to target specific functional genes can help researchers investigate the genetic potential of a microbial population under natural soil

conditions as well as understand the conditions under which gene transcription takes place (Wallenstein and Weintraub, 2008).

In order to address these gaps in the knowledge, laboratory studies were carried out to examine mechanisms of urea hydrolysis under different soil chemical conditions. We combined field sampling of toposequences, including soils from both A and B horizons, in order to evaluate differences in urea hydrolysis across the landscape and with depth in the soil profile. This provided a new and important lens through which to study urea dynamics in soils. If urea is leaching from agricultural soils to the Chesapeake Bay tributaries, it must travel by one of two routes: overland on soil surfaces across agricultural fields and through grassed buffer strips or riparian zones that often border nearby surface waters, or vertically and horizontally through the soil horizons and into the groundwater along a hydraulic gradient. However, no studies have been found that look at urea dynamics across a landscape that includes, but is not limited to, agricultural soils. Field sampling by landscape position and soil horizon allowed us to bridge the disciplines of pedology, soil fertility, microbial ecology and genomics, and soil chemistry (Fig. 1.1). Despite the fact that urea hydrolysis is influenced by many variables, as previously discussed, this kind of multi-discipline approach has not been found in the literature.

We used a laboratory-based approach to study urea hydrolysis because it allowed us to control for many of the factors that influence this process. While some realism is sacrificed in laboratory-based settings compared to field studies, the control over environmental variables that is gained in a laboratory setting allowed for a mechanistic study of urea hydrolysis in our soils. The results of our studies can help inform future

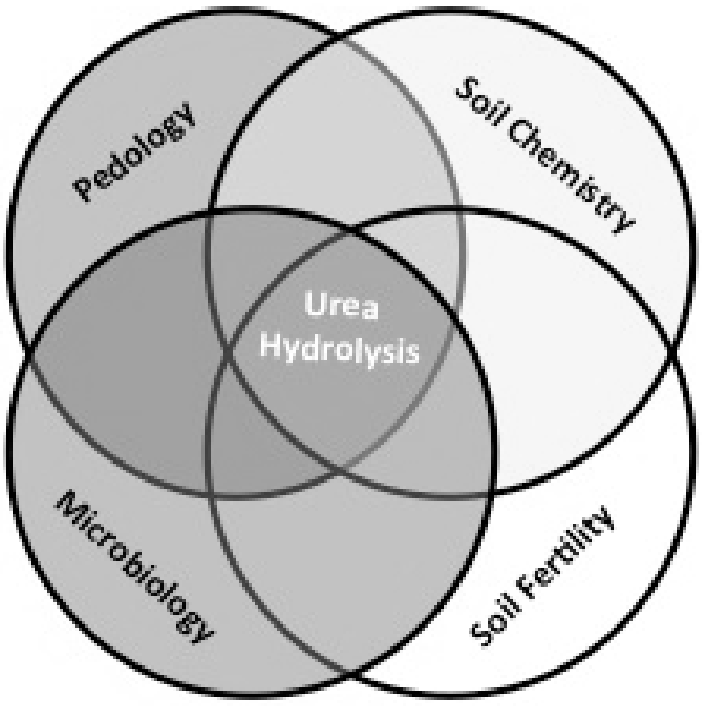


Figure 1.1: The multi-discipline approach used in this research to investigate urea hydrolysis in soils. Pedalogical techniques were used for sampling soil in the field, soil chemical and microbiological methods were employed in experimental designs, and results relevant to soil fertility and water quality have been emphasized.

field-scale agronomic and soil fertility studies to determine whether mechanisms observed under controlled laboratory conditions are measureable against the natural background variability inherent in a field experiment.

In conclusion, we investigated the following broad, working hypotheses in each of the following three chapters:

1. We hypothesized that soil chemical conditions, namely pH, would have significant effects on urea hydrolysis and would explain landscape-scale differences in rates of urea hydrolysis. Toposequences provide a framework for studying urea hydrolysis in soils across an agricultural landscape and with depth in the soil profile. These landscape toposequences provide a gradient of pH and other soil chemical conditions that result from differences in land management.

2. We hypothesized that soil microbial community composition would help to explain differences in rates of urea hydrolysis, both across the landscape and within a pH-adjustment experiment performed in the laboratory. Studies looking at ureolytic microorganisms and urea-N cycling by soil microbes are lacking in the literature. Examination of the microbial community composition and the presence of the urease gene in our soils, using genomic techniques, would provide new information on the microbial communities that are hydrolyzing urea in soils.

3. We hypothesized that the correlations made between urease activity and soil C are related to specific fractions of soil organic matter, and certain labile organic acids would increase rates of urea hydrolysis over others. Soil C and soil organic matter

have both been correlated with urease activity. Different forms of C are known to affect different enzymes differently, with some compounds increasing activity and others decreasing activity. Plants exude different types of organic acids and other C compounds, but it is not known how different kinds of organic acids affect urea hydrolysis, and therefore how different vegetative communities might impact urea hydrolysis.

Research Chapters

Chapter 2: Soil C, N and metal effects on urea hydrolysis in soil profile toposequences of the Coastal Plain and Piedmont regions

Much research has focused on urea hydrolysis in agricultural soils due to the importance of urea as a fertilizer. This research has focused mainly on surface horizons down to a 15 cm depth (Yadev et al., 1987; Khakurai and Alva, 1995; Wali et al., 2003; Antil et al., 2006; Dawar et al., 2011). However, few studies have looked at urea hydrolysis in lower soil horizons (exceptions include Alva (2006) and Khakurai and Alva (1995)), and no studies have been found that have looked at urea hydrolysis across toposequences that include, but are not limited to, agricultural soils. In addition, no studies have been found that look at urea hydrolysis in Maryland soils. This chapter explores these gaps in the literature by looking at a three-point toposequence in the Piedmont region and one in the Coastal Plain region in Maryland. The toposequences each include one soil profile within an active agricultural field, one at the grassed field border, and a third within a transitional/riparian zone adjacent to surface waters. Each

point also includes samples from both the A and B horizons. Studies have shown that urea hydrolysis is affected by organic C content (Kumar and Wagenet, 1984; Gould et al., 1986) and pH (Gould et al., 1986; Hasan, 2000), and these two factors may vary considerably across a toposequence in Maryland. The first research chapter (Chap. 2) of my dissertation investigates how changes in pH affect urea hydrolysis in soils across the toposequences sampled in Maryland, and whether rates of urea hydrolysis can be predicted using soil chemical information including pH, total C and N, and total nutrients (N, P, K) and metals (Ca, Mg, Mn, Al, Fe).

Chapter 3: Urease gene correlation with urea hydrolysis in soils across and agricultural-riparian landscape and pH gradient

Few studies looking at urea hydrolysis in soils have looked closely at the ureolytic microbial community composition. Rothrock et al. (2010) looked at ureolytic microorganisms in poultry litter that had been treated with different acidifying agents and found that the community shifted from bacteria-dominated to fungal-dominated as the pH of the litter declined. Singh et al. (2009) studied the ureolytic microbial community in soils under heavily grazed pastures in the United Kingdom and found that bacterial communities shifted in response to sheep urine deposition, whereas fungal communities did not. The effect on the bacterial communities, however, was only temporary. Since the resident microbial population in soil has an effect on the soil's ability to hydrolyze urea, and this population can change as a result of different soil conditions, it is important to understand how these communities change across a landscape. Chapter 3 of this dissertation addresses the question of how changes in microbial populations may be

correlated with changes in urea hydrolysis in soils. This chapter investigates how microbial community composition, fungal:bacterial ratios, and total bacterial urease gene numbers vary across toposequences in Maryland soils and with the changes in pH and soil characteristics explored in Chapter 2.

Chapter 4: Urea hydrolysis in soils: enhancement and inhibition by ascorbic, gallic, benzoic, and cinnamic acids.

The type and quality of C compounds available in soil affects microbial community composition. Vegetation is a large source of these different C compounds, either through root exudates or detritus. Our study in transects pointed to C and N as predictors of rate of urea hydrolysis, with the riparian zone soils (RZ) responding the most to increases in pH, possibly as a result of C type present in this soil. Water soluble carbohydrates including glucose (Falih and Wainwright, 1996) and some organic acids (p-hydroxybenzoic acid > ferulic acid > vanillic acid > salicylic acid > cinnamic acid) (Lin et al., 2011) are reported to stimulate urease activity, but high levels of phenols and condensed tannins decreased urease activity (Suescun et al., 2012). Stimulation of urease activity in a sandy soil upon release of glucose, glutamate, citrate and oxalate were not statistically significant. This chapter investigates how specific types of C stimulate urea hydrolysis in Coastal Plain soils and at what C concentration these effects are detectable. This provides information on how hydrolysis of urea might be stimulated in degraded soils where urea runoff is a problem or where conversion for plant uptake is important.

Chapter 5: Urea hydrolysis in soils: pedological, chemical, and microbial controls

The research presented in Ch. 2-4 provides evidence that soil horizon, total C and N content, soluble metals, soil pH, number of ureC genes present, and the type of C present can all influence the rate of hydrolysis of urea in Maryland soils. This final chapter summarizes findings and discusses the implications of this research for future studies affecting agriculture and water quality in the Chesapeake Bay watershed and elsewhere.

Chapter 2: Soil C, N, and metal effects on urea hydrolysis in soil profile toposequences from the Coastal Plain and Piedmont regions

Introduction

Urea ($CO(NH_2)_2$) is a form of nitrogen (N) that has both anthropogenic and natural sources. Worldwide consumption of urea fertilizer has increased from approximately 0.3 million tonnes in 1961 to over 40 million tonnes in 2002 (FAO, 2002), and is estimated to currently make up more than 50% of the world's consumption of N fertilizer (Glibert et al., 2014). Aside from fertilizer production and use, urea is also used in some herbicides, pesticides, and de-icers, as well as in the manufacturing of plastics and other products. It is sometimes used in aquaculture to grow algae for shrimp grazing or spread on oil spills to grow bacterial populations capable of degrading the oil (Glibert et al., 2006). Urea is present in the effluent of some sewage treatment plants (Cozzi et al., 2014) as well as in ruminant and poultry manures (Livingston et al., 1962; Nahm, 2003). Urea also occurs naturally in both aquatic and terrestrial environments in the excrement of mammals, some fish, zooplankton, and bacteria, and is a decomposition product of the uric acid produced by birds, some reptiles and amphibians, and most insects as their major nitrogenous waste product (Wright, 1995; Berman and Bronk, 2003). It is also released from the sediments in aquatic systems as a result of microbial decomposition of organic substrates (Berman and Bronk, 2003). The many sources of both anthropogenic and natural urea make this N source an important component of the N cycle in both terrestrial and aquatic environments.

In soils, urea is hydrolyzed to NH_3 by the enzyme urease, a process that can take from 1-14 days depending upon application rate, soil characteristics, and environmental factors (Singh and Yadev, 1981; Yadev et al., 1987; Khakurai and Alva, 1995; Wali et al., 2003; Antil et al., 2006; Krajewska, 2009). The variability in urease activity measured in different soils has been difficult to explain using physical characteristics of the soil, although coarse texture and low organic C content have been correlated with low urease activity (Zantua et al., 1977; Singh and Yadev, 1981; Yadev et al., 1987; Wali et al., 2003). The optimum pH for soil urease activity has been measured between 6-8 in a silt loam soil (Pettit et al., 1976), although others have found no correlation between pH and urease activity in a variety of soils from an area of the Midwestern United States dominated by Alfisols and Mollisols (Zantua et al., 1977). Urease activity increases with plant residue age and degree of decomposition (Hasan, 2000), the addition of N in the form of organic matter, temperature up to 35° C, and water potential up to field capacity (5-10 kPa) (Kumar and Wagenet, 1984; Yadev et al., 1987). Soil urease activity was higher under no-till and reduced tillage agriculture, compared to conventional moldboard plowing (Qin et al., 2010), and has been positively correlated with clay content in soils, although this may have been due to higher organic matter content or cation exchange capacity associated with the clay (Yadev et al., 1987). Low urease activity has been measured in alkaline and saline soils, in some mineral horizons below 30 cm (Gould et al., 1986; Khakurai and Alva, 1995), in flooded soils (Yadev et al., 1987; Wali et al., 2003), at low soil temperatures (Gould et al., 1986; Yadev et al., 1987), after additions of $CaCO_3$ (Kumar and Wagenet, 1984), when soil Ni levels are low (Mobley and Hausinger, 1989), and following long-term N fertilization with NH_4NO_3 (Ajwa et al., 1999).

Although there are disagreements in the literature about optimum pH, temperature, and water content of the soil for urease activity (Gould et al., 1986; Hasan, 2000), in general, warm, moist soils with near-neutral pH result in high urease activity.

Environmental concerns associated with the increased use of urea fertilizer are focused on the eutrophication that can result when excess N reaches aquatic systems (Glibert et al., 2014). The application of urea to soils often results in rapid hydrolysis to NH_4^+ and further oxidation to NO_3^- by soil bacteria and archaea (Yadev et al., 1987; Wessen et al., 2010). Most of the urea-N that is lost from soil is in the form of either NH_3 or NO_3^- (Terman, 1979; Gould et al., 1986), but there is increasing concern that unhydrolyzed urea may be moving out of agricultural fields and contributing to the development of harmful algal blooms (HABs) in surface waters (Glibert et al., 2001; Glibert et al., 2006; Heil et al., 2007; Kudela et al., 2008). Urea is consumed by phytoplankton and is a valuable source of N for these organisms (Altman and Paerl, 2012), averaging 18% of measured N uptake (among nitrate, ammonium, and urea) in the Chesapeake Bay (Lomas et al., 2002). In North Carolina's Neuse River Estuary, urea accounted for 16-45% of the total N taken up by the microbial community, despite its concentration remaining consistently below that of dissolved inorganic N (NH_4^+ and NO_3^-) (Twomey et al., 2005). Increased use of urea fertilizer has been correlated with a higher incidence of HABs throughout the world, and researchers are concerned that urea applied to agricultural fields may be entering surface waters and contributing to organic N enrichment and HABs in these waterways (Glibert et al., 2006).

If urea is moving out of agricultural soils and into surface waters, it must travel by one of two routes: overland on soil surfaces across agricultural fields and through grassed

buffer strips or riparian zones that often border nearby surface waters, or vertically and horizontally through the soil horizons and into the groundwater along a hydraulic gradient. However, no studies were found that look at urea dynamics in the Coastal Plain and Piedmont regions of Maryland across a landscape that includes, but is not limited to, agricultural soils. Some studies have investigated the potential for urea to migrate across agricultural soil surfaces in runoff water, but these values have been low (0.1-0.5% of applied urea-N) (Kibet et al., 2014) unless measured following a high rainfall event (Dunigan et al., 1976). Even in a study that included conditions that would be considered extreme in the area surrounding the Chesapeake Bay (13% slope and an application rate of 450 kg urea-N ha^{-1}) (Moe et al., 1968), only 0.5% of the applied urea was lost in surface runoff. Groundwater losses of unhydrolyzed urea have not been directly investigated on the Coastal Plain of Maryland, although the estimated 2.5 year groundwater residence time in most of the areas of the Coastal Plain immediately adjacent to the Chesapeake Bay suggest that this route of loss may be minor (Sanford et al., 2012). However, to support algal blooms, it does not take more than micromolar levels of urea (Glibert et al., 2001). Further investigations into urea hydrolysis within soil profiles across toposequences are justified due to the proximity of both surface and groundwater in the Coastal Plain region, the uncertainties in the local variability of groundwater residence time (Sanford et al., 2012), the evidence that rates of urea hydrolysis decrease with depth in the soil profile (Myers and McGarity, 1968; Gould et al., 1973; Khakurai and Alva, 1995), and the lack of detailed studies assessing urea hydrolysis in the landscape surrounding the Chesapeake Bay.

The purpose of this research is to study the dynamics of urea hydrolysis in soil profile toposequences sampled from the Coastal Plain and Piedmont regions of Maryland in order to understand the controls governing this process both across a landscape and with depth in the soil profile. The soil master variable, pH, was investigated because of its importance to both agronomic productivity and microbial community composition (Pietri and Brookes, 2008; Pietri and Brookes, 2009). We hypothesized that urea hydrolysis would increase as soil pH increased and that pH, and possibly other factors, could be significant predictors of urea hydrolysis rate.

Materials & Methods

This study was conducted on soil materials sampled from two sites in two different locations in October, 2012 and from multiple sites within one location in October, 2013. In 2012, soils were sampled from the Wye Island Natural Resource Management Area in Queen Anne's County, Maryland (38°54'11.97"N, 76° 8'12.20"W), located within the Coastal Plain (CP) physiographic region (Appendix A), and the University of Maryland's Central Maryland Research and Education Center, Clarksville Facility in Howard County, Maryland (39°15'48.89"N, 76°55'31.78"W) located within the Piedmont (PM) physiographic province (Appendix A). In 2013, soils were sampled from multiple sites within the Wye Resource Management Area.

Soils were sampled along a transect consisting of three points: an agricultural field (AG) actively farmed in a typical Maryland grain rotation of corn, wheat, and soybeans, a grassed field border (GB), and a transitional zone adjacent to surface waters.

The point adjacent to surface water was located within a forested riparian zone (RZ) at the CP site and within an herbaceous wetland edge (WE) at the PM site. The soils were sampled both from the A horizon (0-15 cm) and at a depth representative of the B horizon, which varied from site to site based on differences observed in the soil profile. In 2012, one transect was sampled from the CP site and one was sampled from the PM site. In 2013, three transects were sampled from the CP site.

At the CP sites sampled in both 2012 and 2013, the AG soil sample was from the Ingleside mapping unit (38°54'11.97"N, 76° 8'12.20"W), the GB soil was from the boundary of Ingleside and Longmarsh-Zekiah mapping units (38°54'10.37"N, 76° 8'13.79"W), and the RZ soil was from the Longmarsh- Zekiah mapping unit (38°54'9.98"N, 76° 8'14.70"W); all of which were similar to the Ingleside series (coarse-loamy, siliceous, mesic Typic Hapludult). At the PM site sampled in 2012, the AG soil sample was from the Glenelg unit and the GB soil was from the Glenville-Baile unit; both similar to the Glenelg series (fine-loamy, mixed, semiactive, mesic Typic Hapludults). The WE soil was from the Hatboro-Codorus unit, similar to the Codorus series (fine-loamy, mixed, active, mesic Fluvaquentic Dystrudept).

In 2012, a 7.6-cm open-faced soil auger was used to sample four profiles from four different locations at each transect point. These four samples were combined to form one composite sample from each depth at each point along both transects. The deep sample in the RZ at the CP site, however, consisted of three auger holes because of a proliferation of tree roots that prevented additional sampling. In 2013, sampling followed the same methodology, except that three soil profiles from three different locations at each transect point were combined to form one composite sample for each soil horizon.

Each year, the soils were all sampled on the same day and stored in a cooler for fewer than 5 h before being brought back to the laboratory and allowed to equilibrate to room temperature (21-23 °C). The soils were sieved to pass a 4 mm screen and kept field-moist in double plastic bags in closed plastic buckets for 8 to 10 months during experimentation. Previous work monitored the rate of urea hydrolysis in soils stored over a period of 10 months (Appendix B, Fig. B.3) and found that while there was some variability in the rate of hydrolysis measured during the storage period, a distinct slow-down in the hydrolysis rate was not measured until after 8 to 10 months of storage. These findings are consistent with those of Zantua and Bremner (1977), who determined that urease activity in was not affected by storing field-moist soils for up to six months at temperatures ranging from -10 to 40°C. Sampling soils in the autumn may have resulted in sampling soils with lower seasonal urease activities (Kang et al., 2009) and therefore the rates of hydrolysis discussed in this work may be lower than those that would be measured in soils sampled in the spring. Our method of sampling and storing soils was not designed to represent all soils in all field conditions, but rather to investigate the mechanisms underlying differences in rates of urea hydrolysis in the different landscape positions and soil horizons.

Exchangeable cations, total C and N contents, pH, and soil moisture were determined on three analytical replicates per homogenized soil-horizon from each transect point. Exchangeable cations were operationally defined by ammonium acetate extraction (1.25 M; pH 4.8). For this analysis, the field moist equivalent of 2.5 g oven dried soil were extracted in 37.5-ml polycarbonate centrifuge tubes at a 10:1 solution:soil ratio, placed on an orbital shaker set at 800 cycles min^{-1} for 1 h, and then centrifuged at a

minimum of 17,000 x *g*. The supernatant liquid was diluted as necessary with 1.25 M ammonium acetate for determination of total soluble Al, Mn, Fe, Ca, Mg, and K by flame atomic absorption. The same soil extract was used to measure exchangeable P using the method of Murphy and Riley (1962). Percent C and N were determined using a LECO CHN 2000 Analyzer (LECO, St. Joseph, MI)(Bremner, 1996; Nelson and Sommers, 1996). Field soil pH values were measured in the supernatant liquid of 0.01 M $CaCl_2$ soil slurries (2:1 solution:soil ratio) after soil particles had settled following 10 min of vigorous shaking. The pH values measured during the pH experiments (designated as pH_s) were measured in the supernatant liquid of 0.01 M $CaCl_2$ soil slurries (10:1 solution:soil ratio) after soil particles had settled after 30 min of vigorous shaking in the last h of each urea incubation. Soil moisture was determined after oven drying subsamples at 105° C for 24 h. Soil texture was calculated from duplicate analytical samples using the pipette method (Gee and Or, 2002). The mean of the analytical replicates was used for data analysis.

Treatments for the determination of pH effects on urea hydrolysis were established by weighing out moist soil samples equivalent to 350-g oven-dried soil and equilibrating them with a solution of either 0.5 or 1.0M HCl or reagent grade $CaCO_3$ to achieve a range of four pH values between 3.5 and 7.2. The soils were brought to a moisture content approximately equivalent to -10 kPa using 18 MΩ water or HCl solution, as necessary for the treatment. The soils were stored in the dark at room temperature (21-23° C) in plastic freezer bags and mixed by hand every few days for a month until the pH stabilized.

After pH equilibration, triplicate, moist soil samples equivalent to 2.5 g oven-dried soil from each treatment were weighed into 37.5-mL polycarbonate centrifuge tubes in a randomized complete block design in the laboratory that investigated the variables of transect location, horizon, and pH. The soils were mixed with 22.5 mL of a 7.94×10^{-4} M urea-N solution in a background of 0.01 M $CaCl_2$ and placed on an orbital shaker set at 800 cycles min^{-1} that shook the tubes for 30 minutes each h. This treatment followed the method of Greenan and Mulvaney (1995) with modifications. Specifically, soils were incubated in a solution free of phenylmercuric acetate (PMA) to allow for microbial activity to take place during the incubation. Preliminary rate experiments were used to determine the appropriate time intervals for sampling the different soil treatments. At these time points, triplicate samples were removed from the shaker and 2.5 mL of concentrated PMA was added to halt microbial activity, such that the final concentration of PMA in each centrifuge tube was as described by Greenan and Mulvaney (1995) and each soil sample had received an initial total of 100 mg urea-N kg^{-1} soil. The samples where then shaken again for a minimum of 10 min to ensure distribution of PMA throughout the sample, and were centrifuged at a minimum of 17,000 x g. The supernatant liquid was removed and stored at 4°C for a maximum of three weeks (Douglas and Bremner, 1970). The urea incubations were conducted over a period of 48 – 96 h, depending upon the treatment, and the slope of the line of disappearance of urea over time was used to determine a zero-order rate of hydrolysis at each site. The supernatant liquid of all samples was tested for urea using the colorimetric microplate method of Greenan and Mulvaney (1995).

Statistically significant differences between rates of urea hydrolysis in native (unamended soils in field condition, sieved to 4 mm) soils were determined using one-way ANOVA ($p < 0.05$, and Tukey's test, $p < 0.05$) and differences between A and B horizons were determined using an unpaired t-test in GraphPad Prism (Prism 6, GraphPad Software, Inc., La Jolla, CA). Pearson's correlations and multiple regression (SPSS v.21, SPSS Inc., Chicago, IL) were used to determine the best linear combination of factors predicting rate of urea hydrolysis in soils ($p < 0.05$).

Results & Discussion

Urea hydrolysis in several native soil transects in the Coastal Plain

The changes in the concentration of urea over time in the native soils sampled from the four toposequences in 2012 and 2013 are summarized in Fig. 2.1 and show some variability within each horizon and transect location. When the data were grouped by toposequence position and horizon to provide four replicates for statistical analysis, the rates of urea hydrolysis in A horizon soils, calculated from the slopes of linear regression equations generated from data plotted in Fig. 2.1, were not significantly different among the three toposequence positions (AG, GB, RZ) ($p = 0.28$). However, A horizons did hydrolyze urea significantly faster than B horizons ($p = 0.0002$). Among the B horizons, only the riparian BC soil had a rate of hydrolysis that was significantly faster than the other two B horizon soils ($p \leq 0.05$). The similar A-horizon hydrolysis rates across transect locations, and the faster hydrolysis rates in the A-horizons compared to

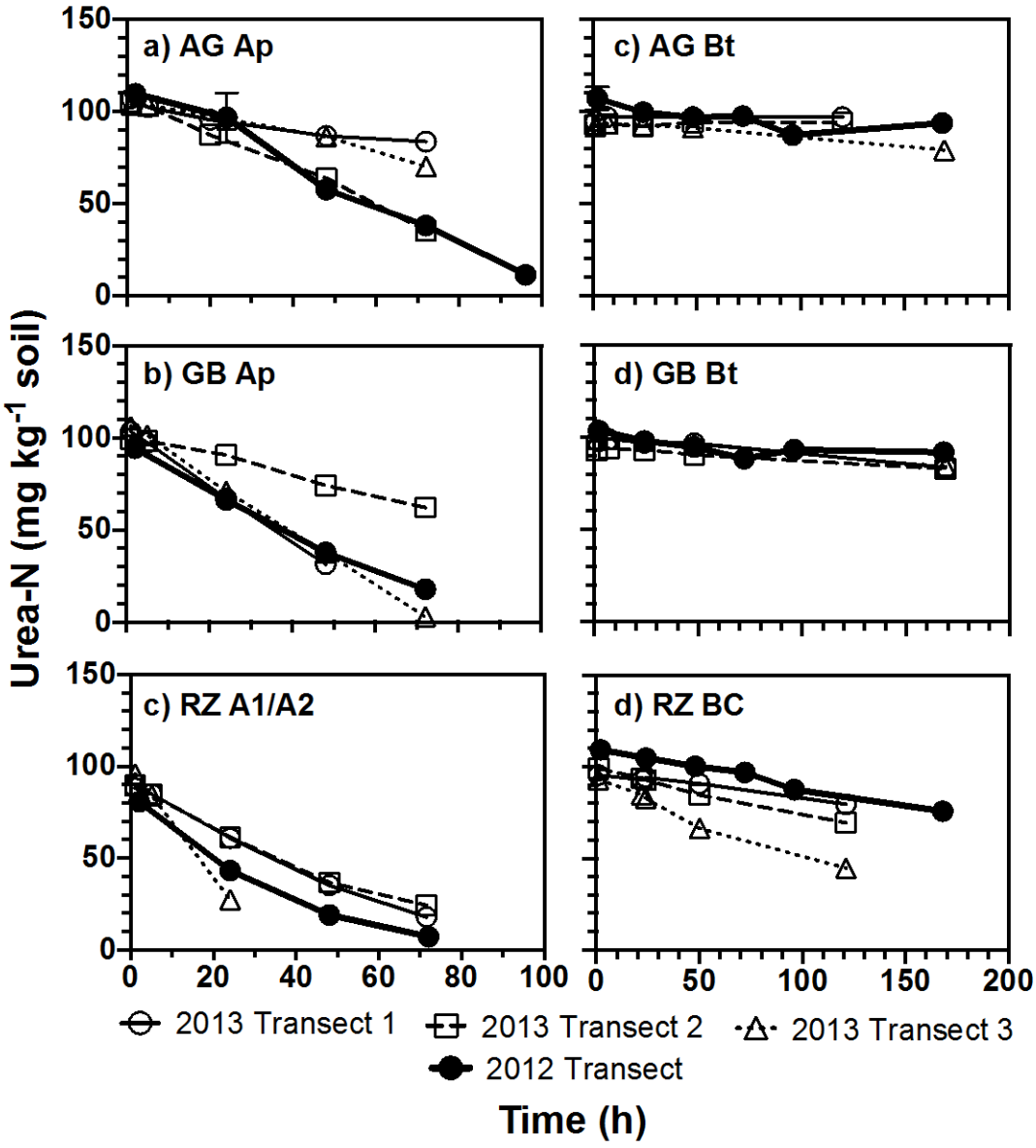


Figure 2.1: Changes in the concentration of urea with time in native soil transects sampled in 2012 and 2013 from the Coastal Plain site. Transects consisted of AG (agricultural field), GB (grassed field border), and RZ (forested riparian zone) A and B horizon soils. SEM plotted, but some error bars fall within the symbol areas (n=3). Soil physical and chemical characteristics for the 2013 soil transects are summarized in Table B.1 in Appendix B.

the B-horizons derived from the four CP transects in Fig. 2.1, are consistent with the findings discussed in the next section, in which analyses were performed on one Coastal Plain transect sampled in 2012 and compared to one Piedmont transect sampled in 2012.

Urea hydrolysis in native soil transects in the Coastal Plain and Piedmont

The native soils sampled in 2012 varied in both their physical and chemical characteristics (Table 2.1). Of particular note are the large ranges in pH (3.6-5.9) and in extractable Mn (0.48-9.8 mg kg^{-1} soil) and Al (8.9-170 mg kg^{-1} soil) concentrations, which can reduce P, Ca, and Mg bioavailability or be toxic in solution, affecting microbial communities (Haynes and Mokolobate, 2001; Lauber et al., 2008). Despite these differences, the native rates of urea hydrolysis in soils from the Coastal Plain (CP) did not differ significantly across the landscape in the A horizon (Fig. 2.2), with a pooled rate of 1.08 mg urea-N kg^{-1} soil h^{-1}. The B horizon data (Fig. 2.3) for the three CP topographic locations show very slow hydrolysis and nitrate accumulation for the AG Bt and GB Bt horizons, while the riparian zone (RZ) BC hydrolysis rate (Table 2.2) and nitrate accumulation was more than twice that of the two other B horizon soils at that site (Fig. 2.3).

In the Piedmont (PM) toposequence soils, the urea hydrolysis rates in the Ap horizon at the wetland edge (WE) was statistically different from the Ap horizons from the two other toposequences points (Fig. 2.2), but this analysis depended heavily on rates derived from the first two data points (at 2 and 24 hr) for both the AG and GB soils. We

Table 2.1: Selected physical and chemical properties of soils sampled in 2012 along two transects consisting of an agricultural field (AG), a grassed field border (GB), and a riparian zone (RZ) in the Coastal Plain (CP) and a wetland edge (WE) in the Piedmont (PM).

Site‡	Horizon	Depth (cm)	Sand	Silt	Clay	PSD †	pH_s	H_2O	C	N	C:N	Mn	Al	Ca	Mg	K	Fe	P
			--- % ---					---- g kg^{-1} soil----				---------- mg kg^{-1} soil ----------						
CP AG	Ap	0-15	64.4	28.9	6.7	SL	5.3	116	7.0	0.57	12	0.79	30	364	118	67	1.0	18
CP GB	Ap	0-15	74.1	21.2	4.8	SL	4.5	96.2	9.4	0.81	12	3.4	62	329	66.9	56	3.7	45
CP RZ	A1/A2	0-15	50.9	40.8	8.3	L	3.6	86.5	22	1.4	16	9.8	170	279	44.8	53	12	73
CP AG	Bt	45-60	56.0	28.2	15.8	SL	5.4	142	1.6	0.15	11	0.5	58	644	120	27	2.7	7.8
CP GB	Bt	65-80	51.2	39.6	9.3	L	5.0	123	3.6	0.22	16	0.5	40	320	53.5	30	1.7	7.3
CP RZ	BC	65-80	69.5	27.5	3.1	SL	3.9	49.7	5.2	0.22	24	1.2	160	28.5	7.6	18	5.7	16
PM AG	Ap	0-15	49.8	23.1	27.2	SCL	5.6	201	18	1.6	11	1.1	18	1,140	147	370	1.0	230
PM GB	Ap	0-15	46.7	32.2	21.1	L	4.7	236	21	1.9	11	3.8	41	944	200	220	21	150
PM WE	Ap	0-15	56.8	22.6	20.7	SCL	5.4	311	17	1.5	11	1.4	9.0	1,140	214	53	5.0	130
PM AG	Bt	35-50	60.3	4.7	35.1	SC	5.9	197	4.8	0.46	10	0.5	77	771	150	150	2.1	12
PM GB	Bt	30-45	42.6	30.6	26.9	L	5.4	165	4.1	0.34	12	1.1	56	806	172	57	8.0	2.2
PM WE	Bw	30-45	40.3	38.4	21.3	L	5.5	217	7.0	0.61	11	1.9	36	839	175	24	8.5	12

†Particle size distribution

‡CP (Coastal plain), PM (Piedmont), AG (agricultural field), GB (grassed border), RZ (riparian zone), WE (wetland edge)

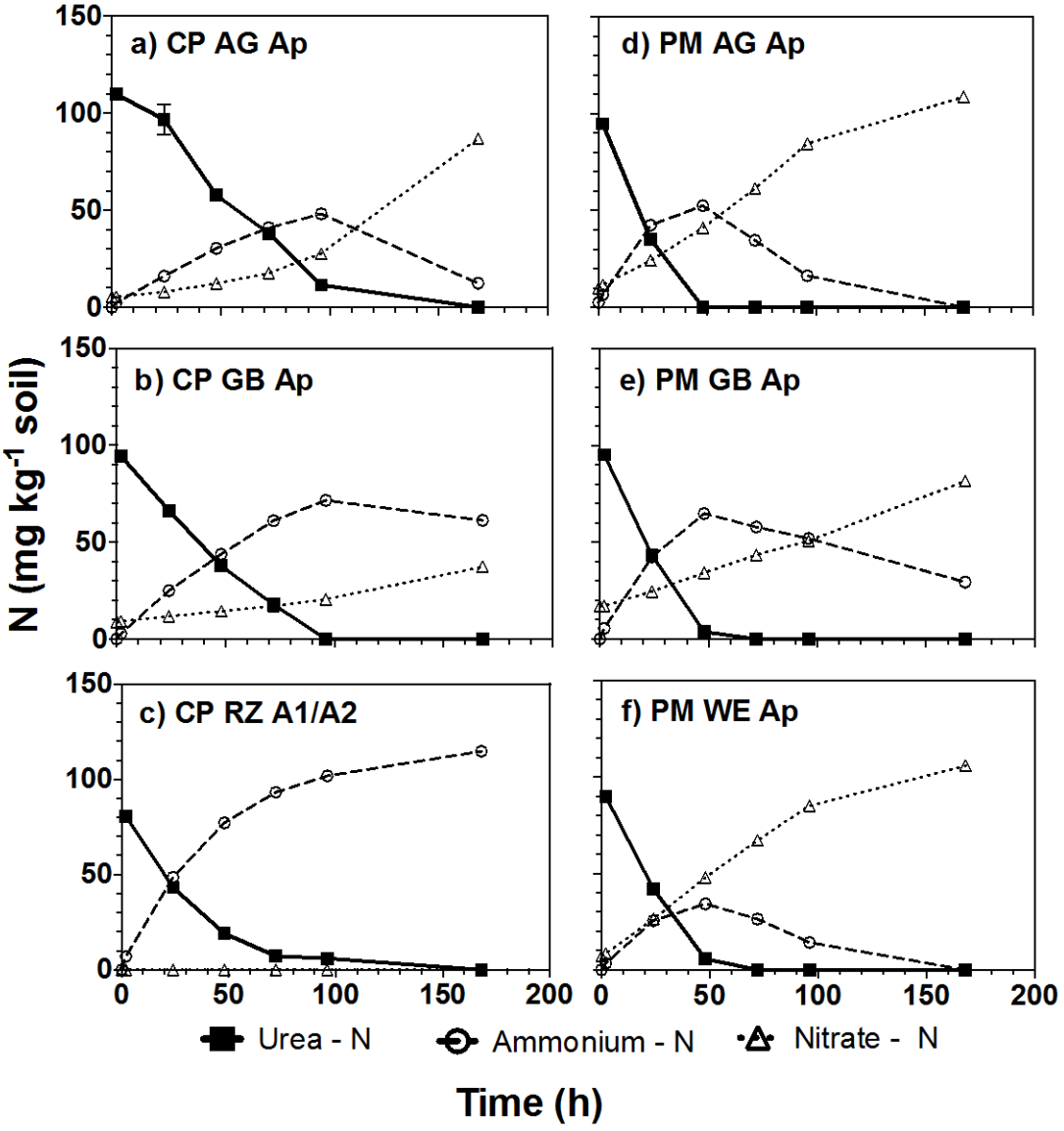


Figure 2.2: Urea hydrolysis, ammonium generation, and nitrification in soils sampled from the A horizons of Coastal Plain (CP) and Piedmont (PM) transects consisting of an agricultural field (AG), a grassed field border (GB), and a riparian zone (RZ) in the CP and a wetland edge (WE) in the PM. SEM plotted (some error bars are within the symbols), n=3.

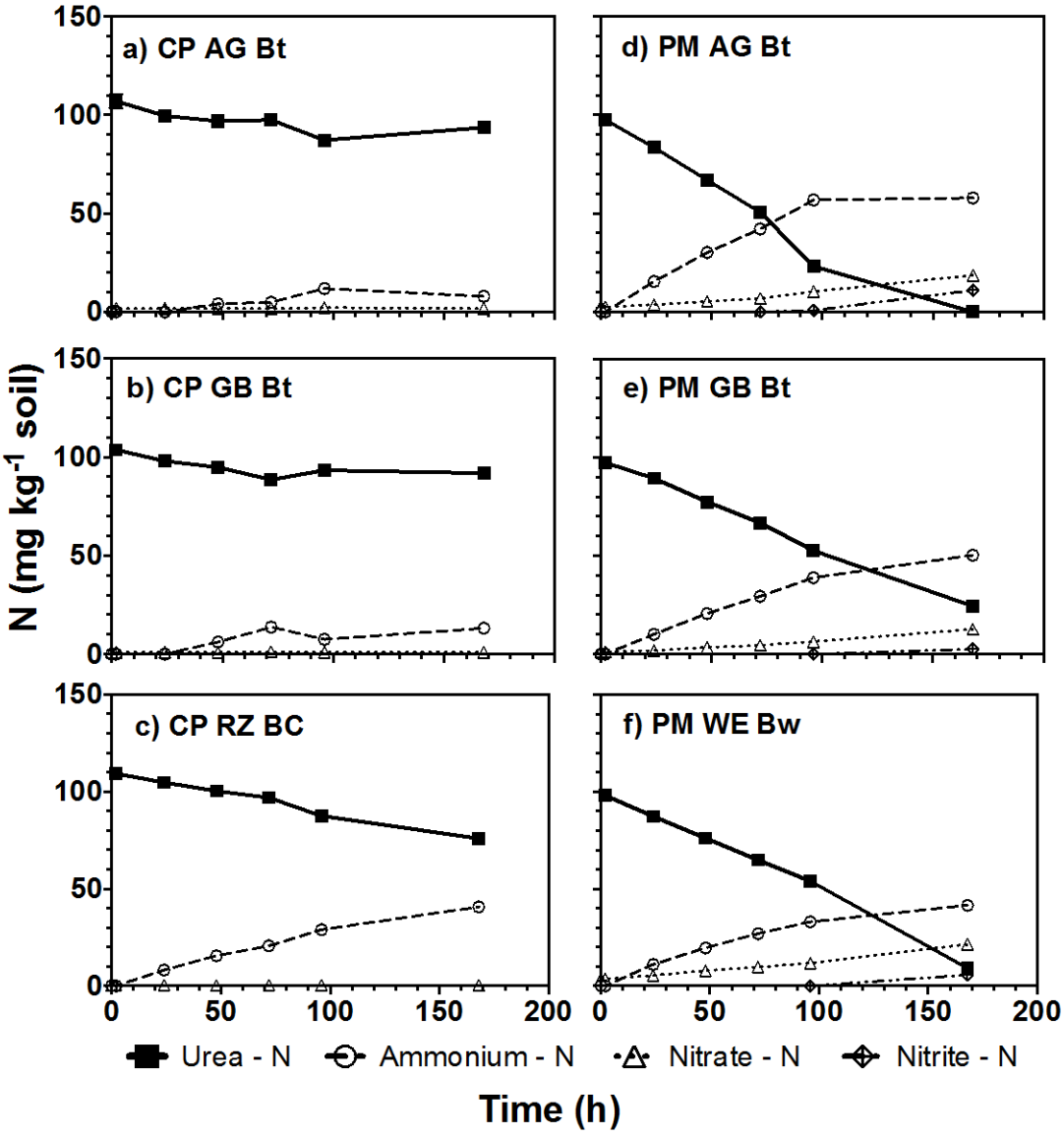


Figure 2.3: Urea hydrolysis, ammonium generation, and nitrification in soils sampled from the B horizons of Coastal Plain (CP) and Piedmont (PM) transects consisting of an agricultural field (AG), a grassed field border (GB), and a riparian zone (RZ) in the CP and a wetland edge (WE) in the PM. SEM plotted (some error bars are within the symbols), n=3.

Table 2.2: Rates of urea hydrolysis in native Coastal Plain and Piedmont transect soils (AG = agricultural field, GB = grassed field border, RZ = riparian zone, WE = wetland edge) based on zero-order kinetics.

Site	Horizon	Rate of Hydrolysis (mg urea-N kg^{-1} h^{-1})	
----------Coastal Plain----------			
AG‡	Ap	1.09	a†
GB	Ap	1.10	a
RZ	A1/A2	1.04	a
AG	Bt	0.0790	b
GB	Bt	0.0619	b
RZ	BC	0.206	c
----------Piedmont----------			
AG	Ap	2.71	d
GB	Ap	2.36	d
WE	Ap	1.83	e
AG	Bt	0.770	f
GB	Bt	0.449	g
WE	Bw	0.531	h

†Rates with the same letter are not significantly different at $p \leq 0.05$

‡AG (agricultural field), GB (grassed border), RZ (riparian zone), WE (wetland edge)

consider the differences between toposequence A horizon locations to not be of practical significance, even though they reach the level of statistical significance. The Bt horizon in the PM soil AG location hydrolyzed 0.770 mg urea-N kg^{-1} soil h^{-1}, which was the fastest rate of all the B horizon soils. However, all three B horizon rates in the PM transect were significantly different from each other (Table 2.2).

The rates of urea hydrolysis in the PM site were faster than at the CP site (Table 2.2), with the Ap horizons at the AG and GB locations more than twice the rates in the corresponding points in the CP transect. The rate of hydrolysis in Ap horizon of the PM WE was 75% faster than that in the A1/A2 horizon of the CP RZ. The same trend was also apparent between the B horizons in the PM compared to the CP transects, but the differences were greater due to the very slow B horizon hydrolysis rates in the CP (Fig. 2.3 and Table 2.2).

Within each toposequence, the rate of urea hydrolysis was substantially higher in the A horizons compared to the B horizons (Table 2.2). The greatest difference in hydrolysis rates between the A and B horizons was in the CP, where the rate in the AG and GB B horizons was less than 10% of that in the corresponding A horizons. The urea hydrolysis rate of the riparian zone BC horizon in the CP was higher, and equal to 20% that of the rate in the riparian zone A1/A2. In the PM toposequence, B horizon soils from all three toposequences positions had hydrolysis rates that were less than 30% of those in the corresponding A horizons (Table 2.2).

Relationships between native urea hydrolysis rates and soil properties

Stepwise multiple regression was used to analyze rate of hydrolysis using pH, C, and N as possible predictors. Since pH influences extractable metal concentrations, pH and metal concentration were not evaluated in the same multiple regression analysis. Instead, the native urea hydrolysis rates were first compared with soil pH, C, and N contents. Then, correlations were run between pH and metals, and metals with significant correlations with pH were run in a separate regression model that excluded pH.

The stepwise regression analysis of the native urea hydrolysis rates from all horizons, with heavy metals excluded, did not find a significant relationship ($p = 0.75$) between pH and urea hydrolysis. Instead, the stepwise regression analysis found that C and N were significantly ($r^2 = 0.90$) related to hydrolysis. This is likely due to soil C and N levels being indirect measurements of soil organic matter, and organic matter has been correlated with urea hydrolysis in other work (Zantua et al., 1977; Kumar and Wagenet, 1984). For example, Zantua et al. (1977) found that soil C and N were significant parameters in the models for predicting levels of urease activity in a range of Iowa soils. But, unlike the current results, Zantua et al. (1977) also found that within those multiple regression models, pH contributed a significant improvement.

The stepwise regression analysis of the native urea hydrolysis rates from all horizons, with pH excluded and pH-correlated metals included (Mn, Al, Ca, Mg), found that N and Mn were significantly ($r^2 = 0.93$) related to hydrolysis. Nitrogen is an indirect measure of organic matter, and organic matter can chelate metals such as Mn and reduce metal toxicity or keep metals from reacting with phosphate (Haynes and Mokolobate,

2001). The high soil C and N concentrations at the lowest pH, particularly in the Coastal Plain RZ soil, are indicative of high total organic matter which is likely buffering these systems against metal toxicities and nutrient limitations that might otherwise develop and affect microbial communities and their ability to hydrolyze urea (Haynes and Mokolobate, 2001).

Urea hydrolysis in pH-adjusted toposequence soils

Adjusting the native pH of the toposequences soils from both sites, by adding HCl or $CaCO_3$, produced several anticipated changes in soil chemical properties that are summarized in Tables 2.3 to 2.6. The soils that were treated with HCl to reduce the pH had higher concentrations of soluble Mn, Al, and Fe in both A and B horizons. Adding $CaCO_3$ to increase pH increased extractable Ca (due to the addition of $CaCO_3$) and soluble P compared to the concentrations of these nutrients found in the native soils. The B horizon soils within each transect point had higher soluble Al concentrations and lower soluble K and P concentrations than the corresponding A horizon soils (Tables 2.3-2.6).

The rate of urea hydrolysis at each pH in all soils followed zero-order kinetics (Appendix B). This indicated that the concentration of urea did not control the rate of hydrolysis and that the process was first order in some other variable. The influence of pH on the rate of urea hydrolysis was summarized by plotting each rate against the pH at which it was measured, which provided a method to estimate the rate of change of urea hydrolysis with changes in pH from the slope of the lines (Fig. 2.4). As with native soil hydrolysis rates, multiple regression analysis was conducted using pH, C, and N as

Table 2.3: Soil chemical characteristics of Coastal Plain A horizon transect soils treated with diluted HCl or $CaCO_3$ to achieve a range of pH values.

		----------NH_4OAc-Extractable (mg kg^{-1} soil)----------						
Treatment	pH_s	Mn	Al	Ca	Mg	K	Fe	P
†AG Acid	3.3	48	340	401	130	76	29	39
AG Native	5.4	0.79	30	364	120	67	1.0	18
AG $CaCO_3$ #1	6.5	1.5	23	604	110	66	0.89	27
AG $CaCO_3$ #2	6.8	1.8	23	704	100	65	0.89	27
GB Native	4.0	3.4	62	329	67	56	3.7	45
GB $CaCO_3$ #1	5.9	1.2	22	721	60	51	1.3	54
GB $CaCO_3$ #2	6.6	2.6	23	941	57	52	1.9	120
GB $CaCO_3$ #3	6.9	3.2	24	1,200	52	48	2.1	140
RZ Native	3.7	9.8	170	279	45	53	12	73
RZ $CaCO_3$ #1	5.1	0.86	76	896	37	42	5.1	62
RZ $CaCO_3$ #2	5.7	0.71	62	1,140	36	43	4.9	72
RZ $CaCO_3$ #3	6.3	1.1	54	1,340	33	45	5.0	80

†AG (agricultural field, GB (grassed field border), RZ (riparian zone)

Table 2.4: Soil chemical characteristics of Coastal Plain B horizon transect soils treated with diluted HCl or $CaCO_3$ to achieve a range of pH values.

		----------NH_4OAc-Extractable (mg kg^{-1} soil)----------						
Treatment	pH_s	Mn	Al	Ca	Mg	K	Fe	P
†AG Acid	3.1	14	450	617	129	31.7	13	9.2
AG Native	5.2	0.5	58	644	120	26.6	2.7	7.8
AG $CaCO_3$ #1	6.6	0.5	75	818	119	23.7	2.5	16.7
AG $CaCO_3$ #2	7.1	0.6	93	1,060	96.3	27.1	2.4	19.6
GB Acid	3.6	24	170	311	49.6	33.5	8.1	14.4
GB Native	5.0	0.5	40	320	53.5	30.0	1.7	7.3
GB $CaCO_3$ #1	6.2	0.6	35	459	46.2	27.7	1.4	20.1
GB $CaCO_3$ #2	6.9	1.1	42	666	39.4	31.2	2.0	22.4
RZ Native	4.1	1.2	160	29	7.6	17.9	5.7	15.8
RZ $CaCO_3$ #1	4.9	0.5	93	216	6.7	17.3	2.6	15.3
RZ $CaCO_3$ #2	5.9	0.5	59	402	5.6	17.9	1.9	13.4
RZ $CaCO_3$ #3	6.6	0.6	49	552	1.7	15.6	2.2	25.7

†AG (agricultural field, GB (grassed field border), RZ (riparian zone)

Table 2.5: Soil chemical characteristics of Piedmont A horizon transect soils treated with diluted HCl or $CaCO_3$ to achieve a range of pH values.

		----------NH_4OAc-Extractable (mg kg^{-1} soil)----------						
Treatment	pH_s	Mn	Al	Ca	Mg	K	Fe	P
†AG Acid	3.9	72.9	52.0	1,180	150	371	3.0	167
AG Native	5.5	1.1	17.9	1,140	147	368	1.0	231
AG $CaCO_3$ #1	6.7	2.5	21.5	1,530	128	293	1.4	384
AG $CaCO_3$ #2	6.9	2.5	20.6	1,960	132	299	2.2	399
GB Acid	3.7	42.3	67.2	966	176	220	33.7	188
GB Native	4.5	3.8	41.2	944	200	219	21.0	153
GB $CaCO_3$ #1	6.2	0.8	22.3	1,260	171	202	9.0	227
GB $CaCO_3$ #2	6.7	1.9	23.2	1,750	207	199	9.9	363
WE Acid	4.2	68.5	30.4	1,100	202	62.0	20.4	183
WE Native	5.2	1.4	8.9	1,140	214	53.2	5.0	132
WE $CaCO_3$ #1	7.0	1.7	5.3	1,470	186	49.5	3.2	239
WE $CaCO_3$ #2	7.2	2.8	9.8	1,920	215	47.2	3.9	256

†AG (agricultural field, GB (grassed field border), WE (wetland edge)

Table 2.6: Soil chemical characteristics of Piedmont B horizon transect soils treated with diluted HCl or $CaCO_3$ to achieve a range of pH values.

		----------NH_4OAc-Extractable (mg kg^{-1} soil)----------						
Treatment	pH_s	Mn	Al	Ca	Mg	K	Fe	P
†AG Acid #1	3.6	8.1	95.9	776	158	145	2.6	60.6
AG Acid #2	4.7	2.0	70.8	799	192	153	2.3	44.7
AG Native	6.1	0.5	77.1	771	150	147	2.1	12.1
AG $CaCO_3$ #1	7.2	0.7	109‡	1,630	159	136	1.9	13.0
GB Acid	4.1	16.4	84.3	853	134	63.3	13.0	7.4
GB Native	5.5	1.1	55.5	806	172	56.6	8.0	8.6
GB $CaCO_3$ #1	7.2	1.4	81.6	1,130	105	53.2	5.9	34.7
GB $CaCO_3$ #2	7.3	1.2	88.7	1,710	133	50.9	6.1	22.8
WE Acid	3.9	73.3	86.1	883	159	28.6	20.0	14.2
WE Native	5.7	1.9	35.8	839	175	23.9	8.5	21.1
WE $CaCO_3$ #1	7.2	1.8	41.2	1,090	124	20.8	5.7	35.1
WE $CaCO_3$ #2	7.3	2.1	47.5	1,690	147	21.8	6.4	32.6

†AG (agricultural field, GB (grassed field border), WE (wetland edge)
‡This value is recognized as an outlier but was re-checked and confirmed, so remains in the data set.

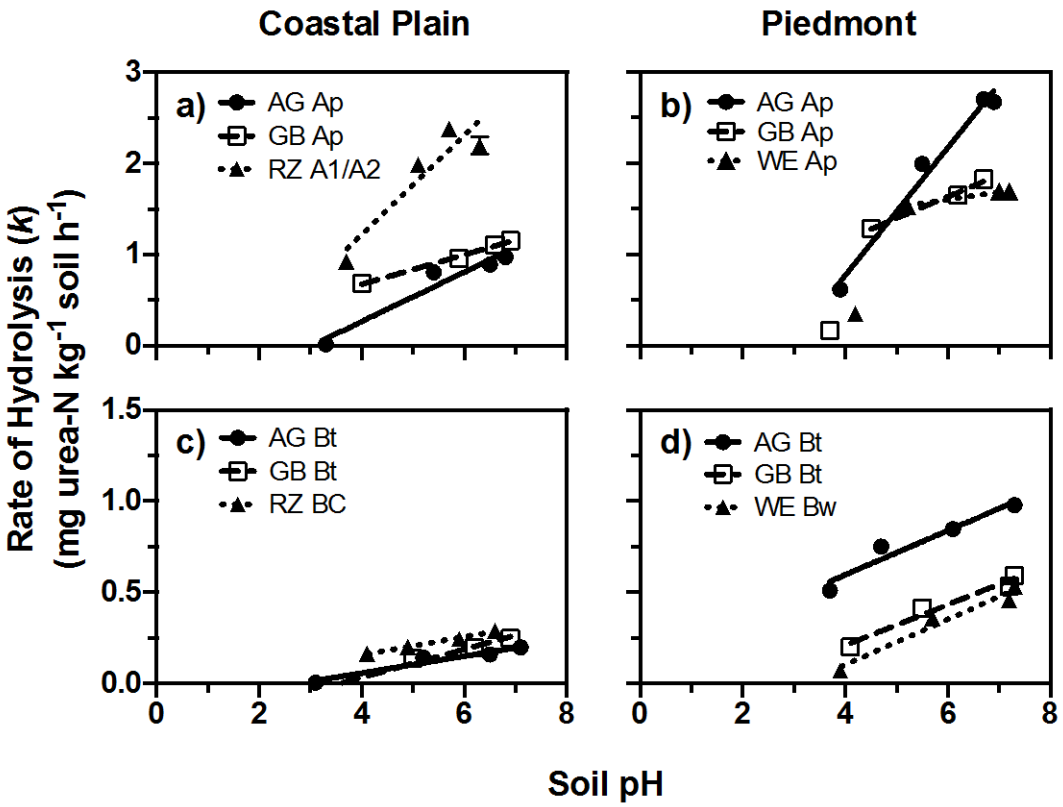


Figure 2.4: Linear regression of rate of urea hydrolysis vs. pH in both the A (panels a and b) and B (panels c and d) horizons of the Coastal Plain (CP) and Piedmont (PM) soil transects consisting of an agricultural field (AG), a grassed field border (GB), and a riparian zone (RZ) in the CP and a wetland edge (WE) in the PM. Hydrolysis rates at the lowest pH values in the PM GB and WE Ap Horizon soils (symbols not connected to linear regression lines) were removed from the linear regression analysis because these data points were not linear. SEM plotted (some error bars are within the symbols), n=3.

predictor components. In contrast to native soils, pH was a significant contributor to the multiple regression models in these pH-adjusted soils. However, the fit of the model was substantially improved with the addition of C and/or N (Table 2.7), which highlights the importance of organic matter-related factors in predicting rates of urea hydrolysis in both native and pH-adjusted soils.

The regression analyses for urea hydrolysis without pH indicated that extractable metals can also affect urea hydrolysis. However, the metal and nutrient ions selected for possible inclusion in the stepwise regressions were those that were significantly correlated with pH. The results of the stepwise multiple regressions without pH, but with metals, are shown in Table 2.7. Six out of the seven models were improved (Table 2.7). Five out of these six improved models included Mn, the same metal ion that was predictive of urea hydrolysis rate in native soils. These results support the view that a combination of soil organic matter (C and/or N) and the extractable metals that might negatively affect soil microbial communities (Al, Mn, Fe) are controlling the rate of hydrolysis in native and pH-adjusted soils.

The rate of change in urea hydrolysis as a result of changes in pH differed in soils from the different landscape positions, but in all cases, an increase in pH resulted in an increase in urea hydrolysis (Fig. 2.4). In the CP A-horizon soils, the hydrolysis rate in the RZ A1/A2 soil responded the most to an increase in pH with its rate of change of 0.54 mg urea-N kg^{-1} soil h^{-1} per pH unit being two to four times faster than in soils from the other two landscape positions (Table 2.8). The large response of the Coastal Plain RZ A1/A2 soil to an increase in pH may be due to a reduction in the toxicity of metals, as shown by the soluble Al decreasing from 170 to 54 mg kg^{-1} soil and Mn decreasing from 9.8 to 1.1

Table 2.7: Multiple regression model components for pH-adjusted soils from the Coastal Plain (CP) and Piedmont (PM) soil transects.

Site	Horizon	pH alone† Adjusted r^2	Model†	Adjusted r^2	Model†	Adjusted r^2
			----Excluding Metals‡----		------Including Metals§------	
CP	Ap + A1/A2	0.12	pH, C	0.89	N, Mn, C, Al	0.96
CP	Bt + BC	0.65	pH, C, N	0.93	Mn, C, Ca, Al	0.98
PM	Ap	0.50	N, pH	0.48	P, Mn, K, C	0.92
PM	Bt + Bw	0.23	pH, C, N	0.98	Fe	0.75
CP + PM	Ap + A1/A2	0.28	C, pH	0.76	Ca, Mn, C, N	0.83
CP + PM	Bt, Bw, BC	0.23	N, pH, C	0.49	N, Fe, C, Al	0.67
CP + PM	Ap, A1/A2, Bt, Bw, BC	0.11	C, pH	0.81	C, Mn, Ca	0.87

†All model components and complete models significant at $p < 0.05$

‡Model excluding metals, but including pH

§Model excluding pH, but including metals that are significantly correlated with pH

Table 2.8: Linear regression models for rate of urea hydrolysis (Y) vs. pH (X) in the pH-adjusted soils from the A and B horizons of the Coastal Plain and Piedmont soil transects consisting of an agricultural field (AG), a grassed field border (GB), and a riparian zone (RZ) in the Coastal Plain and a wetland edge (WE) in the Piedmont.

Site†	Horizon	Linear Regression Model	r^2 Value
Coastal Plain AG	Ap	Y = 0.2705*X - 0.8153	0.94
Coastal Plain GB	Ap	Y = 0.1620*X + 0.02693	0.99
Coastal Plain RZ	A1/A2	Y = 0.5433*X - 0.9512	0.86
Coastal Plain AG	Bt	Y = 0.04631*X - 0.1280	0.95
Coastal Plain GB	Bt	Y = 0.07724*X - 0.2760	0.97
Coastal Plain RZ	BC	Y = 0.04947*X - 0.04133	0.99
Piedmont AG	Ap	Y = 0.7006*X - 2.033	0.98
Piedmont GB	Ap	Y = 0.2373*X + 0.2097	0.99
Piedmont WE	Ap	Y = 0.08264*X + 1.105	1.0
Piedmont AG	Bt	Y = 0.1213*X + 0.1103	0.93
Piedmont GB	Bt	Y = 0.1117*X - 0.2381	0.96
Piedmont WE	Bw	Y = 0.1247*X - 0.3970	0.96

†AG (agricultural field), GB (grassed border), RZ (riparian zone), WE (wetland edge)

mg kg^{-1} soil. The RZ A1/A2 soil also had a starting C content 3.1 times greater than that in the Coastal Plain AG Ap and 2.3 times greater than that in the Coastal Plain GB Ap horizons, which probably buffered the RZ soil from the toxic effects of higher native metal concentrations. In addition, this soil may contain a larger and more diverse microbial community (see Chap. 3). In contrast, the Piedmont AG Ap horizon soil had the greatest response in hydrolysis to an increase in pH at that site, with a rate of change three times that of the GB Ap and more than eight times that of the WE Ap soil at the same site. This may indicate that the Piedmont AG Ap soil contained a greater proportion of ureolytic microbes that were able to respond to urea-N inputs and an increase in pH favored their growth (see Chap. 3). The low rate of change measured in the Piedmont WE Ap soil may be a result of its landscape position and the frequency of flooding. Reductions in rates of urea hydrolysis in flooded soils have been reported (Yadev et al., 1987; Wali et al., 2003), and while the WE soil was not flooded at the time of sampling, its landscape position and the redox features observed in the profile suggest that this soil is periodically flooded. These conditions may limit the establishment of aerobic ureolytic microbes. It is also interesting to note that the two soils that showed the greatest increase in urea hydrolysis with an increase in pH, (the Coastal Plain RZ A1/A2 and the Piedmont AG Ap) are also the two soils with the highest native P levels (Table 2.1). The phosphate anion has been shown to inhibit a form of purified urease in a laboratory experiment (Todd and Hausinger, 1989), but in soils, this nutrient is essential for the growth of microbial biomass. Its availability may have contributed to microbial respiration in these soils or be reflective of general soil fertility and cropping history influences on other soil properties not measured here.

Differences in the native C content of the soils from the two transects may help explain why the rates of urea hydrolysis in some soils were dramatically decreased at low pH, while others were not. In the A horizon soils at both sites, a pH near 4 resulted in a marked decrease in urea hydrolysis, relative to higher pHs (Fig. 2.4). However, a pH of 4 in the Coastal Plain RZ A1/A2 soil did not prevent urea hydrolysis, whereas a similar pH in the Coastal Plain AG Ap, which had a lower C content, nearly stopped hydrolysis. The differences in the responses of these soils to pH may lie in the differences in C and associated organic acids that are capable of chelating metals and preventing the development of toxic Al and Mn concentrations in the soil solution (Haynes and Mokolobate, 2001). A pH of 4 in low-C soils may indicate the point at which organic acids have been saturated with H^+, and Al^{3+} is released from complexing organic ligands and becomes toxic in solution or binds with P and prevents microbial activity. Alternatively, a pH this low may be killing ureolytic microbes or denaturing extracellular urease. Some microbial ureases are reported to denature below pH 5 (Mobley et al., 1995).

The rates of change in urea hydrolysis with an increase in pH were higher in A horizon soils than they were in B horizon soils (Fig. 2.4 and Table 2.8). In the A horizons of soils at both locations, a pH at or below 4 caused a rapid decrease in rates of urea hydrolysis, while the B horizon soils at both locations had smaller and more linear responses to changes in pH. The slow hydrolysis of urea in B horizon soils is in agreement with previously published works (Myers and McGarity, 1968; Gould et al., 1973). There was less C and N in B horizon soils, and this likely supported a smaller microbial community. The hydrolysis that is taking place in B horizons may be

predominantly resulting from extracellular urease that is stabilized on the clay fractions in the soil (Krajewska, 2009).

Conclusions

This study looked at urea hydrolysis in both native and pH-adjusted soil profile toposequences from the Coastal Plain and Piedmont regions of Maryland. In native soils, urea hydrolysis was slower in the soils sampled from the Coastal Plain compared to the Piedmont, but was always significantly faster in A horizons compared to B horizons. These differences were explained ($r^2 \geq 0.90$) by differences in soil C, N and/or Mn. Within the Coastal Plain toposequences, the rate of urea hydrolysis was not significantly different across the landscape in A horizons soils, but was significantly faster in riparian zone BC soils compared to B horizons from the other two landscape positions. In pH-adjusted soils, a combination of organic matter (total C and N), pH and extractable metals significantly predicted rates of change in urea hydrolysis with changes in pH. These findings support the conclusion that increasing soil organic matter content and pH may improve rates of urea hydrolysis in these soils. In addition, urea that is transported from agricultural fields in surface runoff has the potential to be hydrolyzed in soils in the grassed or forested buffer strips that often border agricultural fields. If urea does leach into B horizons, the rate of hydrolysis will be slower. However, the significantly faster hydrolysis in the riparian buffer BC compared to other B horizon soils indicates that landscapes that include riparian buffer zones may hydrolyze more urea than landscapes that do not. Field-scale studies of urea hydrolysis in the Coastal Plain and Piedmont

regions would be valuable to confirm the mechanisms described here and to further address the question of whether unhydrolyzed agricultural urea fertilizer has the potential to contaminate surface waters.

Chapter 3: Urease gene number correlation with urea hydrolysis in soils across an agricultural – riparian landscape and pH gradient

Introduction

Molecular techniques have been used to investigate the link between functional microbial genes and N cycling in many agricultural and non-agricultural soils. Dominant among these studies are investigations into the genes responsible for N_2 fixation (Morales et al., 2010), nitrification (Hallin et al., 2009; Placella and Firestone, 2013), and denitrification (Hallin et al., 2009; Morales et al., 2010; Wu et al., 2012). Potential denitrification rates in soil have been correlated with copy numbers of the gene coding for nitrous oxide (N_2O) reductase (*nosZ*), but not with the genes coding for nitrite (NO_2^-) reductase (*nirS* and *nirK)* (Hallin et al., 2009). Similarly, Wu et al. (2012) found no linear correlation between *nirS and nirK* gene copies and either the rate of nitrate loss or N_2O production. However, Morales et al. (2010) found a significant correlation between N_2O emissions and the difference between *nirS-nosZ* genes, suggesting that the presence of nitrite reducing *nirS* in the absence of nitrous oxide-reducing *nosZ* is a good predictor of the loss of N_2O emissions from soils. Others have found that potential NH_3 oxidation rates have been correlated with the size of the ammonia oxidizing archaeal community, but not with the size of the ammonia oxidizing bacterial community (Hallin et al., 2009). While molecular techniques have helped to uncover some aspects of N cycling in soils, further work is necessary to fully understand the link between functional genes and biogeochemical cycling of N.

In contrast to the relatively large number of studies investigating the genes responsible for nitrification and denitrification, few studies in soils have investigated the functional gene responsible for producing ammonia from urea by the following hydrolysis reaction (for examples of urease work in aquatic systems, see: Collier et al., 1999; Solomon et al., 2010).

$$CO(NH_2)_2 + 2H_2O \rightarrow CO_2 + H_2O + 2NH_3 \qquad \text{(Eqn. 3.1)}$$

Urea is ubiquitous in soil environments as both a natural nitrogenous waste product and as an agricultural fertilizer, and the enzyme urease is responsible for hydrolyzing urea into NH_3 (Mobley and Hausinger, 1989; Glibert et al., 2006). Urease is present in plants, bacteria, fungi, and archaea and exists both within cells and as an extracellular enzyme released into the soil environment upon cell death (Hasan, 2000; Krajewska, 2009; Tourna et al., 2011; Witte, 2011; Lu and Jia, 2013). Outside the cell, soil urease is stabilized by soil colloids, particularly clays and organic matter, and can continue to function in this state (Krajewska, 2009). Some soil organisms have urease activities ranging from 14.5 to 7,100 μmol urea min^{-1} mg $enzyme^{-1}$ (Krajewska, 2009). Despite this wide range, no studies have investigated how differences in the microbial community composition affect urea hydrolysis in soils, and whether changes in community structure can explain variations in observed rates of urea hydrolysis in different soils.

Despite growing concerns regarding agricultural urea fertilizer contamination of surface waters and its role in harmful algal blooms (Glibert et al., 2001; Lomas et al., 2002; Glibert et al., 2005; Heil et al., 2007; Kudela et al., 2008; Li et al., 2010), no studies have been found that investigated ureolytic microbial communities and urea-N

cycling in agricultural soils. Some studies have quantified the bacterial *ureC* gene, which codes for one of three structural subunits in the urease enzyme (Mobley et al., 1995). These studies have investigated the potential for urea hydrolysis to induce calcite precipitation and stabilize liquefiable soils (Burbank et al., 2011) or cause the precipitation or co-precipitation of metals with carbonates (Gresham et al., 2007). However, the only N-cycling studies found were those of Rothrock et al. (2008; 2010), who developed new primers to detect *ureC* genes in a group of microbes present only in poultry litter, and then investigated the ureolytic microbial community composition in acidified poultry litters.

If unhydrolyzed urea is leaching from agricultural soils to surface waters, it must travel by one of two flow paths: overland on soil surfaces across agricultural fields and through grassed buffer strips or riparian zones that often border nearby surface waters; or vertically and horizontally through the soil horizons and into groundwater along a hydraulic gradient. Soil chemical conditions such as pH and organic C content vary across a landscape as a result of agricultural liming and tilling practices (Murty et al., 2002), and the use of toposequences provides an opportunity to investigate community ecology along natural gradients, an approach that has been called for by McGill et al. (2006). In addition, microbial community dynamics below the “plow layer” (i.e., the top 15-20 cm of soil) are not well documented (Kramer et al., 2013), but may be important in explaining the slow rate of urea hydrolysis reported in deeper soil horizons in some studies (see Chap. 2; Khakurai and Alva, 1995).

In a companion experiment, significant differences were found in the response of urea hydrolysis in soils to a pH change imposed in the laboratory (Chap. 2). Specifically,

these soils were sampled along a transect that included a site in an active agricultural field, one in a grassed field border, and one within a riparian zone. Soils from the A horizons hydrolyzed urea more rapidly than soils from the B horizons, and the riparian zone A horizon soil had the greatest increase in hydrolysis rate with an increase in pH. Multiple regression analysis indicated that soil C and N helped to explain how soils brought to the same pH could have different rates of urea hydrolysis. These results indicated that soil organic matter content, and possibly the associated microbial community, are important predictors of urea hydrolysis rates in the soils sampled across these agricultural landscapes.

In this study, we quantified the microbial community composition across an agricultural landscape from an agricultural field through forested or grassed riparian zones adjacent to surface waters, and included soils from both the A and B horizons. We chose a sampling site on the Coastal Plain of Maryland's Eastern Shore because many agricultural fields in this region are composed of sandy soils that are either adjacent to surface waters or have been ditched and drained to lower a high water table. The dynamics of urea hydrolysis in these systems are important to understand because of the high permeability of the soil and the proximity of ground and surface waters leading to the Chesapeake Bay. The soil master variable, pH, was investigated because of its importance to both agronomic productivity and microbial community composition (Pietri and Brookes, 2008; Pietri and Brookes, 2009). We hypothesized that in native soils, 1) patterns of gene type and copy number would be similar among sites with similar urea hydrolysis rates, 2) the fungal:bacterial ratio would be lowest in active agricultural fields due to the near-neutral pH, and would increase in edge of field and riparian zones where

the soils were more acidic, and 3) total urease gene numbers would follow the distribution of bacteria and be highest in agricultural fields, and decrease in grassed field border soils and riparian zones. In pH-adjusted soils, we further hypothesized that bacteria would be highest in high pH treatments, fungi would be higher in the acidic treatments, and that *ureC* gene copy number would be significantly correlated with urea hydrolysis rate. The results of this study were somewhat unexpected: the native soils had similar hydrolysis rates but very different microbial community composition; the highest gene numbers were found in the riparian soil; and bacteria were the main contributors to urea hydrolysis. Of particular note were the strong, positive, statistically significant correlations between *ureC* gene copy number and urea hydrolysis rate, indicating that the presence of this gene may be useful as a biomarker for predicting rates of urea hydrolysis in soils.

Materials & Methods

Site description and soil collection

This study was conducted using soils sampled in October, 2012 at the Wye Island Natural Resource Management Area in Queen Anne's County, Maryland (38°54'11.97"N, 76° 8'12.20"W), located within the Coastal Plain physiographic province of the Mid-Atlantic region of the USA (Appendix A). Soils were sampled along a transect consisting of three sampling locations running from an active agricultural field (AG) used to grow maize (*Zea mays* L.) and soybean (*Glycine max* L.), through a grassed field border (GB) of tall fescue (*Festuca arundinacea* Schreb.), to a forested riparian

zone (RZ) of sassafras (*Sassafras albidum* Nutt.), southern red oak (*Quercus falcata* Michx), black cherry (*Prunus serotina* Ehrh.), red maple (*Acer rubrum* L.), and black walnut (*Juglans nigra* L.), with under story vegetation composed of multiflora rose (*Rosa multiflora* Thunb.), sumac (L.), trumpet creeper (*Catalpa radicans* L. Seem.), common greenbrier (*Smilax rotundilia* L.), Virginia creeper (*Parthenocissus quinquefolia*), and honeysuckle (*Lonicera japonica* Thunb.). The soils were sampled both from the A horizon (0-15 cm) and at a depth representative of the B horizon. In the AG, this depth was between 45-60 cm, and in the GB and RZ, the sample was taken between 65-80 cm due to differences observed in the soil profile. The AG soil was sampled from the Ingleside mapping unit (38°54'11.97"N, 76° 8'12.20"W), the GB soil sampled from the boundary of Ingleside and Longmarsh & Zekiah mapping units (38°54'10.37"N, 76° 8'13.79"W), and the RZ soil was sampled from the Longmarsh & Zekiah mapping unit (38°54'9.98"N, 76° 8'14.70"W); all of which were similar to the Ingleside series (coarse-loamy, siliceous, mesic Typic Hapludult). Sampling soils in the autumn may have resulted in sampling soils with lower seasonal urease activities (Kang et al., 2009) and therefore the rates of hydrolysis discussed in this work may be lower than those that would be measured in soils sampled in the spring.

A 7.6-cm diameter, open-faced soil auger was used to obtain four subsamples which were combined to form one composite sample from each depth at each point along the transect. The BC horizon sample in the RZ, however, consisted of three auger holes because a proliferation of tree roots prevented additional sampling. The soils were sampled on the same day and stored in a cooler for fewer than 5 h before being brought back to the laboratory. Subsamples weighing a minimum of 80 g and providing analytical

replicates for DNA analysis were then immediately frozen in separate bags at -20°C for a period of less than 11 months. Each individual sample was homogenized by hand mixing before DNA analysis. The rest of the soil was allowed to equilibrate to room temperature (21-23 °C), sieved to pass a 4 mm screen, and kept field-moist in double plastic bags in closed plastic buckets until used.

Determination of pH effects on urea hydrolysis

The determination of pH effects on urea hydrolysis is described in the Materials & Methods section of Chap. 2. After pH equilibration, the moist equivalent of 2.5-g oven-dried soil from each pH treatment was frozen at -20°C for future DNA extraction. Each urea rate incubation experiment also included a set of triplicate samples that were not treated with phenylmercuric acetate at the end of the incubation, but rather were brought to volume with 0.01M $CaCl_2$, centrifuged, and the resulting soil pellet was frozen at -20°C for DNA extraction.

DNA extraction

Total genomic DNA was operationally defined by extraction from each soil using a PowerSoil DNA isolation kit (Mo Bio Laboratories, Carlsbad, CA, USA) following the manufacturer's instructions with the exception that the soils were homogenized using a FastPrep-24 (45 sec at 6 m s^{-1}; MP Biomedicals, LLC, Solon, OH). The DNA in all extracted solutions was quantified using a Qubit 1.0 Fluorometer (Life Technologies,

Grand Island, NY, USA). Extracted DNA was stored at -20˚ C for fewer than three months while analyses were completed. DNA extracts were diluted to 2.5 ng μL^{-1} in preparation for quantitative PCR (qPCR) amplification of bacterial *ureC* gene copy numbers and 1.25 ng μL^{-1} for qPCR amplification of bacterial and archaeal 16S rRNA and fungal ITS.

Standard preparation and quantitative-PCR amplification (QPCR)

Quantitative PCR was used to estimate bacterial and archaeal 16S rRNA, fungal ITS, and bacterial *ureC* genes. Standard clones for Eub16S rRNA, Arc16S rRNA, ITS, and *ureC* were created from stock cultures of *Escherichia coli*, *Sulfolobus solfataricus*, *Haematonectria haematococca*, and *Pseudomonas aeruginosa*, respectively, and total genomic DNA was isolated using the DNEasy Tissue and Blood Extraction kit (Qiagen Sciences, Germantown, MD, USA). Target inserts were amplified using forward and reverse primers for Eub16S (Eub338F, Eub518R)(Pietri and Brookes, 2008; Pietri and Brookes, 2009), Arc16S (A915, Arc1059) (Fierer et al., 2005), ITS (5.8S, ITS1f)(Yu et al., 2005) and *ureC* (ureC-F, ureC-R)(Fierer et al., 2005). Isolates were generated using a Topo TA cloning kit (Invitrogen, Carlsbad, CA) according to the manufacturer's instructions. Plasmids were purified using GenElute Plasmid Miniprep Kit (Sigma Aldrich, St. Louis, MO, USA). Prior to analysis, standards were linearized using ECO-RV (Promega, Madison, WI, USA) and cleaned up using the UltraClean PCR clean-up kit (Mo Bio Laboratories, Carlsbad, CA). Standards were quantified using a Qubit 1.0 Fluorometer (Life Technologies, Grand Island, NY, USA) and a 10-fold dilution series

was generated. An equimolar soil standard dilution series was used to relativize plasmid curves for sample-specific inhibition (Hargreaves et al., 2013).

Soil DNA extracts and standards were run in triplicate 20 μL reactions with 10.0 μL of SYBR Green qPCR mastermix (Life Technologies), 0.5 μM final concentration of the forward and reverse primer each, and 2.5 ng template DNA for community composition or 5 ng of template DNA for functional gene quantification. In some cases, soil DNA was below these concentrations, and these were run at stock DNA concentrations and calculations were adjusted accordingly. All reactions were run on the StepOne Plus real-time PCR instrument (Applied Biosystems, Foster City, CA). With two exceptions, thermocycler conditions were the following for amplification of all genes: heat inactivation at 95°C for 5 min, followed by 40 cycles of 95°C for 5 s (denaturation), 55°C for 15 s (annealing), and 72°C for 10 s (extension and acquisition). SYBR Green was quantified during the 72°C elongation step. The amplification of archaeal 16S rRNA required an annealing temperature of 57°C to avoid non-specific amplification in samples with low copy numbers. The amplification of bacterial *ureC* genes used a 58°C annealing temperature. Melt curve analysis was performed following every run to confirm product specificity. A double melt curve was observed with some ITS runs, but specificity was confirmed by gel electrophoresis. Data was extracted from runs with standard curves having r^2 values >0.98 and efficiency values between 100-101% for *ureC*, between 92-96% for ITS, and between 96-101% for bacterial and archaeal 16S rRNA.

Soil properties

Exchangeable cations, total C and N, pH, and water content were calculated as described in Chapter 2.

Statistical analyses

Statistically significant differences among the rates of urea hydrolysis in soils and gene abundances were determined using one-way ANOVA ($p < 0.05$, and Tukey's test, $p < 0.05$) in GraphPad Prism (Prism 6, GraphPad Software, Inc., La Jolla, CA). Multiple regression analysis (SPSS v.21, SPSS Inc., Chicago, IL) was used to determine the best linear combination of factors predicting the rate of urea hydrolysis in soils ($p > 0.05$). Correlations and Pearson correlation coefficients ($p < 0.05$) were computed using GraphPad Prism (Prism 6, GraphPad Software, Inc., La Jolla, CA).

Results & Discussion

Analyses of freshly sampled soil

The native soils sampled in this study varied in both their physical and chemical characteristics (Coastal Plain soils data listed in Table 2.1). Of particular note are the large ranges in pH (3.6-5.4) and in extractable Mn (0.48-9.8 mg kg^{-1} soil) and Al (30 -170 mg kg^{-1} soil) concentrations, which can reduce P, Ca, and Mg bioavailability or be toxic in solution, affecting microbial communities (Haynes and Mokolobate, 2001; Lauber et

al., 2008). In addition, gene copy numbers for archaea, fungi, bacteria, and *ureC* varied significantly across the landscape (Table 3.1). In particular, the RZ A1/A2 soil contained significantly more fungi, bacteria, and *ureC* than all other A and B horizon soils. Despite this variation in soil chemical properties and gene copy numbers across the landscape, the native rates of urea hydrolysis did not differ significantly in the A horizons (Table 3.2).

These findings do not support our first hypothesis, that patterns in gene type and copy number would be similar among sites with similar urea hydrolysis rates. Since we quantified DNA rather than RNA, we can only measure genetic potential in the soil, rather than the real-time expression of genes by the microbial community (Saleh-Lakha et al., 2005). As a result, we can conclude that in these native soils, the urea hydrolysis rate is not easily explained by the genetic potential of the microbial community as measured by the numbers of bacterial (Eub 16S), archaeal (Arc 16S), fungal (ITS), and *ureC* (urease) genes. In measuring *ureC*, we measured all organisms carrying the gene, even those who were not actively expressing it. Therefore, it is possible that only a subset of the population carrying the *ureC* gene is active and contributing to the measured rates of urea hydrolysis. The wide rates of urease activity measured in some soil organisms (14.5 to 7,100 μmol urea min^{-1} mg $enzyme^{-1}$) (Krajewska, 2009) could explain how relatively few microbes could be doing the majority of the urea hydrolysis measured in these soils.

The RZ BC soil had significantly faster urea hydrolysis than did the AG Bt and GB Bt soils (Table 3.2). This may be a result of the higher C content and higher C:N ratio in the RZ soil indicating a N-deficient environment that quickly responded to N inputs

Table 3.1: Gene abundances in native Coastal Plain transect soils. AG (agricultural field), GB (grassed field border), RZ (riparian zone), n = 3.

	Arc 16S‡		ITS		Eub 16S		*ureC*	
	----------genes g^{-1} soil----------							
AG Ap	6.73E+08	b†	5.96E+07	bcd	1.08E+10	c	2.00E+08	c
GB Ap	8.32E+08	a	8.15E+07	b	1.65E+10	b	2.96E+08	b
RZ A1/A2	3.27E+08	d	9.22E+08	a	2.19E+10	a	3.32E+08	a
AG Bt	6.66E+07	e	1.88E+06	d	6.02E+08	e	1.05E+07	e
GB Bt	4.54E+08	c	4.51E+06	cd	2.78E+09	de	3.97E+07	d
RZ BC	4.17E+08	c	7.00E+07	bc	4.16E+09	d	4.16E+07	d

†Means within the same column sharing a letter are not significantly different at $p<0.05$
‡Arc 16S (total archaea), ITS (total fungi), Eub 16S (total bacteria), *ureC* (urease gene)

Table 3.2: Rates of urea hydrolysis in native Coastal Plain transect soils. AG (agricultural field), GB (grassed field border), RZ (riparian zone).

Site	Horizon	Rate of Urea Hydrolysis (mg urea-N kg-1 h-1)†	
AG	Ap	1.1	a
GB	Ap	1.1	a
RZ	A1/A2	1.0	a
AG	Bt	0.079	c
GB	Bt	0.062	c
RZ	BC	0.21	b

†Rates with the same letter are not significantly different at $p < 0.05$.

when urea was added during the experiment.

The calculated fungal-to-bacterial gene copy ratios (Table 3.3) supported our second hypothesis that this ratio would be lowest in the AG soil and would increase in the RZ soil. The RZ A1/A2 soil had a significantly higher fungal-to-bacterial ratio than the other A horizon soils, and the RZ BC soil had a significantly higher fungal-to-bacterial ratio than the other two B horizon soils. The fact that the AG and GB soils did not differ from each other in this ratio may indicate a legacy of agricultural management and nutrient inputs at these two landscape positions. Bacteria are thought to have higher nutrient requirements than fungi, as a result of the lower C:N ratio found in their biomass (3-6), as compared to fungi (5-15) (Strickland and Rousk, 2010). In soils where N is not limiting, a shift toward bacterial dominance in soil is expected. In contrast, the RZ soils had the highest fungal-to-bacterial gene copy ratios and the highest C:N ratios (Table 2.1). The high numbers of fungi in the RZ are likely a result of mycorrhizal associations with the plants in the riparian area, including documented arbuscular mycorrhizal associations with black walnut (*Juglans nigra* L.) and red maple trees (*Acer rubrum* L.) (Wang and Qiu, 2006). This hypothesis is supported by a more than 80% decrease in ITS copy numbers measured in stored RZ A1/A2 horizon soil relative to freshly sampled soil (Appendix C), a change that could be explained by the disappearance of mycorrhizal fungi dependent on living plants that are not present in stored soils. The high concentration of bacteria in RZ soils compared to the other transect points was unexpected since bacterial numbers are thought to decrease as pH decreases (Rousk et al., 2010; Rousk et al., 2011), but some studies have found that bacterial communities shift in

Table 3.3: Fungal-to-bacterial gene copy ratios in native transect soils. AG (agricultural field), GB (grassed field border), RZ (riparian zone).

Site	Horizon	Fungal:Bacterial	±SD	
AG	Ap	5.52E-03	2.69E-04	c†
GB	Ap	5.16E-03	3.57E-05	c
RZ	A1/A2	4.13E-02	3.84E-03	a
AG	Bt	3.13E-03	5.81E-06	c
GB	Bt	1.60E-03	6.00E-05	c
RZ	BC	1.68E-02	5.21E-04	b

†Means with same letter are not significantly different at $p<0.05$

composition at low pH, with some taxa increasing in number (Lauber et al., 2008; Pietri and Brookes, 2009). Some authors have hypothesized that the increase in extractable Al as pH decreases explains some observed decreases in microbial biomass and activity (Pietri and Brookes, 2008), while others have found Al toxicity effects on bacteria to be minimal (Rousk et al., 2010). Nevertheless, the high C levels measured at the lowest pH in the transect may be providing a C source for heterotrophic microorganisms and buffering these systems against metal toxicities and nutrient limitations that might otherwise develop (Haynes and Mokolobate, 2001). Therefore, nutrient inputs from agricultural management in the AG and GB locations contrasted with high soil C in the RZ may explain the patterns of bacterial and fungal dominance measured across this transect.

The RZ A1/A2 soil had the lowest pH, lowest field-sampled moisture content, and highest concentrations of soluble Al, Mn, and Fe (Table 2.1), but also contained the highest numbers of *ureC* genes among the A horizon soils transect points (Fig. 3.1 and Table 3.1). The pattern is the same in the B horizon soils, except that the RZ BC horizon and GB Bt do not differ in the number of *ureC* genes extracted. These data do not support our third hypothesis; while *ureC* gene copy numbers did follow the distribution of Eub 16S genes, both sets of genes were highest in the RZ soil and lower in the GB and AG soils, which was the opposite of what we predicted. This may be a result of a large microbial community capable of growing in the RZ soil due to the presence of available C and the associated ameliorating effects against metal toxicity and high (H^+) discussed previously.

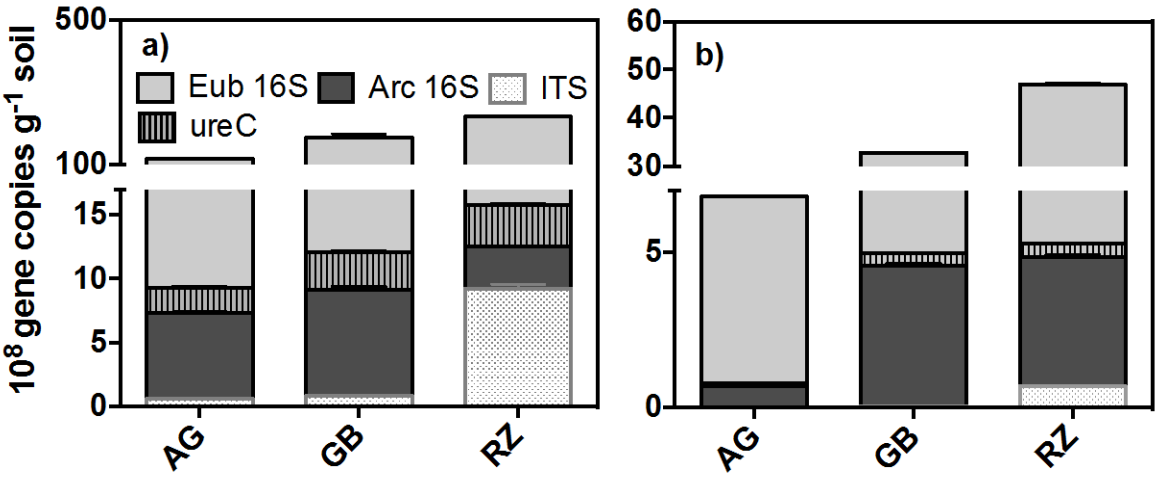


Figure 3.1: Genes extracted from the native agricultural field (AG), grassed field border (GB), and riparian zone (RZ) soils in both the a) A and b) B horizons. Error bars represent the SEM among triplicate analytical replicates, and in some cases are within the margins of the bar graph.

Archaea were most prevalent in the GB Ap soil. In soils, some archaea are capable of urea hydrolysis and ammonia oxidation (Hatzenpichler, 2012), and have been found across a gradient of soil pH from 3.75 to >8 (Hu et al., 2013; Lu and Jia, 2013). For example, the ammonia-oxidizing archaea (AOA) phylum *Thaumarchaeota* has been reported to decrease with increasing soil pH, but its relative abundance, compared with other measured archaea, increased across the same range, indicating the potential importance of this phylum over a wide range of soil pH (Hu et al., 2013). In other work, AOA abundance was negatively correlated with the availability of organic C and the C:N ratio, possibly as a result of competition with heterotrophs (Wessen et al., 2010). Based on our findings, we can speculate that the intermediate C:N ratio and pH of the GB soil in this transect may provide a combination of soil conditions that is preferable for these organisms.

The A horizon soils contained more bacteria, archaea, and *ureC* genes than did B horizon soils across all three transect points (Table 3.1). ITS gene copies were more varied, with fewer statistical differences between A and B horizons (Table 3.1). However, the most striking difference was the significantly higher numbers of ITS in the RZ A1/A2 soil compared to all other soils (Fig 3.1). These findings agree with other studies that have found a decrease in both C concentration and microbial abundance with depth in the soil profile (Eilers et al., 2012; Kramer et al., 2013), presumably due to less total and bioavailable C in lower soil horizons.

DNA extracted from the native soils indicated that between 1.5 – 20% of the bacterial community contains the *ureC* gene and is capable of urea hydrolysis, assuming that each bacterium carries between 1 and 10 copies of the 16S rRNA gene (Klappenbach

et al., 2000) and one copy of the *ureC* gene (Srivatsan et al., 2008; Burbank et al., 2011). This proportion of ureolytic bacteria was consistent across the different landscape positions and in both the A and B horizon soils sampled, and is similar to the range of 17-30% found by Lloyd and Sheaffe (1973), who used a dilution-plate method and urea agar containing the indicator phenol red to calculate the proportion of ureolytic bacteria in their soils. The faster rate of urea hydrolysis measured in the RZ B horizon (Table 3.2) compared to other B horizon soils may therefore be due to a greater proportion of ureolytic bacteria actively expressing the *ureC* gene relative to the soils from the other landscape positions. However, field replicates and mRNA analyses were not available to test these different possibilities. The RZ A1/A2 and BC soils had the highest C:N ratio of their respective transect points (Table 2.1), indicating that this soil may be N-limited and that the urea-N supplied during the experiment to determine hydrolysis rate provided a limited nutrient and resulted in rapid uptake and metabolism.

Analyses of soils treated in the laboratory to achieve a range in pH

Adjusting the native pH of the toposequences soils from both sites, by adding HCl or $CaCO_3$, produced several anticipated changes in soil chemical properties that were likely to affect the activity of the microbial community (Chap. 2, Tables 2.3 to 2.6). The soils that were treated with HCl to reduce the pH had higher concentrations of potentially toxic soluble metals (Mn, Al, and Fe) in both A and B horizons. Adding $CaCO_3$ to increase pH increased extractable Ca (due to the addition of $CaCO_3$) and soluble P compared to the concentrations of these essential nutrients found in the native soils. The

B-horizon soils within each transect had less favorable conditions for microbial growth, including higher soluble Al concentrations and lower soluble K and P concentrations than the corresponding A-horizon soils (Tables 2.3-2.6). Multiple regression analysis determined that a model that included both pH and C explained 89% of the variability in the rates of urea hydrolysis in pH-manipulated Coastal Plain A horizon soils, and a model including pH, C, and N explained 93% of the variability in the rate of urea hydrolysis in pH-adjusted Coastal Plain B horizon soils (Chap.2, Table 2.7).

An increase in pH corresponded to an increase in the rate of urea hydrolysis in all soils and all horizons (Chap. 2, Fig 2.2). The most dramatic increase in urea hydrolysis with increasing pH occurred in the RZ A1/A2 soil, possibly due to this soil having the largest ureolytic microbial community of all the field soils sampled (Table 3.1), and both the highest C content and the highest C:N ratio. These factors may indicate an N-limited environment in which microbes were able to quickly metabolize added urea-N. The RZ A1/A2 soil was also the only A horizon soil that had a significant increase in Eub16S genes in the $CaCO_3$ treatments relative to the control pH treatments (Fig. 3.2). Lowering pH resulted in a large and significant increase in ITS in the acidified AG Ap soil and the acidified GB Bt soil (Fig. 3.3). Although not consistent across all soils, these data support our fourth hypothesis, that bacteria would be highest in high pH treatments and that fungi would be most numerous in low pH treatments. The optimum range of pH for fungi has been reported to be wide, while the pH optima for bacteria tends to be constrained by the soil conditions in which the population has become established (Strickland and Rousk, 2010), indicating that large changes in pH imposed by the addition of acid and base likely affected bacteria more than they did fungi. Low fungal growth at high pH has been

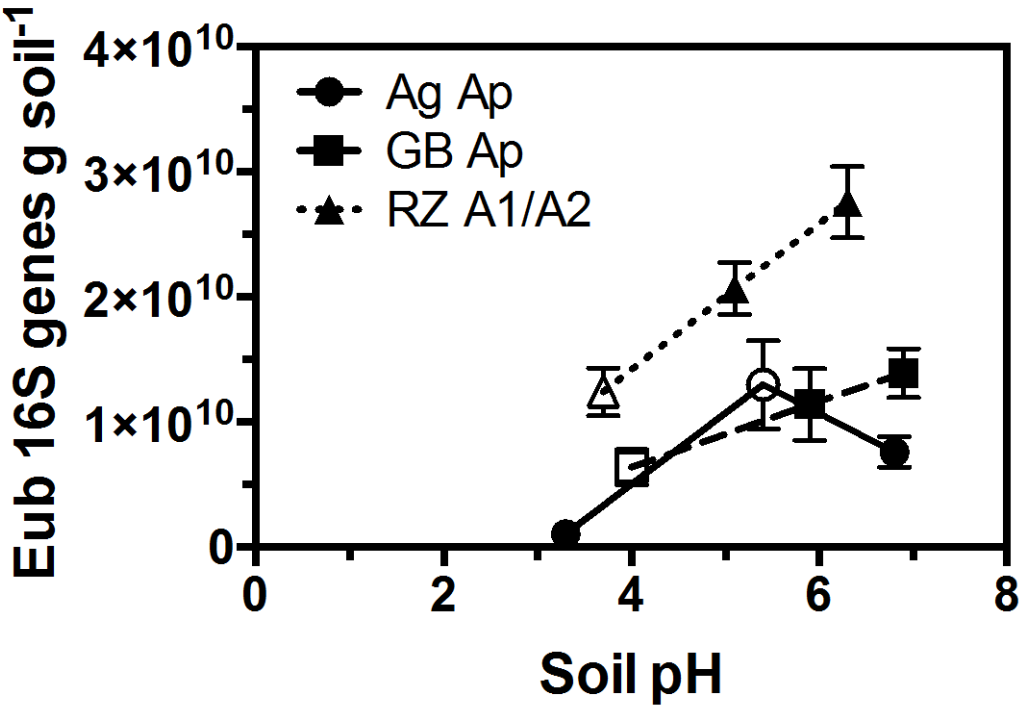


Figure 3.2: Eub 16S genes in pH-adjusted A horizon soils following the addition of urea during the urea hydrolysis rate experiment. Hollow symbols denote native soils, and error bars indicate ± SEM (n=3).

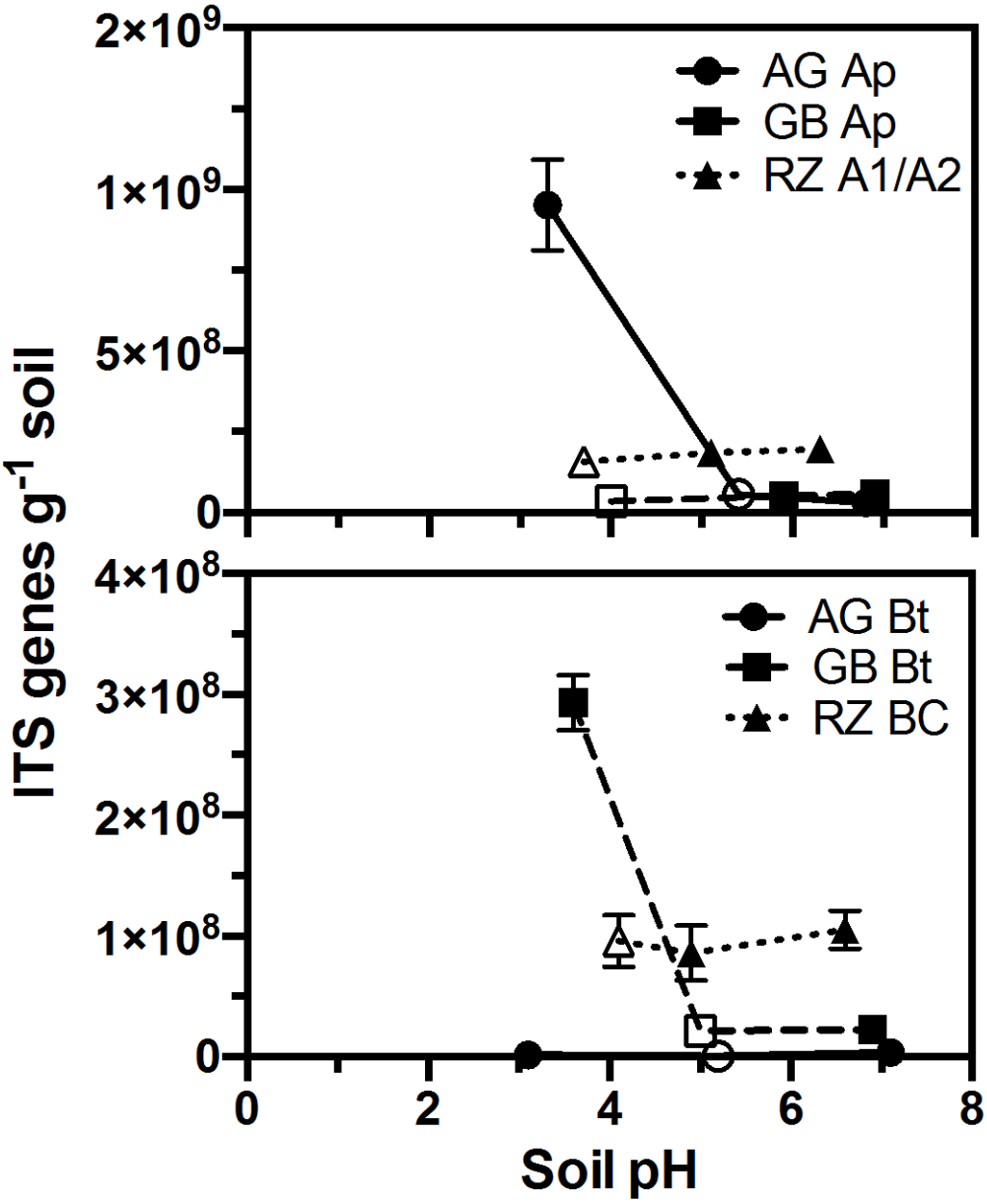


Figure 3.3: ITS genes in pH-adjusted A and B horizon soils following the addition of urea during the urea hydrolysis rate experiment. Hollow symbols denote native soils, and error bars indicate ± SEM (n=3).

reported, and has been found to be due to bacterial competition rather than lack of substrate for the fungi (Rousk et al., 2010).

An increase in the rate of urea hydrolysis was significantly correlated with an increase in Eub 16S gene copies in the pH A horizon soils both before and after urea was added (Fig. 3.4). The correlation improved after urea was added to the system (r^2 of 0.79 increased to 0.91). This suggests that the presence of urea resulted in the growth of an ureolytic bacterial population, and therefore hydrolysis is occurring mainly through the activity of the live microbial biomass in A horizon soils. In contrast to the A horizon soils, the B horizon soils did not show a significant correlation between urea hydrolysis rate and Eub16S numbers. This suggests that either the bacterial community in the B horizon was composed mostly of non-ureolytic bacteria, the *ureC* primers used in this study did not capture all urease enzyme present in the soil, or that some other sub-population of microorganisms, or possibly extracellular urease, is responsible for urea hydrolysis in these lower mineral horizons. In some soils, extracellular urease has been reported to contribute to over half of the observed activity. In this form, it is protected by soil clays and colloids against thermal and proteolytic degradation and continues to function (Pettit et al., 1976; Klose and Tabatabai, 1999). The higher clay content of both the AG and GB Bt soils (Table 2.1) may therefore contain greater amounts of bound and active extracellular urease, or greater amounts relative to the corresponding Ap soils. Neither A nor B horizon soils showed significant correlations between urea hydrolysis rate and ITS or Arc16S genes, indicating that bacteria are the principal microbial taxon responsible for urea hydrolysis in these soils.

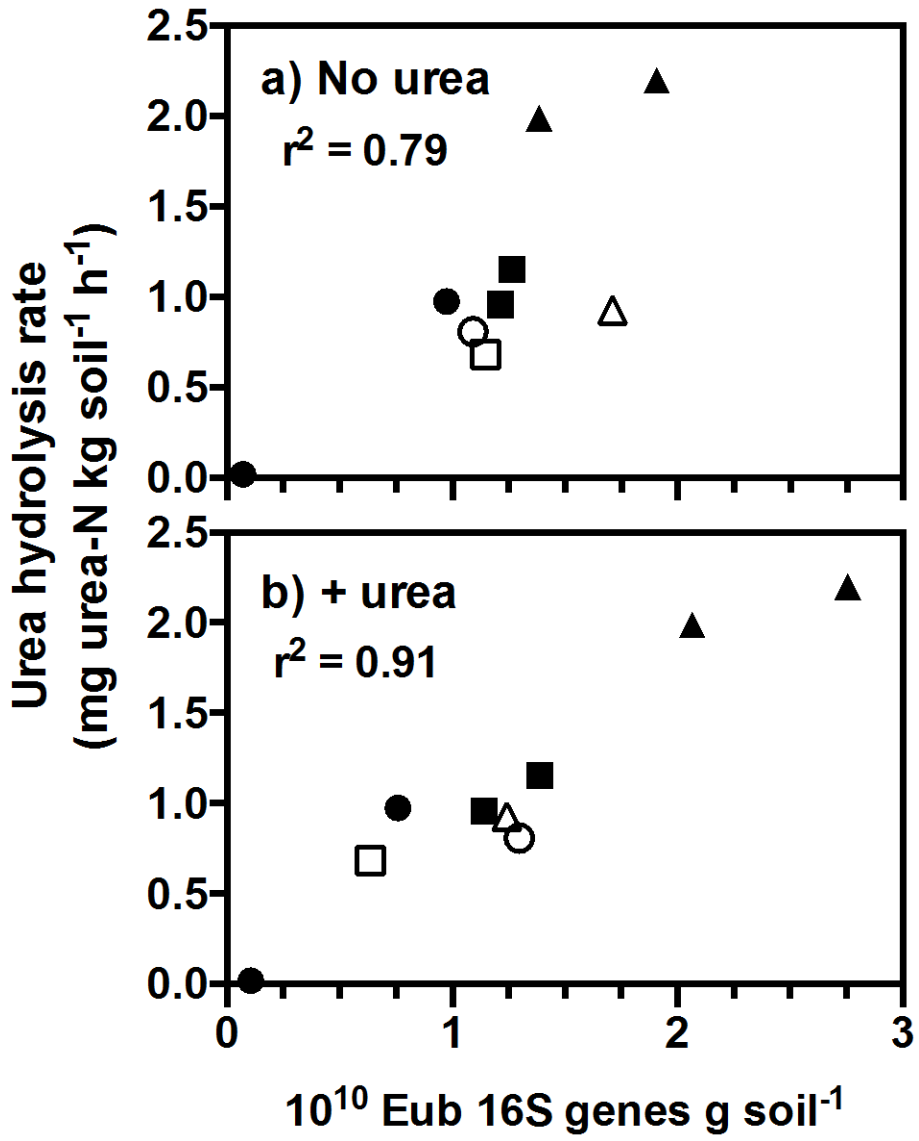


Figure 3.4: An increase in the rate of urea hydrolysis was significantly correlated with Eub 16S gene copies in the pH-adjusted A horizon soils both a) before and b) after incubation with urea ($p < 0.05$). AG Ap (circles), GB Ap (squares), and RZ A1/A2 (triangles) plotted. Hollow symbols denote native soils (n=3).

The correlation between urea hydrolysis rate and *ureC* genes was significant in both A and B horizon soils, supporting our fifth hypothesis in this study, that *ureC* gene copy number would be significantly correlated with urea hydrolysis rate. The correlation coefficient remained consistent both before and after urea was added to the system (Fig. 3.5), potentially indicating that a consistent proportion of the urease activity is attributable to live biomass, as opposed to extracellular enzyme activity. In addition, there were significant correlations between Eub16S and *ureC* genes in both A and B horizons (Fig. 3.6). The correlation improved in the A horizon after urea was added (r^2 of 0.70 improved to 0.91), but this pattern was not apparent in B horizon soils. These findings further support the conclusion that bacteria were the greatest contributors to urea hydrolysis in A horizon soils.

Conclusions

Analysis of microbial community composition and *ureC* genes across an agricultural field-riparian zone landscape toposequence, in A and B horizons, and over a range of soil pH, allowed us to investigate the influence of soil chemical factors on both microbial community composition and urea hydrolysis. The most important result was that the native RZ A1/A2 soil contained the greatest abundance of bacteria, fungi, and *ureC* genes, despite what might be considered an unfavorable environment of low pH, low field-sampled moisture content, and high extractable Al and Mn. The high C content and high C:N ratio of the RZ soil likely mediated these potentially toxic characteristics and supported a microbial community that could hydrolyze urea when it was supplied

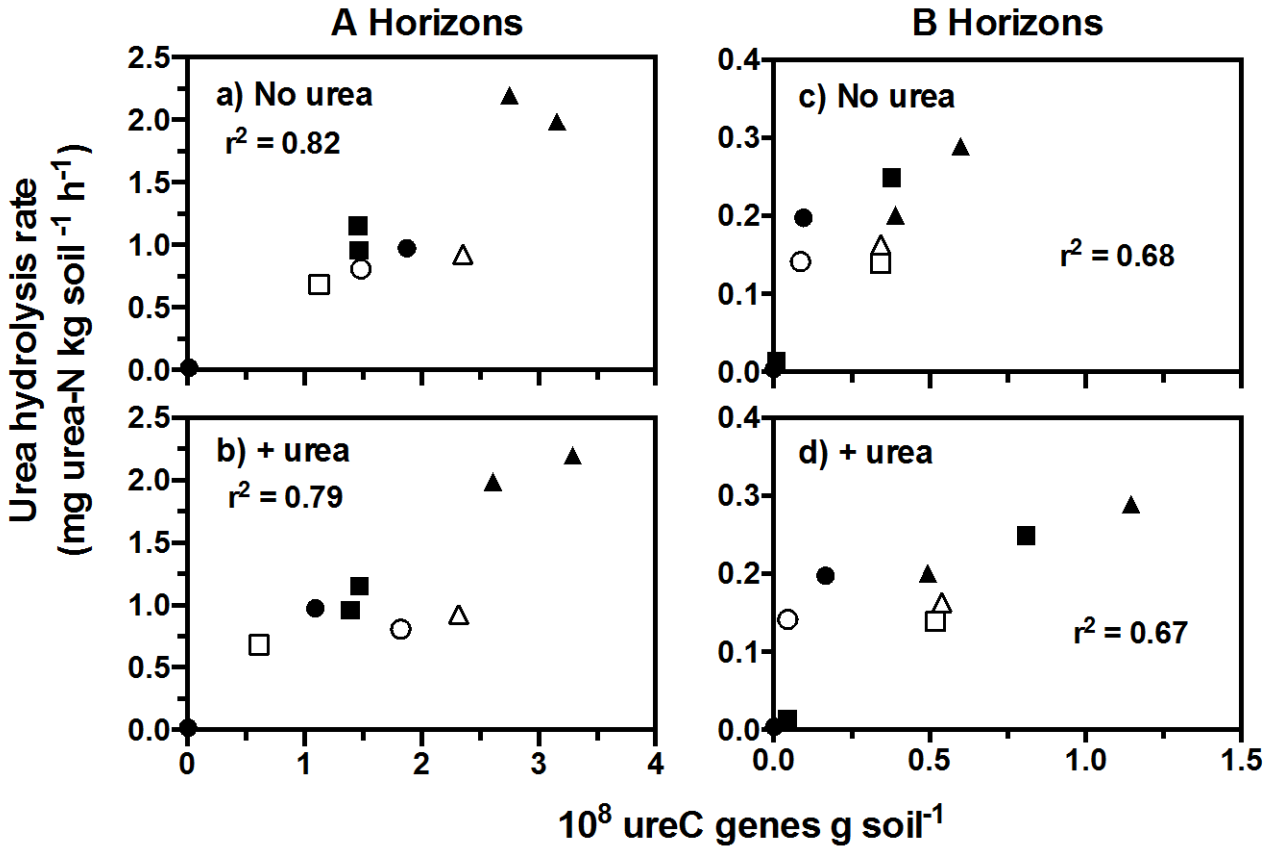


Figure 3.5: Significant correlations between urea hydrolysis rate and genes in both A (a and b) and B (c and d) horizons, before (a and c) and after (b and d) urea added ($p < 0.05$). AG Ap (circles), GB Ap (squares), and RZ A1/A2 (triangles) plotted. Hollow symbols denote native soils (n=3).

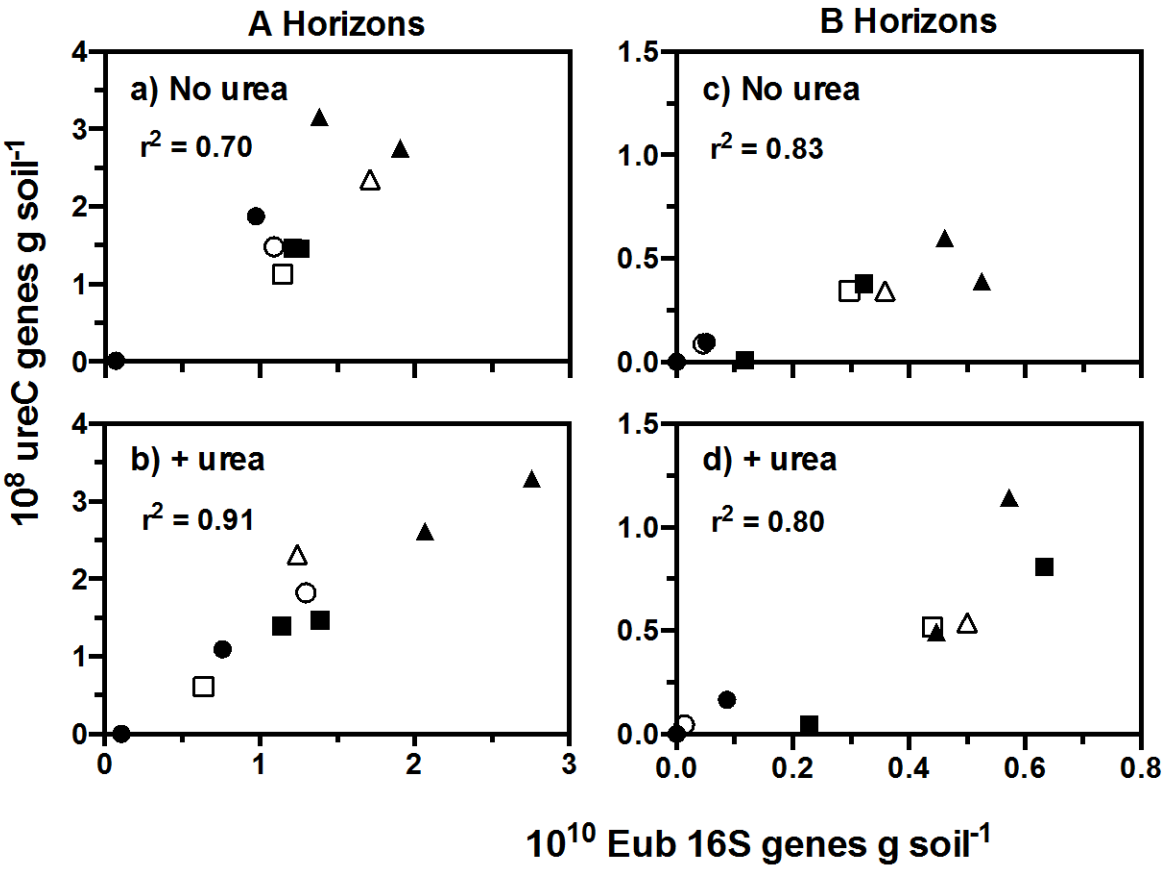


Figure 3.6: Significant correlations between Eub 16S and *ureC* genes in both A (a and b) and B (c and d) horizons, before (a and c) and after (b and d) urea added ($p < 0.05$). Addition of urea improved r^2 in A horizon (a and b). AG Ap (circles), GB Ap (squares), and RZ A1/A2 (triangles) plotted. Hollow symbols denote native soils (n=3).

from an external source. We found that the *ureC* gene was significantly correlated with urea hydrolysis rate in an incubation experiment with soil pH lowered or raised. Further studies of urea hydrolysis across a wider soil landscape are necessary to determine whether this gene can be used as a biomarker for ureolytic microbial activity in soils. A better understanding of ureolytic microbial composition in soils, and the factors that influence its activity across an agricultural-riparian landscape, will help researchers make recommendations related to urea fertilizer application so that urea-N can be efficiently used by crops and urea movement across the landscape and into surface waters can be minimized.

Chapter 4: Urea hydrolysis in soils: enhancement and inhibition by ascorbic, gallic, benzoic, and cinnamic acids.

Introduction

Urea is an organic N-containing fertilizer that is hydrolyzed to NH_4^+ by soil microbes and extracellular urease (Krajewska, 2009). The rate of this hydrolysis reaction varies across both natural soil landscapes and in laboratory studies of pH-adjusted soils, and soil C has been identified as an important variable for predicting urease activity (Zantua et al., 1977; Saviozzi et al., 2001; Yin et al., 2014). However, these studies have not looked at the components of soil C, especially reducing and metal-complexing organic acid fractions of soil C, that are influential in increasing or decreasing the rate of urea hydrolysis. The type of C incorporated into soils through plant material deposition, agricultural soil amendments and root exudates has been found to influence microbial community composition and affect enzyme activities, including that of urease (Zaman et al., 2002; Renella et al., 2007; Chen et al., 2014; Yin et al., 2014). Whether the stimulatory effects of soil C are due to increased substrate for heterotrophic microorganisms, improved metal chelation and associated reductions in toxicity, or other factors, is poorly understood. However the increased urease activity following the addition of glucose to soils appears to be a straightforward result of increased microbial use of an accessible C source (Falih and Wainwright, 1996). Since microbial communities, and bacteria specifically (Ch. 3), are important mediators of urea hydrolysis in soils, further investigation into the effect of different C amendments on urea hydrolysis is warranted.

Carbon enters the soil environment through C-fixation by photosynthetic plants, plant root exudates, and the decomposition of organic materials (Kalbitz et al., 2000; McDowell, 2003). These processes and materials provide a complex mixture of C compounds to the soil environment, including cellulose, hemicellulose, lignin, condensed and hydrolysable tannins, phenols, organic acids, amino acids, and water-soluble carbohydrates (Cook and Allan, 1992a; Kraus et al., 2003; Suescun et al., 2012) that vary in their bioavailability. Renella et al. (2007) measured a significant increase in double-stranded DNA in a clayey soil treated with glucose and glutamate, and in a sandy soil treated with the organic acid oxalate, indicating that these C compounds stimulated microbial growth. Lin et al. (2011) found that the application of the phenolic compounds *p*-hydroxybenzoic acid, ferulic acid, vanillic acid, salicylic acid, and cinnamic acid increased microbial biomass C and microbial respiration, in the order listed. Other types of C have differing effects on microbial growth based on the complexity of the C structure; tannin monomers and dimers can be used as microbial C substrates, whereas polymerized condensed tannins can inhibit growth (Kanerva et al., 2006).

The dynamic complexity of soil organic C, its variability across soil types, vegetative cover, and bioavailability to different microorganisms, has made it difficult to completely understand the complex interactions between soil C and the soil biotic community, and the effects of different types of soil C on biogeochemical cycling (Chantigny, 2003; Chen et al., 2014; Yin et al., 2014). A clear understanding of the effects of organic C on urease activity in soils is complicated by the use of different methodologies for extracting and measuring soluble C in soils. Concentrations of dissolved organic matter (DOM), dissolved organic C (DOC), water extractable organic

matter (WEOM), and water extractable organic C (WEOC) are all reported in the literature and vary in their components based on the size of the membrane used to filter soil extracts, whether C or organic matter were quantified, and the method by which the leachate was extracted from the soil (Chantigny, 2003). Nevertheless, a review of just those papers reporting DOC or WSOC indicates that the level of C in soil extracts can vary by several orders of magnitude depending on past land management and vegetative cover. Soils sampled from previously cultivated agricultural fields after 12 to 62 yr of secondary succession contained between 6 – 16 g total C kg^{-1} soil, of which 0.07 - 0.1 % was dissolved organic C (9 to 12 mg kg^{-1} soil) (Cook and Allan, 1992b). In a grassland soil, tillage resulted in water extractable organic C concentrations ranging from 50-90 mg kg^{-1} soil (Chantigny, 2003). In one study, the rooting zones of legumes contained greater concentrations of WSOC than those of non-legumes, although all measured values were in the range of 40-75 mg kg^{-1} soil (Chantigny, 2003). The application of sugar beet to soils at a rate simulating that of pockets of decomposing root left in the soil after harvest and equivalent to 20,000 mg C kg $soil^{-1}$, stimulated enzyme and microbial activities in an agricultural soil (Falih and Wainwright, 1996). Renella et al. (2007) studied the effects of glucose, citrate, oxalate or glutamate added to soils at a rate of 300 mg C kg^{-1} soil to simulate plant root exudation into the rhizosphere. WSOC can be quite high in different types of manures and composts. Prost et al. (2013) studied the effects of composting manure with different types of biochar, a type of organic material that is pyrolyzed to produce a carbonaceous compound that is used to improve soil fertility and sequester C when added to the soil. They found that WSOC increased as a result of the added biochar, presumably due to sorption effects, with the final product containing 3,500-7,000 mg

water-extractable organic C kg^{-1} compost. The application of composts and manures to soils will of course result in the dilution of these nutrients, but stratification can result from surface application, resulting in locally high concentrations of WSOC. Following 10 yr of a long-term poultry litter and chemical fertilizer application experiment, WSOC concentrations were measured at 780 mg kg^{-1} soil between 0-2.5 cm depth, 517 mg kg^{-1} soil between 2.5-5 cm depth, decreasing to 168 mg kg^{-1} soil at a depth of 15-30 cm (Zhang et al., 2011).

Knowledge of the naturally or agriculturally relevant concentration range of soluble C in soils is not enough; different components of total soluble C can have varying effects on soil biological properties. In a study comparing cultivated, forested, and grassland soils, the concentration of WSOC was not different among the sites (900 mg C kg^{-1} soil), but the quality of the different fractions of WSOC differed, with greater concentrations of labile carbohydrates and phenolics in the non-cultivated soils (Saviozzi et al., 2001). This correlated with higher enzyme activities (including urease) in the grassland site and lower activities in the agricultural site. Investigations into the fractions of soluble organic C that are most highly correlated with urease activity may help explain conflicting reports in the literature regarding the correlation of urease activity with total soil C. The results of Chap. 2 and the work of others (Yin et al., 2014) found total C to be predictive of urease activity in soils, but others have not found the same correlation (Hassan et al., 2013).

Despite soluble C concentrations ranging from 10-20,000 mg C kg^{-1} soil, several studies investigating the effects of C on enzyme activities were based on only one level of added C. Lin et al. (2011) studied the application of different phenolic acids to soil at a

rate of 500 mg phenolic acid kg^{-1} soil and found that these compounds stimulated urease activity in the order: p-hydroxybenzoic acid > ferulic acid > vanillic acid > salicylic acid > cinnamic acid. The addition of glucose from sugar beets was investigated only at the concentration of 20,000 mg C kg^{-1} soil and found to stimulate urease activity (Falih and Wainwright, 1996). Others have found that unidentified components of the residues of the aromatic herbs basil and savory stimulate soil enzyme activities, including that of urease (Chen et al., 2014). However, no literature has been found that looks at ranges of specific components of added C sources to obtain information about the threshold at which stimulatory effects may be seen. Since C can be used by heterotrophic microorganisms, can be involved in metal chelation and solubility (Haynes and Mokolobate, 2001), can influence pH (Saviozzi and Cardelli, 2014), and since all of these effects may be concentration-dependent, additional information is needed on the concentration effects of added fractions of soil organic C.

The objective of this study, therefore, was to evaluate the effects of specific types of added C on urea hydrolysis in soils. Sites with different soil and plant management histories were chosen to understand how this factor might influence our results; soils were sampled from the Ap and Bt horizons of an active agricultural field and from the A1/A2 and BC horizons of a forested riparian buffer adjacent to the agricultural field. To address previously mentioned gaps in the literature, we evaluated a range of added C from 0 – 10,000 mg C kg^{-1} soil that was representative of the values already discussed and found in natural and agricultural settings. The C sources chosen for evaluation were benzoic (BA), *trans*-cinnamic (CA), ascorbic (AA), and gallic acids GA), all of which occur naturally in plants (Hoskins, 1984; Brewer, 2011). BA and CA have been found to

serve as C substrates for soil microorganisms (Hoskins, 1984; Lin et al., 2011). Cinnamates are deamination products of phenylalanine and its derivatives and can be used by plants to synthesize lignin (Hoskins, 1984). CA can also be microbially metabolized to BA before being converted to other metabolic compounds (Gibson, 1968). There are both aerobic and anaerobic pathways for the catabolism of BA by microbes (Ismail and Gescher, 2012). In contrast to these more labile C sources, AA and GA were chosen to investigate the effects of added C sources that are known antioxidants and reducing agents (Alamed et al., 2009; Brewer, 2011). Understanding the link between these types of organic C and rates of urea hydrolysis can provide valuable information for land managers to use when making decisions related to improving N cycling and C dynamics in agricultural settings or in degraded, low C soils.

Materials & Methods

This study was conducted on soil materials sampled from one transect in October, 2013 from the Wye Island Natural Resource Management Area in Queen Anne's County, Maryland (38°54'11.97"N, 76° 8'12.20"W), located within the Coastal Plain (CP) physiographic region (Fig. A.1). The sampling consisted of a transect of two locations: one in an agricultural field (AG) and the other in a forested riparian zone (RZ) adjacent to surface water. The soils were sampled both from the A horizon (0-15 cm) and the B horizon (45-60 cm). The AG soil sample was from the Ingleside mapping unit (38°54'11.97"N, 76° 8'12.20"W) and the RZ soil was from the Longmarsh- Zekiah mapping unit (38°54'9.98"N, 76° 8'14.70"W); both of which were similar to the

Ingleside series (coarse-loamy, siliceous, mesic Typic Hapludult). A 7.6-cm open-faced soil auger was used to obtain three subsamples, which were combined to form one composite sample from each depth at each point from the two sampling locations. The soils were all sampled on the same day and stored in a cooler for fewer than 5 h before being brought back to the lab and allowed to equilibrate to room temperature (21-23 °C). The soils were sieved to pass a 4 mm screen and kept field-moist in double plastic bags in closed plastic buckets until used.

Exchangeable cations, total C and N contents, pH, soil moisture and texture were determined as previously described in Chap. 2.

Treatments for the determination of organic acid effects on urea hydrolysis were established using solutions of ascorbic ($C_6H_8O_6$), benzoic ($C_7H_6O_2$), *trans*-cinnamic ($C_9H_8O_2$), and gallic ($C_7H_6O_5$-H_2O) acids made equivalent on a C-basis. Moist soil samples equivalent to 2.5 g oven-dried soil from each location/horizon were weighed into 37.5-mL polycarbonate centrifuge tubes in a randomized complete block design in the laboratory that investigated the variables of transect location, horizon, organic acid, and organic acid concentration. Triplicate samples of each soil from each location and horizon received 0, 100, 400, 1000, 4000, or 10,000 mg C kg^{-1} soil from each organic acid C source and a final concentration of 100 mg urea-N kg^{-1} soil in a background of 0.01 M $CaCl_2$. One exception to this occurred in the case of cinnamic acid; because it is only sparingly soluble in water, the treatments were limited to concentrations of 100, 400, and 1,000 mg C kg^{-1} soil. The samples were placed on an orbital shaker set at 800 cycles min^{-1} that shook the tubes for 30 min each hour for a total of 24 h, and were analyzed for urea-N as previously described in Chap. 2. The pH and Eh values measured during the

experiments were measured in the supernatant liquid of 0.01 M $CaCl_2$ soil slurries (10:1 solution:soil ratio) after soil particles had settled after 30 min of vigorous shaking in the last h of each urea incubation. The Mn, Al, and Fe measured in the different organic acid treatments were measured using flame atomic absorption in the 0.01M-$CaCl_2$-PMA extract described in Chap. 2.

Statistically significant differences among treatments were determined using one-way ANOVA ($p < 0.05$). Tukey's test ($p < 0.05$) was used for identifying significant differences among all treatments, while Dunnett's test ($p < 0.05$) was used for comparing individual treatments with the control (Prism 6, GraphPad Software, Inc., La Jolla, CA).

Results & Discussion

The soils sampled in this study varied in both their physical and chemical characteristics (Table 4.1). All soils sampled were sandy loams with the exception of the RZ A1/A2, which had a finer silt loam texture. The finer texture in the RZ A1/A2 may be a result of decades of soil eroding from the upslope agricultural field and settling in the vegetated riparian buffer. The RZ A1/A2 and BC horizons had field pH values of 4.5 and 4.0, respectively, which were lower than those of the AG Ap (5.3) and Bt (4.8) horizons. The lower pH values in the RZ soils are likely a result of greater concentrations of organic acids in the RZ soils and the absence of regular liming practices. Sandy soils with low C content are poorly buffered against the acidifying effects of rainfall and N fertilizer application, which helps to explain the relatively low pH that is present over the entire

Table 4.1: Selected physical and chemical properties of soils sampled along a transect consisting of an agricultural field (AG) and a riparian zone (RZ) in the Coastal Plain region of Maryland's Eastern Shore.

Site‡	Horizon	Depth (cm)	Sand	Silt	Clay	PSD†	pH_s	H_2O	C	N	C:N	Mn	Al	Fe	Ca	Mg	K
			-------- % --------					---- g kg^{-1} soil----				---------- mg kg^{-1} soil ----------					
AG	Ap	0-15	59.9	30.0	10.2	SL	5.3	154	7.7	0.59	13	0.89	52	0.87	393	125	61
RZ	A1/A2	0-15	24.1	56.1	19.9	SiL	4.5	249	21	1.8	12	0.0	110	4.0	782	200	91
AG	Bt	45-60	58.3	22.3	19.4	SL	4.8	156	1.7	0.091	19	3.2	96	3.4	587	122	28
RZ	BC	45-60	54.8	37.0	8.2	SL	4.0	76.7	6.2	0.39	16	2.0	170	7.3	173	41	34

†Particle size distribution

‡AG (agricultural field), RZ (riparian zone)

landscape. The field-sampled moisture content of the RZ BC soil, at 76.7 g kg^{-1} soil, was much lower than that of the other three horizons (150-250 g kg^{-1} soil). Since low water content was also observed in this horizon in a previous sampling in the fall of 2012 (Chap. 2, Table 2.1), we suggest that the combination of coarse soil texture and the prevalence of live trees roots in the RZ BC soil resulted in rapid percolation and uptake of water from this soil horizon. The concentrations of soil C measured in the RZ A1/A2 (21 g kg^{-1}) and BC (6.2 g kg^{-1}) were much higher than those measured in the AG Ap (7.7 g kg^{-1}) and Bt (1.7 g kg^{-1}) soils, likely due to the presence of perennial vegetation in the forested riparian zone. However, the C:N ratio of the A horizons (12 and 13) and B horizons (16 and 19) were similar across both soil sampling sites. Soluble Al and Fe were highest in the RZ A1/A2 (110 mg kg^{-1} and 4.0 mg kg^{-1}, respectively) and lowest in the AG Ap (52 mg kg^{-1} and 0.87 mg kg^{-1}, respectively). This is probably due to lower pH in the RZ A1/A2, which results in greater solubility of Al and Fe. The concentrations of other measured cations varied by horizon (Table 4.1).

The addition of the four organic acids (Fig. 4.1) to all of the soils resulted in a steady decline in pH as added C increased (Appendix D). The effect was most pronounced for ascorbic and gallic acids in the AG soils, probably as a result of lower soil C content and associated buffering capacity in the AG soils, compared to the riparian zone soils.

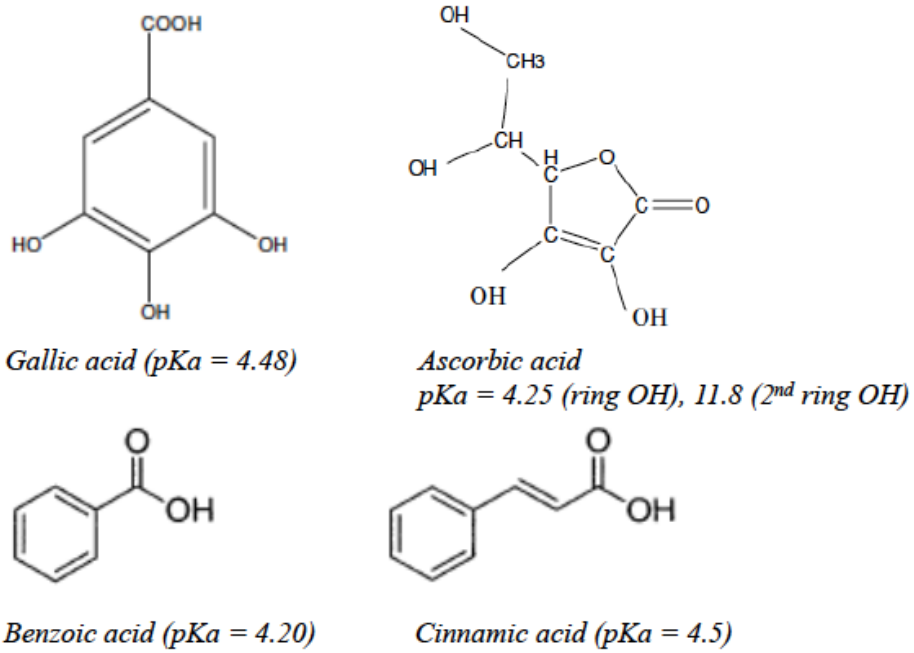


Figure 4.1: Structural formulas for the four organic acids tested in this study. Gallic and ascorbic acids contain resonance-stabilized –OH groups, which makes them effective reducing agents and anti-oxidants. Benzoic and cinnamic acids do not donate electrons and therefore do not act as reducing agents (Alamed et al., 2009; Brewer, 2011).

Ascorbic and Gallic Acids

Ascorbic acid (AA)-treated A horizon soils from both locations and the AG Bt soil (Fig. 4.2 a, b, c) had similar responses to increasing levels of this added C source. The lowest levels of added AA (100 and 400 mg C kg^{-1} soil) resulted in an increase in urea hydrolysis over the control, but the intermediate level of 1000 mg C kg^{-1} soil either inhibited urea hydrolysis (Fig. 4.2 a and c) or was not different from the control (Fig. 4.2b). The highest levels of added AA (4,000 and 10,000 mg C kg^{-1} soil in AG Ap and Bt and 10,000 mg C kg-1 soil in RZ A1/A2) resulted in an increase in hydrolysis over the no C-added control. Others have observed similar concentration-dependent effects of AA. Yen et al. (2002) reported that increasing concentrations of AA between 0.004 – 0.24 mM AA (equivalent to 3 – 160 mg C kg^{-1} soil in our study) corresponded with increasing oxidative damage to DNA. Damage peaked at 1.65 mM AA (1,070 mg C kg^{-1} soil), above which damage decreased with increasing concentration of AA. They postulated that this resulted from the AA-induced reduction of Fe^{3+} to Fe^{2+}, which stimulated •OH formation and free radical damage to DNA at low concentrations, but which was overcome by the anti-oxidant •OH-scavenging activity of AA at higher concentrations. The concentrations at which Yen et al. (2002) report oxidative damage by AA correspond to our treatments of up to 1,000 mg C kg^{-1} soil, which was the treatment with the most inhibition in the AG Ap and Bt, and the treatment that was not different from the control in the RZ A1/A2 (Fig. 4.2). The damaging oxidative effects of AA observed by Yen et al. (2002) below this threshold were not observed in our study; in fact, urea hydrolysis was enhanced at 100 and 400 mg C kg^{-1} soil (Fig. 4.2 a, b and c). However, it is important

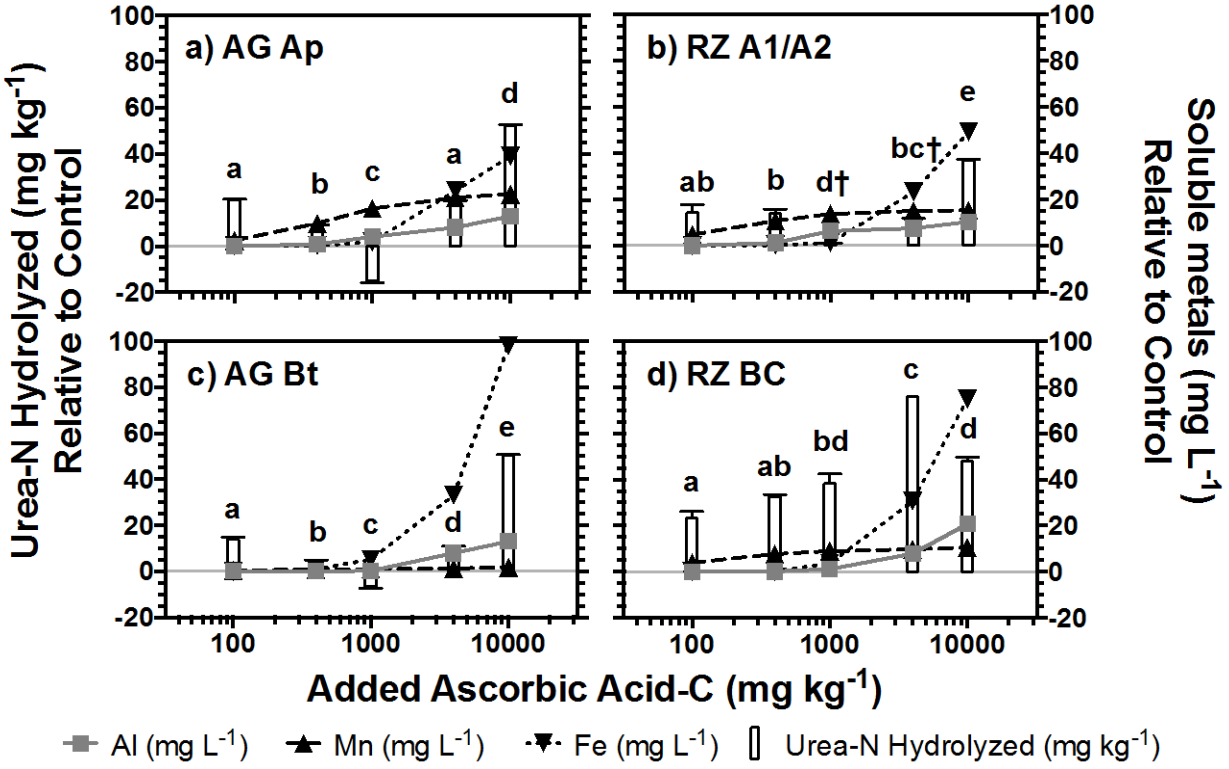


Figure 4.2: Effects of added ascorbic acid-C on urea hydrolysis and soluble metal concentration, relative to the control treatment, in each soil and horizon. Letters that are not different within the same soil horizon are not significantly different in the amount of urea-N hydrolyzed ($p < 0.05$). †Indicates treatment is not significantly different from the control treatment for that soil horizon. AG: agricultural field, RZ: riparian zone. SEM plotted (n = 3; n = 2 in urea-N in RZ BC 4,000 mg C kg^{-1} soil).

to note that our study took place in unbuffered soil suspensions with varying concentrations of metals, whereas that of Yen et al. (2002) was conducted using a controlled and buffered deoxyribose assay. Fenton reactions (Eqn. 4.1), in which Fe^{2+} catalyzes the decomposition of hydrogen peroxide to OH^- and hydroxyl radical (•OH) with the production of ferric iron (Fe^{3+}), are the basis for the oxidative damage that can result from low concentrations of AA (Decker and Hultin, 1992; Bradshaw et al., 2011):

$$Fe^{2+} + H_2O_2 \rightarrow Fe^{3+} + OH^- + \bullet OH \qquad \text{(Eqn. 4.1)}$$

The presence of a reducing agent, such as AA, reduces Fe^{3+} back to Fe^{2+} and maintains the Fenton reaction in a continuous cycle (Eqn. 4.2):

$$2Fe^{3+} + \text{ascorbate } (C_6H_7O_6^-) \rightarrow 2Fe^{2+} + \text{dehydroascorbic acid } (C_6H_6O_6) + H^+ \qquad \text{(Eqn 4.2)}$$

While low concentrations of AA are known to promote lipid oxidation by reducing metals in this manner and higher concentrations can prevent damage by scavenging free radicals, the concentrations at which these thresholds are met are dependent upon the concentration of Fe (Decker and Hultin, 1992). The low concentrations of soluble Fe present at 100-400 mg kg^{-1} added AA-C (Fig. 4.2 a, b, and c) may have resulted in low levels of this Fe-dependent oxidative damage. Instead of causing problems these concentrations, AA may have been chelating other metals (Brewer, 2011), such as the

soluble Mn^{2+} that was present (Fig. 4.2 and 4.3a). The position of all C-treated soils below the MnOOH-Mn^{2+} equilibrium lines (Fig 4.3) indicates that the reduced species, Mn^{2+}, is likely in soil solution, either as a result of pH- or ligand- induced reductive dissolution (Rajapaksha et al., 2012). AA is capable of chelating bivalent metals (Brewer, 2011) and may be binding with Mn^{2+} to reduce its toxicity and provide a net benefit to the system that allowed for enhanced hydrolysis to take place. Excess Mn^{2+} can be toxic to plants as a result of increased oxidative stress and the production of reactive oxygen species, increased lipid peroxidation, and protein oxidation (Srivastava and Dubey, 2011). Similar toxicities may develop for soil microorganisms and lead to lowered activity and reduced urea hydrolysis.

Why the RZ BC soil exhibited an essentially opposite response to increasing levels of AA remains unclear (Fig 4.2d). Based on previous work (see Chap. 3), we concluded that the majority of the urea hydrolysis that takes place in the A horizons of our soils is the result of the activity of live microbial biomass, whereas the hydrolysis in B horizons may be influenced to a greater degree by extracellular urease that is sorbed to and stabilized by clay materials (Krajewska, 2009). Microbial cell membranes and enzymatic DNA would all be hypothetically susceptible to oxidative damage, and the concentration-dependent inhibition of urea hydrolysis in both A horizons and the AG Bt horizon soils (Fig. 4.2 a, b, and c) can be postulated to result from interactions between live cells, DNA, and the pro- (causing oxidative damage) and antioxidant effects of added AA at different concentrations described by Yen et al. (2002). The input of C into the RZ BC may be composed of different organic acids than is found in the other soil horizons, since this horizon is the only one with a prevalence of perennial tree roots. Some organic

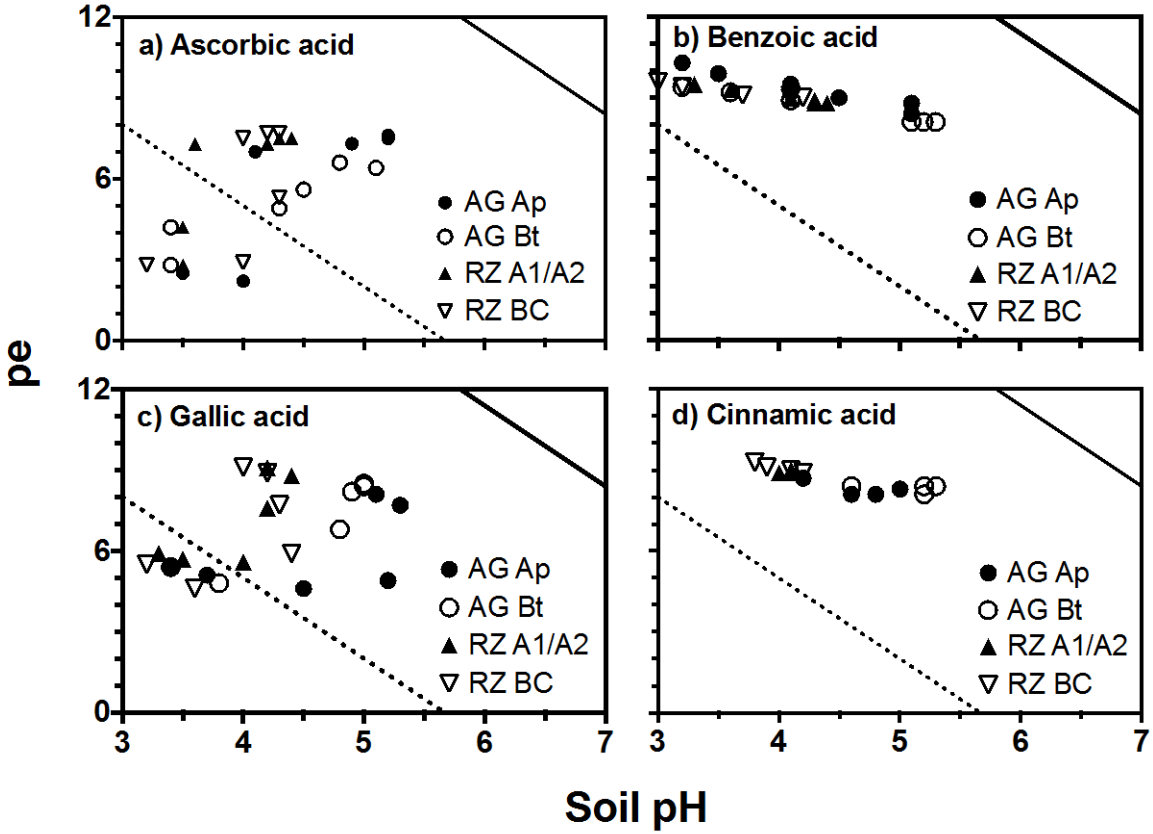


Figure 4.3: pe-pH diagrams for each organic acid showing FeOOH-Fe^{2+} ((red)/(ox)=10^{-4}; dashed line) and MnOOH-Mn^{2+} ((red)/(ox) = 10^{-4}; solid line) equilibria. AG: agricultural field, RZ: riparian zone. Treatments corresponding to those below the FeOOH-Fe^{2+} equilibrium line are listed in Appendix D.

acids, such as tartaric and malic acids, preferentially chelate Fe(III) over Fe(II), which would affect the redox equilibrium between these two forms of iron and encourage the oxidation of Fe(II) to the less soluble Fe(III) form (Bradshaw et al., 2011). If this redox shift occurred in the RZ BC, the organic acids in solution may have reversed the negative effects of the AA-induced reduction of Fe(III) such that the damaging effects of added AA up to 4,000 mg kg^{-1} C were not apparent, and enhancement of urea hydrolysis took place as a result of both Fe and Mn chelation, as discussed previously. At the highest concentration of added AA (10,000 mg C kg^{-1} soil), the Fe(II) in solution increased and the ameliorating effects of natural organic acids may have been overcome by the reductive power of AA. Indeed, the enhancement of urea hydrolysis in the RZ BC at the highest level of added AA is not different from that found at the same level of added AA in the AG Bt ($p < 0.05$).

Gallic acid (GA)-treated soils had similar patterns of enhancement and inhibition of urea hydrolysis (Fig 4.4) as was found in AA-treated soils (Fig. 4.2). Yen et al. (2002) also investigated the concentration-dependent pro- and antioxidant effects of GA and found that damage to DNA increased with increasing concentrations of GA between 0.004 – 0.82 mM (equivalent to 3 – 620 mg C kg^{-1} soil in our study). Damage peaked above 1.65 mM GA (1,250 mg C kg^{-1} soil) but then decreased with further increases in GA concentration. However, the damage induced by GA was less than that induced by AA. The explanation for these concentration effects was again based on the reduction of Fe^{3+} to Fe^{2+} stimulating •OH formation and free radical damage to DNA at low concentrations, with the anti-oxidant •OH-scavenging activity of GA overcoming this process at higher concentrations. The concentrations at which Yen et al. (2002) report

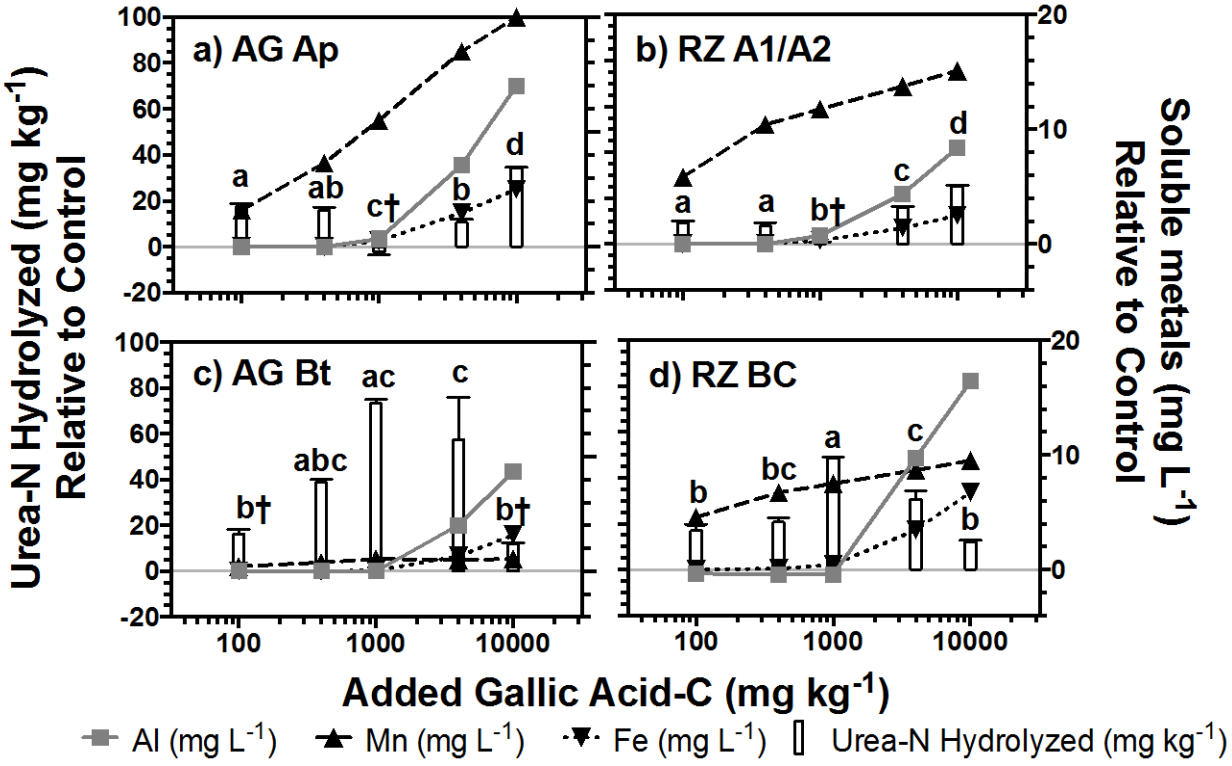


Figure 4.4: Effects of added gallic acid-C on urea hydrolysis and soluble metal concentration, relative to the control treatment, in each soil and horizon. Letters that are not different within the same soil horizon are not significantly different in the amount of urea-N hydrolyzed ($p < 0.05$). †Indicates treatment is not significantly different from the control treatment for that soil horizon. AG: agricultural field, RZ: riparian zone. SEM plotted (n = 3).

oxidative damage resulting from added GA again corresponded to our treatments of 100-1,000 mg C kg^{-1} soil. Again, the addition of 1,000 mg GA-C kg^{-1} soil was the treatment with the least urea hydrolysis in the AG Ap and RZ A1/A2 (not different from the control), which supports the conclusion that GA caused free radical formation and damage to microbial membranes and enzymatic DNA that resulted in decreased urea hydrolysis in these soils. Interestingly, both B horizons responded similarly to the addition of GA, in a manner that was opposite to the patterns measured in the A horizon soils. This may be explained by the substantially lower concentrations of Fe in solution in the GA-treated soils (25-70 mg kg^{-1} soil) compared with the AA-treated soils (390-1,000 mg kg^{-1} soil), and less corresponding oxidative damage associated with Fenton reactions (Eqn. 4.1). As a further benefit, GA has a greater free radical scavenging ability than AA (Alamed et al., 2009; Brewer, 2011). Less reductive dissolution of Fe(III) to Fe(II) would correspond to less free radical formation and less damage to DNA, with greater scavenging of the free radicals that did form. This may have resulted in enhancement of hydrolysis in the B horizons because there is a smaller microbial community in B horizon soils (Chap. 3), and less respiration would result in less free radical formation. In addition, Fe(III) is an inhibitor of jack bean urease (Zaborska et al., 2004). Jack bean urease is not an ideal model for soil urease (see Chap. 1), but it may indicate that extracellular urease could be more active in soil environments with low levels of free Fe(III). Therefore, while pro-oxidant effects may be the explanation for patterns of urea hydrolysis measured in the middle concentrations of added C in the A horizons, the enhancement in urea hydrolysis measured at the same concentrations of added GA-C in the B horizon soils may be a result of less overall Fe in solution compared to AA

treatments. The measured enhancement in urea hydrolysis may be the result of less Fe(III) in solution affecting extracellular urease or less Fe(II) in solution forming fewer free radicals. Since GA is a better free radical scavenger than AA, the net effect may be a system in which GA is able to lend other benefits to the system, such as metal chelation (Brown and Kelly, 2007), including that of potentially toxic Mn^{2+} that was in solution (Fig. 4.3).

Benzoic and Cinnamic Acids

In contrast to the resonance-stabilized O functional groups of ascorbic and gallic acids (Fig. 4.1), which makes them effective as reducing agents, benzoic (BA) and cinnamic acids (CA) are not redox-active. The BA and CA-treated soils maintained positions above the FeOOH-Fe^{2+} equilibrium line irrespective of added C (Fig. 4.3 b and d), indicating that the treatments did not result in the reductive dissolution of Fe^{2+} (Figs. 4.5 and 4.6). Neither BA nor CA are capable of acting as antioxidants (Kim and Lee, 2004). The enhancement of urea hydrolysis measured with additions of these C sources therefore appears to be a simple interaction between the increased availability of a bioavailable C source for heterotrophic microbes and the negative effects associated with decreased pH at the highest levels of added C (Appendix D; Figs. 4.5 and 4.6). Additions of BA to the AG Ap soil resulted in an increase in urea hydrolysis (Fig. 4.5). This was also true for the RZ A1/A2 soil, except that at the highest level of C added (10,000 mg C kg^{-1} soil), the enhancement decreased to that measured upon addition of 1,000 mg C kg^{-1} soil (Fig. 4.5b). This maximum rate of urea hydrolysis at 4,000 mg kg^{-1} added BA-C in

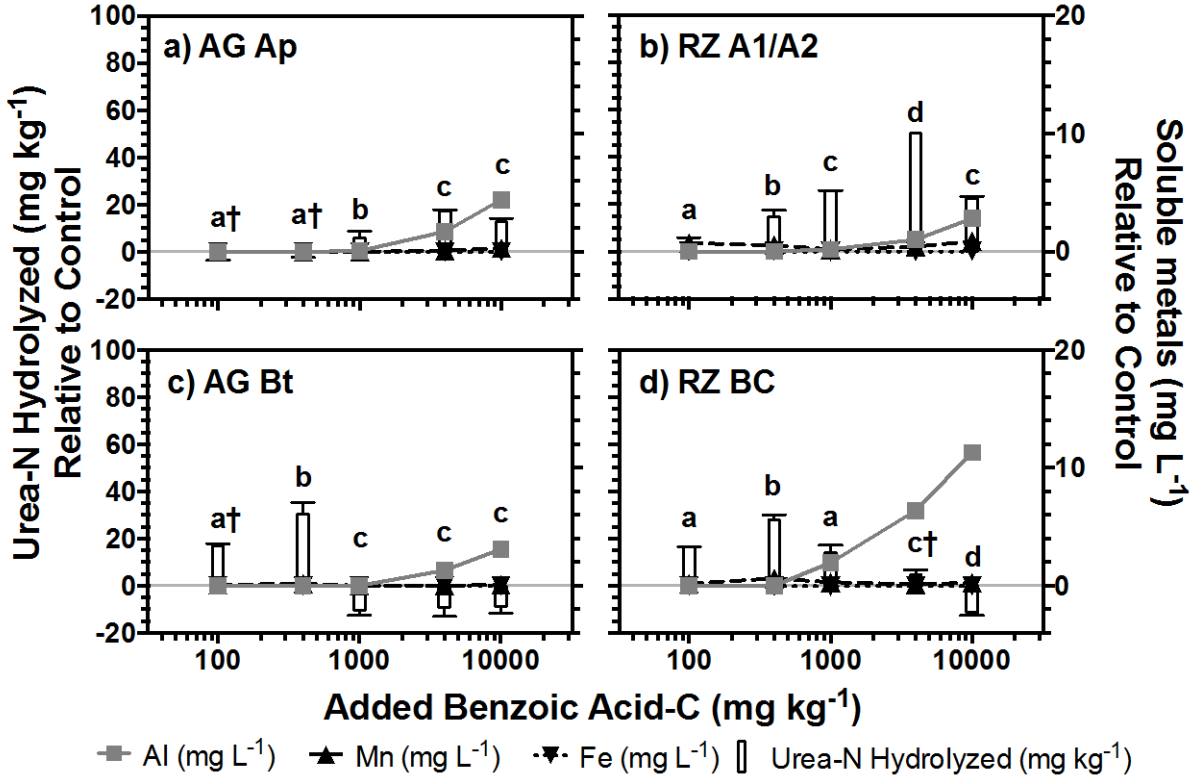


Figure 4.5: Effects of added benzoic acid-C on urea hydrolysis and soluble metal concentration, relative to the control treatment, in each soil and horizon. Letters that are not different within the same soil horizon are not significantly different in the amount of urea-N hydrolyzed ($p < 0.05$). †Indicates treatment is not significantly different from the control treatment for that soil horizon. AG: agricultural field, RZ: riparian zone. SEM plotted (n = 3).

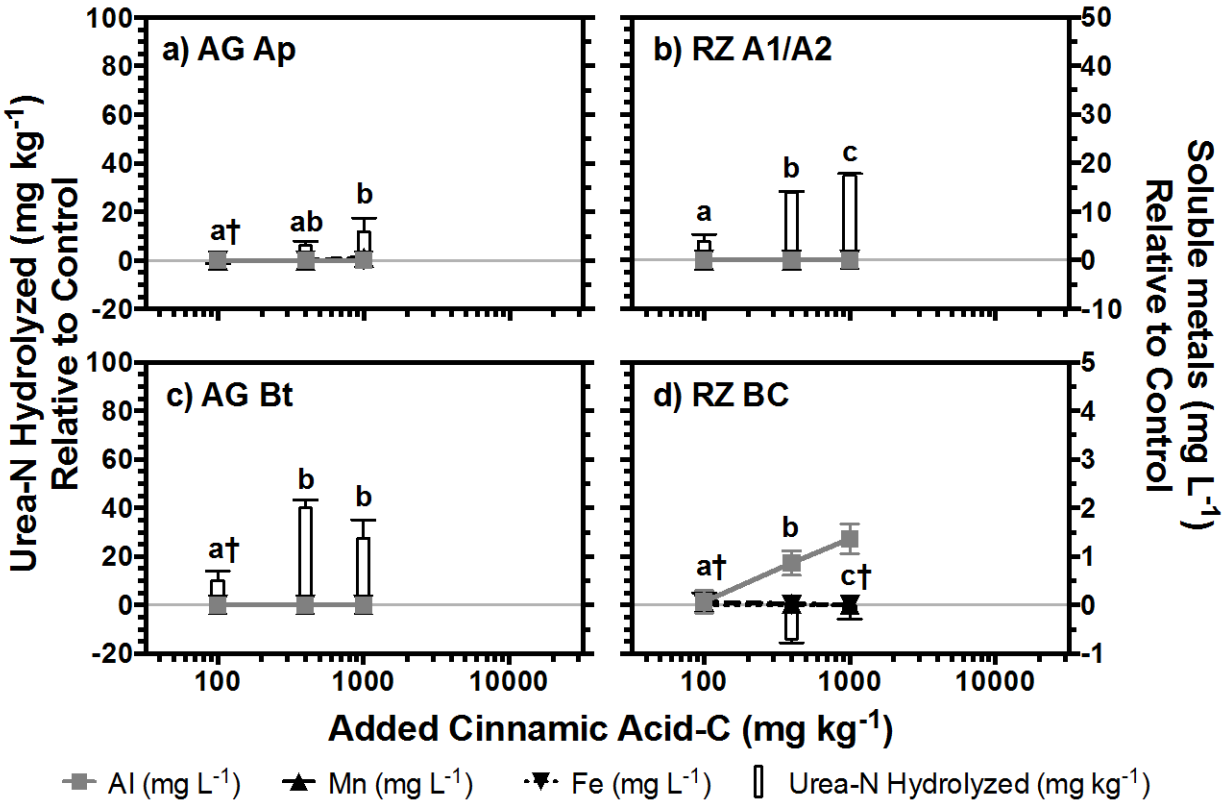


Figure 4.6: Effects of added cinnamic acid-C on urea hydrolysis and soluble metal concentration, relative to the control treatment, in each soil and horizon. Letters that are not different within the same soil horizon are not significantly different in the amount of urea-N hydrolyzed ($p < 0.05$). †Indicates treatment is not significantly different from the control treatment for that soil horizon. AG: agricultural field, RZ: riparian zone. SEM plotted (n = 3).

the RZ A1/A2 may have been due to a pH that fell to 3.0 (Appendix D), the lowest pH of all the BA-treated soils. At this level of soil acidity, the bacteria primarily responsible for urea hydrolysis in these soils (see Ch. 3) may be unable to function as a result of high concentrations of H^+ that protonate organic acid functional groups or cation exchange sites on soil and force unchelated Al^{3+} into solution. The lower levels of Al present in this horizon (Fig. 4.5b) compared to the others tested (Fig. 4.5) may explain why hydrolysis continued, even under these conditions of low pH. At soil pH values below 5.5, unchelated Al^{3+} present in the soil solution can bind with soil P and reduce the bioavailability of this essential nutrient (Haynes and Mokolobate, 2001). While ascorbic and gallic acids both are capable of metal chelation (Brown and Kelly, 2007; Brewer, 2011), the chelating ability of benzoic and cinnamic acids were not found in the literature. Therefore, the soils treated with cinnamic and benzoic acids reached pH values below 3.5, possibly without the addition of a C source capable of chelating the Al that became soluble under these conditions. The greater enhancement in urea hydrolysis in the RZ A1/A2 compared with the AG Ap, may be due to a larger and more diverse microbial population (see Chap. 3) capable of metabolizing BA, or a broader range of natural organic acids present in the soil that conferred some protection against metal toxicities (Haynes and Mokolobate, 2001).

In B horizon soils (Fig. 4.5 c and d), the enhancement in urea hydrolysis occurred at lower levels of added BA-C (400 mg kg^{-1} in AG Bt and 100-1000 mg kg^{-1} in RZ BC). This effect may be the result of adding a labile C source to soil with lower total C (Table 4.1), the stimulatory effects of which reached a threshold after 400 mg C kg^{-1}, when increased free Al^{3+} in solution slowed or inhibited hydrolysis (Fig. 4.5 c and d). These

effects may not have been observed in the A horizon soils as quickly due to the Al-chelating effects of higher total C in those soils. BA is metabolized by microbes and can be converted into several other metabolic compounds including succinate and acetyl CoA, pyruvic acid, and acetaldehyde (Gibson, 1968). There are both aerobic and anaerobic pathways for the microbial catabolism of BA (Ismail and Gescher, 2012).

The addition of CA to soil resulted in a similar pattern of urea hydrolysis enhancement (Fig. 4.6) as seen with added BA (Fig. 4.5). Although the highest concentrations of added C could not be added in a solution of CA due to its low solubility, the addition of 400 and 1,000 mg C kg^{-1} soil increased hydrolysis in the AG Ap and Bt as well as the RZ A1/A2 soils (Fig. 4.6 a, b, and c). The only difference observed in the RZ BC soil was some inhibition of hydrolysis with the addition of 400 mg C kg^{-1} soil. This was the only soil with measureable soluble Al, and the lowest pH range (3.8-4.0) across the range of C additions, which could have adversely affected microbial hydrolysis of urea by the presence of free Al^{3+} and possibly a reduction in the availability of P, as discussed earlier. Again, there was greater enhancement in the AG Bt (Fig. 4.6c) compared to the AG Ap (Fig. 4.6a), possibly due to the addition of a labile C source to the low-C AG Bt soil.

Our findings for cinnamic acid agree with those of Lin et al. (2011), who found that the application of cinnamic acid at a rate of 360 mg C kg^{-1} soil stimulated urease activity 56% over the control. This stimulation was much higher than that measured in our soils at approximately the same C application rate (Fig 4.6), although they used re-wetted air-dried soils, which may have resulted in the amplification of the effect (Bartlett and James, 1980). The soils studied by Lin et al. (2011) increased in both microbial C

and microbial respiration as a result of CA treatment. CA is a byproduct of the deamination of phenylalanine, which is mediated by the enzyme phenylalanine ammonia lyase, which is found in many higher plants, fungi, and yeasts. CA can be utilized in the synthesis of lignin (Hoskins, 1984), or metabolized to benzoic acid before being converted to catechol and other metabolic compounds (Gibson, 1968).

Conclusions

Urea-N fertilizer is used extensively in agricultural settings, and the rate of its hydrolysis in soils has been tied to the total organic C content of the soil. Analysis of soils from A and B horizons of both agricultural and riparian soils treated with a range of added C in the form of ascorbic, gallic, benzoic, or cinnamic acid allowed us to investigate the effects of specific C fractions and concentrations on urea hydrolysis. We found that ascorbic and gallic acids exhibit both oxidative and antioxidant effects in soils, which resulted in both increased and decreased urea hydrolysis, depending upon concentration. The effects were similar across both agricultural and riparian soils, but differed between A and B horizons. These differences were likely due to interactions between native C content of the soil and soluble metal activity. In contrast to the complicated effects of added ascorbic and gallic acids, benzoic and cinnamic acids appeared to have less complex effects in the soils. The measured enhancement of urea hydrolysis for these sources of C were likely a result of the addition of a labile C source that enhanced microbial activity and urea hydrolysis up to a threshold at which pH dropped and metals came into solution. Further studies will need to be completed to

confirm the mechanisms behind the observed patterns, but this study provides important preliminary information for explaining how different fractions of soil C could have differing effects on important microbial processes in soils.

Chapter 5: Urea hydrolysis in soils: pedological, chemical, and microbial controls

Maryland's Coastal Plain soils: an exception to the general knowledge on urea hydrolysis?

Urea is the most popular type of N fertilizer used worldwide, in part because it has a high N content (46% by weight) and is easy to transport. Plants can take up urea in its unhydrolyzed form or after its conversion to NH_3/NH_4^+ by soil microorganisms. This conversion, or hydrolysis reaction, takes place in soils through the action of both extracellular urease enzyme and the live microbial community. Most agronomic research has focused on the rate of urea hydrolysis in agricultural soils to better understand and control for the potential loss of N by volatilization of NH_3 or the loss of NO_3^- following nitrification by soil microorganisms. In addition, there has been extensive research into developing urease inhibitors that are able to slow the action of the urease enzyme, thereby reducing the rate of urea hydrolysis and allowing for increased plant uptake of the N supplied by this type of fertilizer. The paucity of information in the literature on the activity of unhydrolyzed urea in soils reflects this focus on N movement as NH_3 or NO_3^-. In many soils, this focus is reasonable because the rates of urea hydrolysis are high enough to result in conversion to ammonia in 1-3 days. This appears to be the case for the soils used in this dissertation research that were sampled from the Piedmont region in Maryland. However, coastal areas that are dominated by coarsely textured sandy soils may behave differently. Not only do these soils tend to be lower in carbon, lower in surface area, and possibly support smaller microbial communities, they can have high infiltration rates as well as poor buffering capacity that result in soil pH values between 3

and 6. Agricultural fields in these areas are often farmed and fertilized within a few dozen meters of surface waters or drainage ditches leading to surface waters. Grassed or riparian buffers often lie between farm fields and surface waters, but rates of urea hydrolysis soils from these landscape positions have not been investigated in detail. Therefore, it is unknown whether urea that moves off of agricultural fields would be subject to faster, slower, or comparable rates of hydrolysis in these bordering landscape soils. Since micromolar concentrations of urea can result in harmful algal blooms in surface waters, concentrations of N that agronomists consider inconsequential in agricultural applications have the potential to negatively affect aquatic environments. Therefore, it is imperative that the disciplines of agronomy and aquatic ecology learn to better understand the subtleties of each other's discipline so that the importance and implications of these kinds of differences can be appreciated. In particular, the dynamics of urea fertilizer in sandy soils in humid temperate regions such as the mid-Atlantic region of the United States deserve special attention and should not be directly compared to soils from other regions that have different physical and chemical characteristics.

Addressing gaps in the literature: important findings of this dissertation research

The research presented in this dissertation addresses gaps in the already extensive literature on urea hydrolysis by providing data on how hydrolysis changes across an entire agricultural landscape, including those positions outside of the agricultural field and along the possible flow paths to nearby surface waters. Surface runoff from an agricultural field would have to cross grassed and/or forested riparian buffers before

reaching surface waters, and any water that leaches through the A horizons and into B horizons would have to move laterally through these deeper soil horizons along flow paths to surface waters. Much research in agronomy and soil science has focused on the dynamics of urea hydrolysis in the upper 15 cm of agricultural soils. This is appropriate for many areas in which rapid infiltration is not a concern and where surface waters are not in close proximity to agricultural fields. However, sandy Coastal Plain soils such as those on the Eastern Shore of Maryland can exhibit rapid infiltration, and the research presented in this dissertation shows that urea that leaches below the microbially rich agronomic root zone (0-15cm) has a greater potential to remain in the soil without being converted to NH_3/NH_4^+. This is because both the C content and the size of the microbial community decreases with depth in the soil profile. Cooler soil temperatures found deeper in soil profiles can also slow this process. Since this dissertation research was conducted in the laboratory and focused on mechanisms associated with rates of urea hydrolysis in soils, field-based research is necessary to confirm these mechanisms and to determine whether rates of runoff or groundwater movement are likely to result in the delivery of unhydrolyzed urea to nearby surface waters.

To address the question of overland flow of urea-containing runoff, we investigated dynamics of hydrolysis in A horizons across agricultural landscapes. Our data indicate that rates of hydrolysis in A horizons do not vary significantly across the landscape in the Coastal Plain, but are higher in A horizons than in B horizons (Chap. 2). In addition, riparian zone BC horizon soils hydrolyzed urea faster than soils sampled from the B horizons in the other two landscape positions, likely as a result of the presence perennial vegetation and the higher numbers of associated microbes in this soil. These

findings indicate that landscapes that include forested riparian zones may be hydrolyzing more urea than landscapes that do not. In addition to providing erosion control, the faster rates of hydrolysis in the lower mineral horizons of these zones may provide a buffer strip in which urea that runs off of or percolates out of agricultural fields is converted to NH_4^+. The riparian zone A horizons soils had delayed nitrification (Fig. 2.2c), probably as a result of low pH. Therefore, the conversion to NH_4^+ would mean that N would have the potential to be retained, at least temporarily, on cation exchange sites, possibly allowing time for perennial plants to take up NH_4^+ and incorporate it into more complex and less-easily leached forms of organic N. Field-based research investigating surface runoff and groundwater leaching from agricultural fields, as well as studies investigating nutrient uptake dynamics in riparian buffers, the age at which newly planted buffers begin to provide these benefits, the types of trees or plants that are most effective at building a ureolytic microbial community, and ways in which nutrients may be harvested and removed from the system to reduce N movement to surface waters, would be important follow-up work.

Despite the fact that urea hydrolysis in soils is the product of past and present microbial communities, very little work has been done to understand how microbial community dynamics influence urea-N cycling in soils. While many organisms are known to carry the urease enzyme, little is understood about which groups of microbes are responsible for the majority of the urease activity in soils. Since members of the bacterial, archaeal, and fungal communities are known to contain the urease gene, knowledge about which microbial community is most influential for this process is important for improving N cycling in degraded soils and managing landscapes for the

efficient use of this type of popular fertilizer. This work found that ureolytic bacteria were largely responsible for rates of hydrolysis in native and pH-adjusted soils sampled from a soil profile toposequences on the Coastal Plain of Maryland, and that the *ureC* gene may be a useful biomarker for predicting rates of urea hydrolysis in soils. This work will hopefully open the door for additional studies of microbially mediated urea hydrolysis in more soils from different landscapes. If found to apply on a larger scale than investigated in this dissertation research, the quantification of *ureC* may be a useful biomarker for predicting rates of urea hydrolysis in soils and could potentially be used to identify "agricultural fields of concern" in which urea hydrolysis is slow. With proper field studies to develop it, a quick test could be established using *ureC* as the variable-of-interest to identify the "problem sites" most likely to be leaching urea to surface waters. Recommendations could then be tailored specifically to the operation managing those hectares to improve urea hydrolysis within the bigger picture of N biogeochemical cycling in those soils. In addition, further work is necessary to understand the conditions under which ureolytic microbes express the urease gene. In soils, it is largely unknown whether urease is induced by the presence of urea as a substrate, repressed by elevated concentrations of its product, NH_3/NH_4^+, or continually expressed irrespective of environmental conditions. Knowing more about these processes would help managers understand how to manage soils to promote or reduce the activities of certain broad groups of microorganisms, and possibly how to manage agricultural fertilizer applications for specific outcomes. For example, knowing that bacteria are mostly responsible for urea hydrolysis in soils could lead to management of pH or plant species known to promote the growth and activity of these organisms.

Soil C is an important predictor of rate of urea hydrolysis, but whether this is tied to microbial biomass C, labile C fractions, antioxidant C, good chelating C, or other factors is unknown. Chap. 4 showed that both labile C and antioxidant C stimulate urea hydrolysis, likely by different mechanisms, but that the effect is concentration-dependent. The combined influence of plant exudates and other rhizosphere C sources, below-ground biomass decomposition, above-ground C inputs through leaf litter, fallen wood, animal excrement and biomass decomposition provide a complex mixture of C to the soil environment and makes analysis of C effects on urea hydrolysis difficult to parse out. The research in this dissertation singled out four different C sources to understand their individual effects on urea hydrolysis in natural soils. With further research on other C sources, we will begin to have a database for concentrations at which enhancement and inhibition of urea hydrolysis is likely, which may be helpful in applied settings. For example, in housing developments where A horizons have been stripped back for construction purposes and only partially or incompletely restored, B horizon soils are exposed and expected to grow lawns and other perennial vegetation. As presented in this dissertation, B horizons hydrolyze urea much more slowly than A horizons. However, the amendment of these soils with labile C fractions, such as the gallic acid treatments shown in Chap. 4, can greatly enhance urea hydrolysis in B horizon soils, and possibly start to build back the microbial biomass C that is essential for proper N cycling. Knowledge of which types of C will promote these processes most rapidly will help landscape architects and managers restore these landscapes, which will result in greater plant establishment, less soil erosion, and the restoration of biogeochemical N cycling. These principles can also apply to mine reclamation sites or other projects in which soils have been severely

impacted and are in the process of being restored to functional ecosystems. Knowledge of C exuded by plants or that are present in decomposing plant material would also help guide decisions for the types of crops to plant in agricultural crop rotations or in vegetated buffer strips adjacent to agricultural fields to improve urea-N cycling across an agricultural landscape.

Implications of this research for agronomy and water quality

Assuming that the mechanisms explored in this dissertation research apply on a field scale, the agronomic implications for Coastal Plain-type agriculture would vary depending on the goals and field conditions of different agricultural operations. As previously mentioned, a urea hydrolysis "quick test" could be investigated in more detail for future development, which would enable landscape nutrient managers to identify "fields of concern" for targeted application of new technology and best management practices. Assuming that the application of urea fertilizer to sandy soils adjacent to surface waters would be the fields in which these recommendations would be focused, increasing hydrolysis without losing N to NH_3 volatilization would be a goal. Recommendations for this type of situation would likely include 1) applying urea-N as late as possible in the spring, once soil had warmed up and the rate of hydrolysis had increased (see Appendix E for temperature effects on urea hydrolysis), 2) splitting applications of urea fertilizer as much as possible to reduce loss of N as both urea and NH_3, 3) liming poorly buffered sandy agricultural fields more often to increase both pH and the rate of urea hydrolysis, 4) occasional liming of grassed buffers to keep hydrolysis

in this zone high, 5) injecting urea into the soil instead of applying it to the surface of soils where hydrolysis is fastest and risk of NH_3 volatilization is greatest, 6) possibly installing or expanding grassed or riparian buffer strips between agricultural fields and surface waters, 7) surface-applying fertilizer before light, but not heavy, rain, to increase movement of urea into the soil and below the surface where NH_3 loss is a concern, 8) increasing C content of soils by planting high residue crops and planting cover crops regularly, and 9) increasing specific fractions of soil C based on future research into the C content of both plants and their root exudates. While each of these recommendations may not be practical for all producers, it is important for nutrient management advisors, farmers, and agronomists to incorporate the dynamics of urea into the larger N cycle to understand how management changes would impact the hydrolysis and movement of this form of N in addition other, more commonly managed forms of N such as nitrate-N.

Conclusions

Maryland's sandy Coastal Plain soils are dominated by agriculture, and their physical and chemical properties may make them more susceptible to loss of unhydrolyzed urea-N fertilizer than other, more intensively studied soils from other regions of the U.S. In addition, the proximity of surface waters in this region means that heavy rain may result in the loss of urea via surface runoff. The high infiltration rates of these sandy soils may also make it more possible for urea to percolate into B horizons without being hydrolyzed. Once in B horizons, the movement of unhydrolyzed urea to surface waters may be possible. The likelihood of these routes of urea movement cannot

be confirmed without field-based studies that include the actual measurement of percolation, infiltration, leaching, and runoff from agricultural fields. However, the data in this dissertation indicate that the mechanisms influencing urea hydrolysis in different soil horizons make it possible for hydrolysis to be delayed, and provides justification for future field-based studies. Whether these mechanisms continue to function against a background of variable temperature, microbial community distribution, macropore flow, pH, infiltration, wetting and drying cycles, and organic matter decomposition, remains to be seen. However, greater attention to regional differences in soil chemical and physical factors is warranted for evaluating urea-N dynamics in soils. In addition, the proximity of sensitive aquatic environments and the rates of movement of both surface and groundwater should be evaluated on a field scale. It would appear that "one size does not fit all" in terms of the dynamics of urea fertilizer application to soils, and careful comparisons must be made between studies to maximize our understanding of these dynamics.

Appendix A: Soil sampling locations

Figure A.1: Transect sampling points at the Wye Island Natural Resource Management Area within the Coastal Plain physiographic province, consisting of an agricultural field (AG), a grassed field border (GB), and a riparian zone (RZ). The red transect was sampled in October, 2012 and the blue transects were sampled in October, 2013. AG soil sample was from the Ingleside mapping unit (IgB) (38°54'11.97"N, 76° 8'12.20"W), the GB soil was from the boundary of Ingleside and Longmarsh-Zekiah (LZ) mapping units (38°54'10.37"N, 76° 8'13.79"W), and the RZ soil was from the Longmarsh- Zekiah mapping unit (38°54'9.98"N, 76° 8'14.70"W); all of which were similar to the Ingleside series (coarse-loamy, siliceous, mesic Typic Hapludult). Photo obtained using Google Earth (Version 7.1.2.2041).

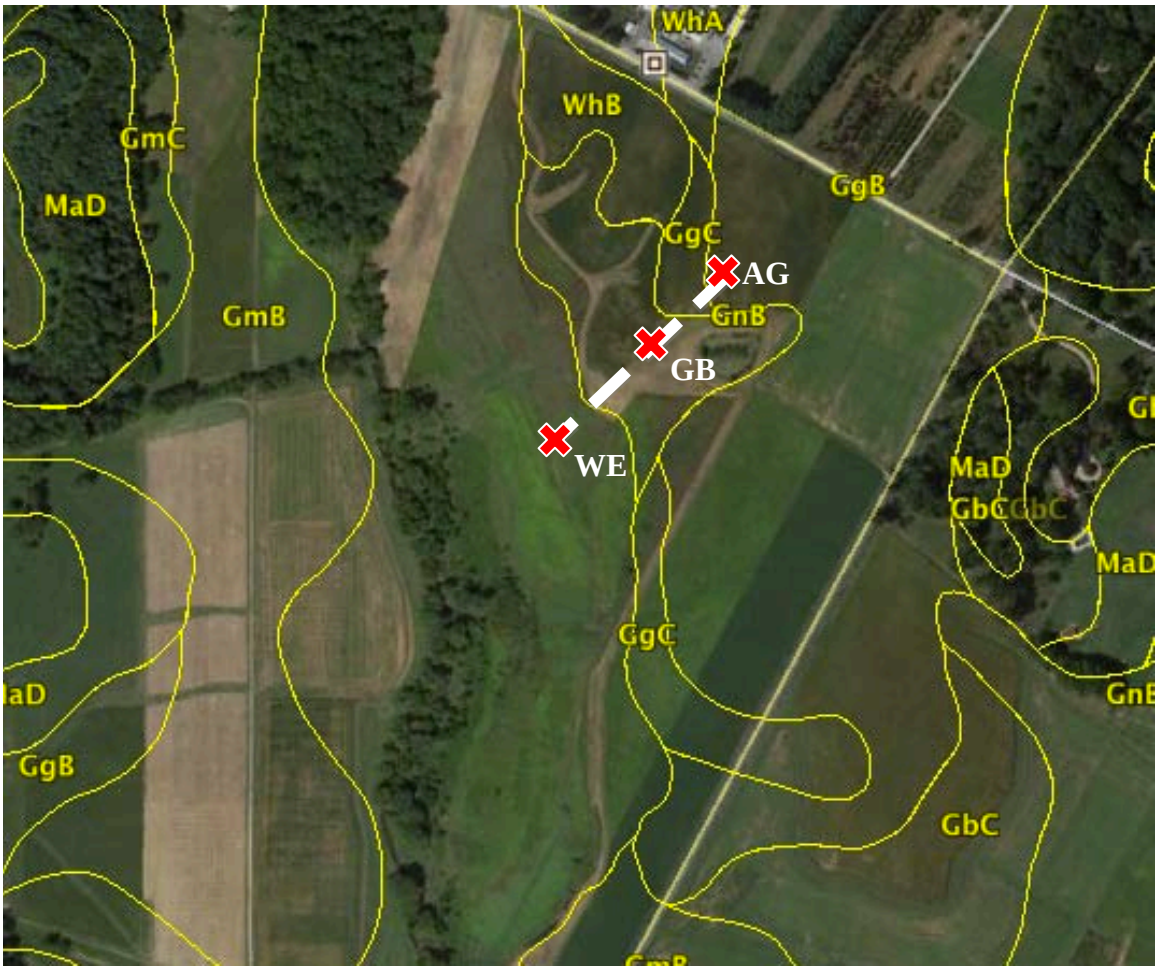


Figure A.2: Transect sampling points at the Central Maryland Research and Education Center, Clarksville Facility, in the Piedmont physiographic province consisting of an agricultural field (AG), a grassed field border (GB), and a wetland edge (WE). The AG soil sample was from the Glenelg unit (GgB) and the GB soil was from the Glenville-Baile unit (GnB); both similar to the Glenelg series (fine-loamy, mixed, semiactive, mesic Typic Hapludults). The WE soil was from the Hatboro-Codorus unit, similar to the Codorus series (fine-loamy, mixed, active, mesic Fluvaquentic Dystrudept). Photo obtained using Google Earth (Version 7.1.2.2041).

Appendix B: Additional data and information related to findings in Chapter 2

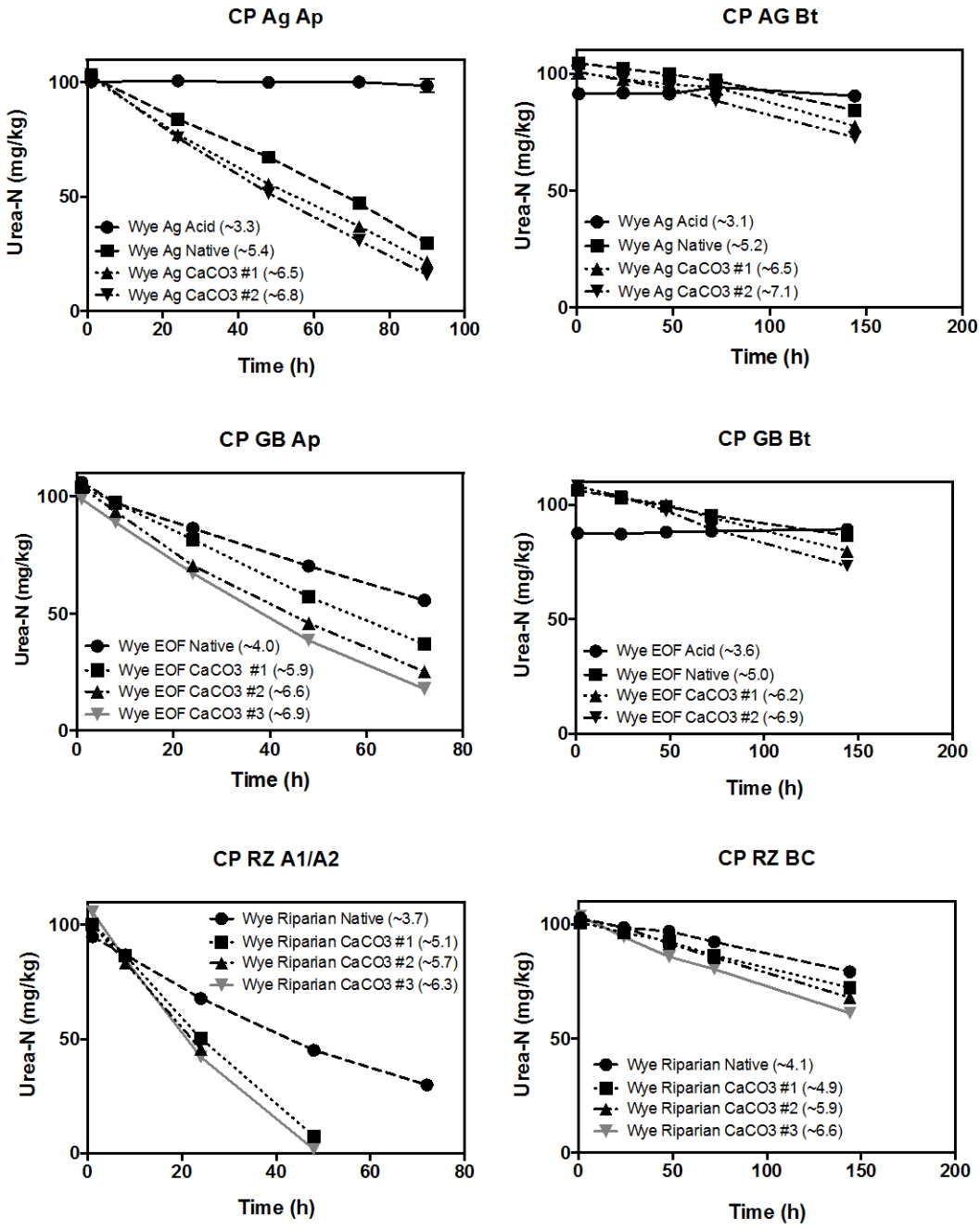


Figure B.1: First-order kinetics in Coastal Plain (CP or Wye) pH-manipulated transect soils, consisting of an agricultural field (AG), a grassed field border (GB), and a riparian zone (RZ) sampled from both A and B horizons. SEM plotted (some error bars are within symbols), n=3.

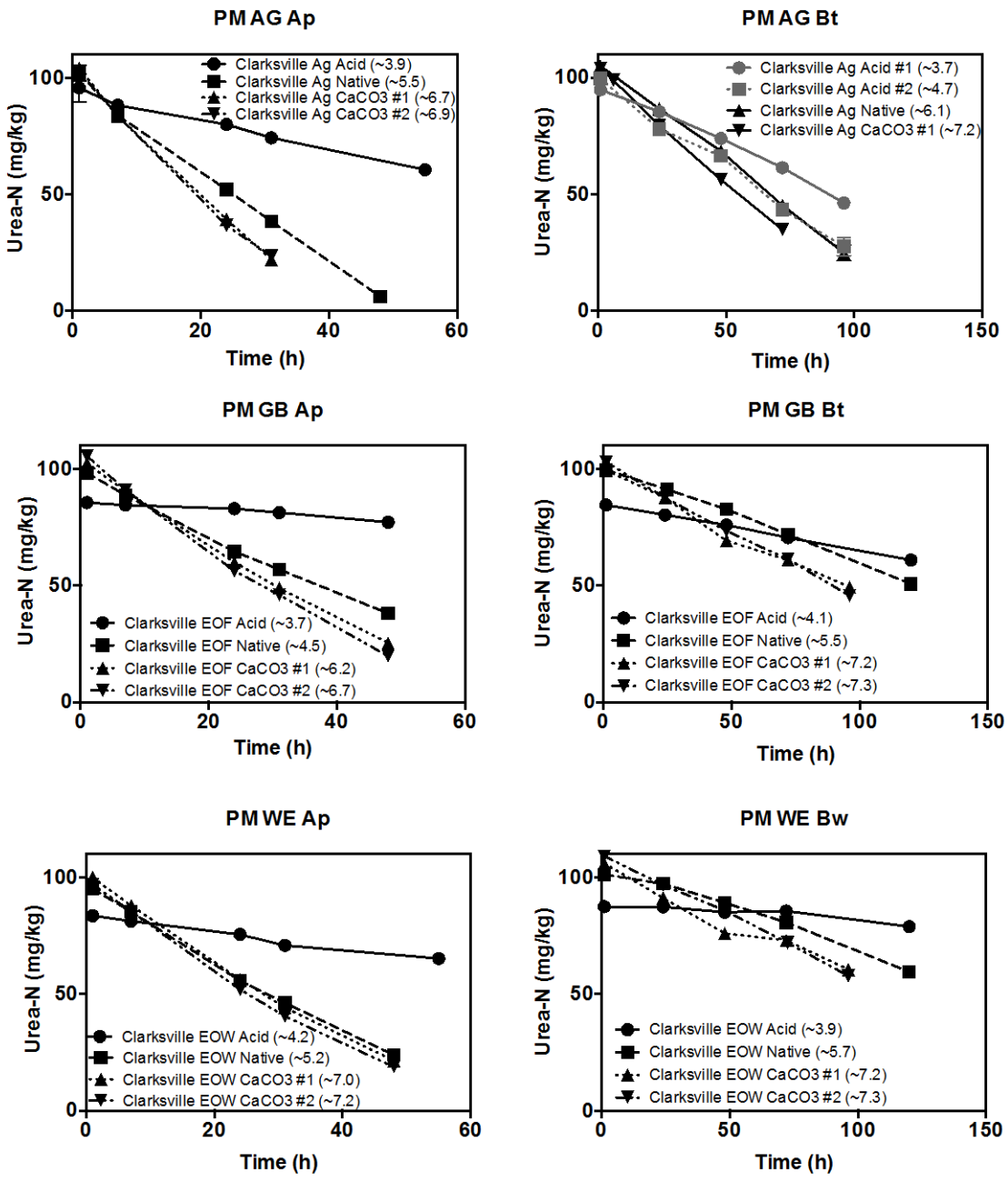


Figure B.2: First-order kinetics in Piedmont (PM or Clarksville) pH-manipulated transect soils, consisting of an agricultural field (AG), a grassed field border (GB), and a wetland edge (WE) sampled from both A and B horizons. SEM plotted (some error bars are within symbols), n=3.

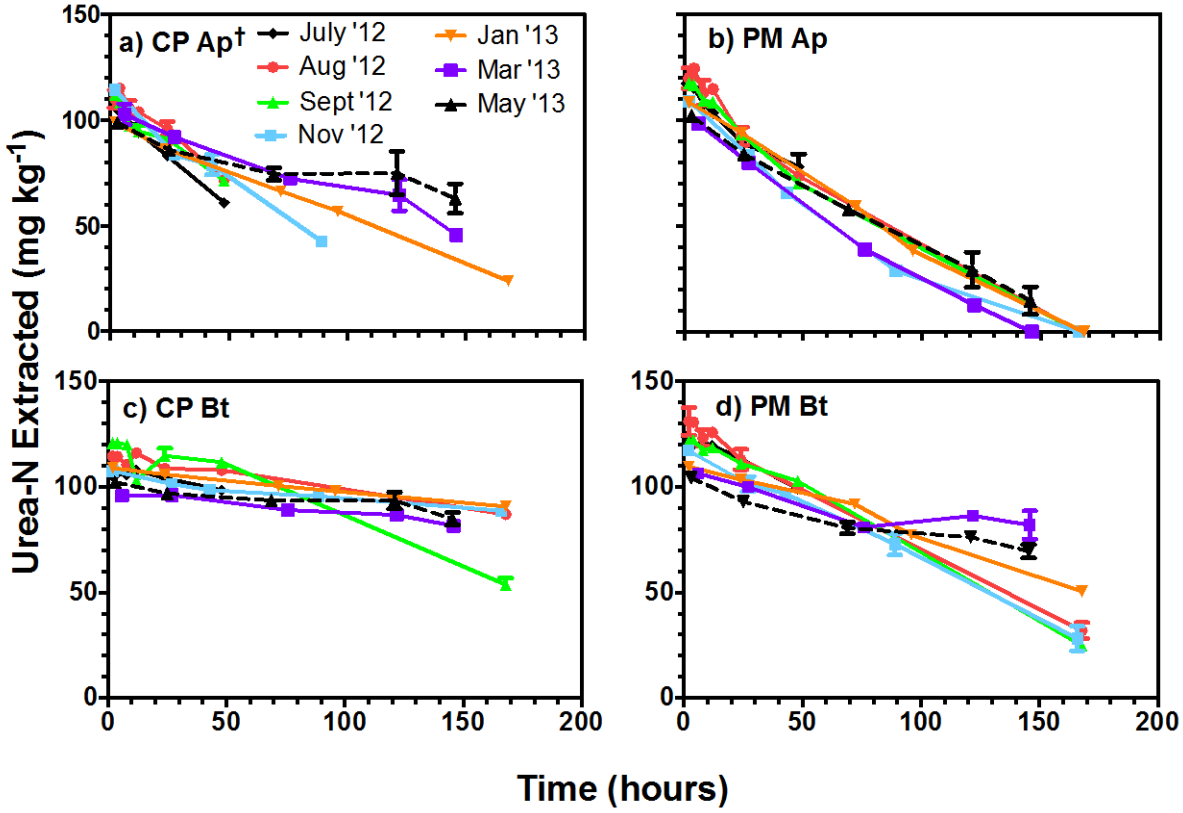


Figure B.3: Soils sampled in July 2012 from the Ap and Bt horizons of grassy areas near agricultural fields at both the Piedmont (PM) and Coastal Plain (CP) sites were used to monitor the stability of urea hydrolysis rate in soils stored field-moist in the laboratory (†Due to differences in field sampled moisture content, final concentrations of added N varied; CP Ap received 113 mg urea-N kg^{-1} soil, CP Bt received 110 mg urea-N kg^{-1} soil, PM Ap received 114 mg urea-N kg^{-1} soil, and CP Bt received 121 mg urea-N kg^{-1} soil

Table B.1: Selected physical and chemical properties of soils sampled in 2013 along three transects consisting of an agricultural field (AG), a grassed field border (GB), and a riparian zone (RZ) in the Coastal Plain.

Transect†	Horizon	Depth (cm)	Sand	Silt	Clay	PSD ‡	pHs	H2O	C	N	C:N	Mn	Al	Ca	Mg	K	Fe	P
			----------%----------					-----g kg-1 soil----				----------mg kg-1 soil----------						
AG 1	Ap	0-15	64.4	25.4	10.3	SL	4.3	127	4.9	0.30	16	1.0	61	242	71	61	1.7	16
GB 1	Ap	0-15	52.2	34.0	13.8	L	4.6	178	11	0.80	14	1.1	52	532	138	100	5.6	21
RZ 1	A1/A2	0-15	51.2	34.9	13.9	L	3.7	195	20	1.5	14	8.2	179	359	89	42	20	35
AG 2	Ap	0-15	57.3	32.2	10.6	SL	5.3	160	7.7	0.50	15	1.0	34	415	122	100	0.6	10
GB 2	Ap	0-15	71.8	19.6	8.7	SL	3.7	130	8.6	0.60	14	4.0	100	195	61	28	13	18
RZ 2	A1/A2	0-15	54.5	34.4	11.2	SL	3.3	138	21	1.2	17	3.3	210	164	27	49	23	48
AG 3	Ap	0-15	59.9	30.0	10.2	SL	5.3	154	7.7	0.59	13	0.9	52	393	125	61	0.9	25
GB 3	Ap	0-15	48.4	36.0	15.7	L	4.7	161	9.0	0.70	13	1.2	45	579	140	57	3.1	41
RZ 3	A1/A2	0-15	24.1	56.1	19.9	SiL	4.5	249	21	1.8	12	3.2	110	782	200	91	4.0	28
AG 1	Bt	45-60	68.4	16.1	15.6	SL	4.9	134	1.3	0.03	43	0	56	541	88	25	2.5	17
GB 1	Bt	45-60	48.4	31.6	20.0	L	4.8	170	1.7	0.10	17	0	86	715	144	32	3.0	32
RZ 1	BC	45-60	55.2	29.9	15.0	SL	4.1	104	3.7	0.28	13	1.2	119	543	170	18	3.8	26
AG 2	Bt	45-60	48.6	29.4	22.1	L	5.0	162	1.5	0.07	21	0	77	696	128	25	2.6	17
GB 2	Bt	45-60	63.8	21.0	15.3	SL	4.1	140	1.7	0.05	35	0.7	150	459	163	33	3.5	22
RZ 2	BC	45-60	73.9	20.4	5.8	SL	3.6	68.3	4.2	0.16	26	0.8	170	18	16	21	7.9	48
AG 3	Bt	45-60	58.3	22.3	19.4	SL	4.8	156	1.7	0.13	13	0	96	587	122	28	3.4	110
GB 3	Bt	45-60	50.9	30.8	18.4	L	5.4	157	1.8	0.09	20	0	65	660	143	27	2.9	46
RZ 3	BC	45-60	54.8	37.0	8.2	SL	4.0	76.7	6.2	0.39	16	2	170	173	41	34	7.3	44

†Transects sampled in 2013 consisting of an agricultural field (AG), grassed field border (GB), and riparian zone (RZ) and numbered 1 – 3.
‡Particle size distribution

Table B.2: Date for rates of urea hydrolysis in native Coastal Plain A and B horizon soils plotted in Figure 2.1. Three replicates per soil for each time are listed from the AG (agricultural field), GB (grassed field border), and RZ (riparian zone) transect points sampled in 2013. Data for the 2012 transect is included in Tables B.2 and B.3.

	2013 AG Ap								
Time (h)	Transect 1			Transect 2			Transect 3		
	----------mg urea-N kg-1 soil----------								
1	104.7	103.9	112.9	106.3	105.3	103.4	103.0	103.5	104.8
5	105.9	100.0	101.3	104.7	102.8	101.3	100.2	104.7	105.6
20	96.6	95.3	94.8	89.1	86.9	85.8			
24							94.598	96.581	96.146
48	87.4	86.8	86.2	61.0	65.6	64.8	82.6	87.1	89.5
72	92.8	76.3	81.9	38.3	34.0	33.5	68.6	68.1	74.0
	2013 GB Ap								
1	102.1	104.5	103.7	95.2	101.6	101.3	105.1	107.2	106.3
5	97.6	97.4	97.3	98.0	101.3	95.7	97.2	104.9	102.1
24	62.8	71.0	67.3	87.9	94.4	89.8	69.7	73.0	70.1
48	32.8	29.7	31.7	72.2	77.8	72.7	34.7	37.3	41.2
72	0.0	0.0	0.0	59.6	64.5	62.4	0.0	0.0	8.7
	2013 RZ A1/A1								
1	90.4	89.3	84.9	92.4	88.7	87.7	96.2	96.2	95.4
5	87.9	84.0	83.1	85.7	82.5	86.1	84.2	85.4	83.0
24	63.5	63.1	56.2	60.5	63.8	59.8	27.7	27.0	27.4
48	38.4	32.8	34.5	35.1	37.3	37.6	0.0	0.0	0.0
72	17.4	19.2	17.0	26.2	22.2	24.4			

Table B.3: Data for rates of urea hydrolysis in native Coastal Plain A horizon transect soils sampled in 2012 and plotted in Fig. 2.2. Three replicates per soil at each time are listed from the AG (agricultural field), GB (grassed border), and RZ (riparian zone) A horizon soils.

Time (h)	AG Ap			GB Ap			RZ A1/A2		
	----------mg urea-N kg^{-1} soil----------								
0	0	0	0	0	0	0	0	0	0
2	109	108	112	95.9	92.0	95.4	81.6	82.4	77.9
24	112	88.9	89.1	67.8	66.1	65.1	40.9	43.5	45.2
48	57.0	58.3	57.8	36.8	36.8	39.8	18.2	18.5	20.6
72	37.0	37.4	40.0	18.5	16.9	17.9	5.7	7.0	9.0
96	11.9	11.7	10.8	0.0	0.0	0.0	6.4	5.9	5.7
168	0	0	0	0	0	0	0	0	0
	----------mg NH_4^+-N kg^{-1} soil----------								
0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
2	2.5	1.7	2.8	2.7	2.8	2.9	10.4	5.2	5.6
24	15.6	16.9	15.7	24.8	24.6	25.4	51.1	48.2	46.6
48	30.0	30.4	30.8	45.3	43.5	42.3	73.4	77.1	81.1
72	40.5	41.7	40.6	61.9	60.6	60.8	95.4	92.9	91.4
96	48.3	48.1	48.1	70.7	70.1	73.8	98.3	103.5	103.7
168	11.4	12.6	13.1	61.8	59.2	62.5	110.6	119.9	113.7
	----------mg NO_3^--N kg^{-1} soil----------								
0	5.3	5.2	5.5	8.9	8.9	8.9	0.0	0.0	0.0
2	5.4	5.2	5.3	9.3	8.9	9.3	0.0	0.0	0.0
24	7.7	7.9	8.3	11.7	11.4	12.1	0.0	0.0	0.0
48	12.4	12.1	12.1	14.3	14.2	14.6	0.0	0.0	0.0
72	17.4	17.8	17.6	17.3	17.2	16.9	0.0	0.0	0.0
96	27.3	27.1	28.8	21.0	21.3	19.2	0.0	0.0	0.0
168	88.5	86.4	85.4	39.0	36.8	36.0	0.0	0.0	0.0

Table B.4: Data for rates of urea hydrolysis in native Coastal Plain B horizon transect soils sampled in 2012 and plotted in Fig. 2.3. Three replicates per soil at each time are listed from the AG (agricultural field), GB (grassed border), and RZ (riparian zone) B horizon soils.

Time (h)	AG Bt			GB Bt			RZ BC		
	----------mg urea-N kg^{-1} soil----------								
0	0	0	0	0	0	0	0	0	0
2	114	105	102	105	102	103	112	110	107
24	100	99.0	99.4	99.3	97.1	97.7	108	103	104
48	95.9	97.3	97.3	97.1	94.1	93.4	99.8	101	101
72	98.3	97.3	96.7	89.5	87.7	88.4	98.2	95.6	96.9
96	88.9	85.9	86.7	95.2	91.9	92.8	86.7	90.5	85.4
168	93.7	91.0	96.3	92.0	93.1	90.8	75.7	73.7	78.2
	----------mg NH_4^+-N kg^{-1} soil----------								
0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
2	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
24	0.0	0.0	0.0	0.0	0.0	0.0	7.8	8.9	8.0
48	6.3	2.8	3.4	6.1	6.0	6.5	15.4	15.4	15.9
72	5.4	4.3	5.1	14.1	13.1	14.1	19.9	22.4	19.7
96	11.6	12.0	12.0	7.2	8.4	6.9	29.6	27.2	30.2
168	7.8	8.3	7.7	12.9	12.8	13.7	41.2	43.4	37.6
	----------mg NO_3^--N kg^{-1} soil----------								
0	1.8	1.8	1.7	1.2	1.1	1.0	0.5	0.4	0.4
2	1.8	1.8	1.7	1.0	0.9	1.0	0.4	0.4	0.3
24	1.7	1.7	1.7	1.0	1.0	1.0	0.4	0.4	0.3
48	1.8	1.8	1.7	1.0	0.9	1.0	0.4	0.4	0.4
72	1.7	1.7	1.8	1.2	1.2	1.3	0.4	0.4	0.4
96	2.3	2.2	2.1	1.0	1.0	1.0	0.4	0.4	0.4
168	1.7	1.8	1.8	1.0	1.0	1.1	0.4	0.4	0.4

Table B.5: Data for rates of urea hydrolysis in native Piedmont A horizon transect soils, plotted in Fig. 2.2. Three replicates per soil at each time are listed from the AG (agricultural field), GB (grassed border), and WE (wetland edge) A horizon soils.

Time (h)	AG Ap			GB Ap			WE Ap		
	----------mg urea-N kg^{-1} soil----------								
0	0	0	0	0	0	0	0	0	0
2	92.9	96.4	95.2	94.2	93.4	98.3	89.3	90.2	90.1
24	32.3	36.5	36.7	46.1	42.0	41.9	41.2	39.7	44.9
48	0	0	0	0	0	0	5.41	6.42	5.11
72	0	0	0	0	0	0	0	0	0
96	0	0	0	0	0	0	0	0	0
168	0	0	0	0	0	0	0	0	0
	----------mg NH_4^+-N kg^{-1} soil----------								
0	7.6	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
2	5.8	6.5	7.1	6.0	5.2	5.4	3.2	3.3	3.6
24	43.1	41.9	42.4	40.7	43.6	42.8	27.2	24.8	24.5
48	52.3	50.1	55.1	65.7	64.6	64.5	33.9	33.8	35.1
72	35.6	34.0	34.4	56.3	58.3	59.0	25.0	26.1	28.1
96	15.4	14.3	19.2	51.4	52.1	52.8	15.5	13.5	13.7
168	0.0	0.0	0.0	27.3	29.1	31.9	0.0	0.0	0.0
	----------mg NO_3^--N kg^{-1} soil----------								
0	9.6	10.0	10.1	17.5	17.0	17.2	7.3	7.0	7.1
2	11.8	11.9	10.8	17.6	17.0	17.0	8.4	8.5	8.5
24	24.6	23.6	24.6	23.6	25.4	24.9	26.6	26.9	25.6
48	39.4	42.3	41.5	32.9	36.1	33.8	49.6	46.3	47.8
72	59.5	62.2	62.4	43.5	43.1	43.6	68.6	66.7	66.4
96	85.1	88.9	79.9	51.0	49.6	51.8	88.1	83.7	83.6
168	108.5	109.6	108.3	80.9	78.5	85.5	106.9	106.0	104.9

Table B.6: Data for rates of urea hydrolysis in native Piedmont B horizon transect soils, plotted in Fig. 2.3. Three replicates per soil at each time are listed from the AG (agricultural field), GB (grassed border), and WE (wetland edge) B horizon soils.

Time (h)	AG Bt			GB Bt			WE Bw		
	----------mg urea-N kg^{-1} soil----------								
0	0	0	0	0	0	0	0	0	0
2	98.3	97.5	96.8	96.3	96.4	99.2	97.4	97.1	99.9
24	82.9	84.8	82.9	87.6	89.6	90.8	87.9	85.6	88.6
48	68.7	67.7	64.6	76.3	75.9	79.8	77.1	75.6	75.7
72	55.9	50.2	45.5	69.0	66.3	64.9	66.0	66.5	62.1
96	22.7	22.7	24.3	53.3	53.3	51.3	55.5	54.6	51.9
168	0	0	0	26.4	20.2	26.8	8.06	12.8	6.41
	----------mg NH_4^+-N kg^{-1} soil----------								
0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
2	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
24	15.3	14.7	16.5	10.4	9.8	10.1	11.2	11.3	10.9
48	29.1	30.3	31.0	21.0	20.7	20.3	18.8	19.7	20.3
72	38.3	42.3	45.9	27.4	29.9	31.0	26.5	26.1	28.3
96	55.9	57.6	57.0	38.3	38.9	39.4	32.4	32.8	33.9
168	53.3	62.7	57.7	49.5	52.3	49.1	41.4	41.6	41.7
	----------mg NO_3^--N kg^{-1} soil----------								
0	2.7	2.9	2.9	1.2	1.3	1.3	3.6	3.5	3.4
2	2.5	2.4	2.3	1.2	1.1	1.2	3.4	3.8	3.5
24	3.7	3.7	3.8	2.0	1.7	1.8	5.2	5.7	5.3
48	5.2	5.5	5.3	3.9	3.3	3.1	7.6	7.7	8.4
72	6.6	6.8	7.1	4.1	4.9	4.6	9.7	9.0	10.4
96	10.8	10.1	10.3	6.6	6.2	6.5	11.8	12.2	11.3
168	19.7	17.2	19.0	11.3	13.8	12.9	23.3	19.3	21.6

Table B.7: Data for rate of urea hydrolysis in pH-manipulated Coastal Plain A horizon transect soils plotted in Fig. 2.4a. AG (agricultural field), GB (grassed border), and RZ (riparian zone) soils listed. Each mean and SEM derived from three replicates.

	AG Ap		GB Ap		RZ A1/A2	
	----------Rate of Urea Hydrolysis---------- (mg urea-N kg^{-1} soil h^{-1})					
pH	Mean	SEM	Mean	SEM	Mean	SEM
3.3	0.0172	0.0252				
3.7					0.926	0.0356
4.0			0.683	0.0290		
5.1					1.99	0.0388
5.4	0.807	0.0151				
5.7					2.38	0.0475
5.9			0.956	0.0120		
6.3					2.20	0.0933
6.5	0.891	0.0175				
6.6			1.11	0.0362		
6.8	0.974	0.0248				
6.9			1.15	0.0312		

Table B.8: Data for rate of urea hydrolysis in pH-manipulated Coastal Plain B horizon transect soils plotted in Fig. 2.4c. AG (agricultural field), GB (grassed border), and RZ (riparian zone) soils listed. Each mean and SEM derived from three replicates.

	AG Bt		GB Bt		RZ BC	
	----------Rate of Urea Hydrolysis----------					
	(mg urea-N kg^{-1} soil h^{-1})					
pH	Mean	SEM	Mean	SEM	Mean	SEM
3.1	0.004026	0.0159				
3.6			-0.0136	0.004115		
4.1					0.1632	0.0108
4.9					0.2008	0.006483
5.0			0.139	0.006216		
5.2	0.141	0.0133				
5.9					0.2453	0.005176
6.2			0.198	0.005868		
6.5	0.159	0.0264				
6.6					0.2891	0.0104
6.9			0.249	0.006093		
7.1	0.198	0.00922				

Table B.9: Data for rate of urea hydrolysis in pH-manipulated Piedmont A horizon transect soils plotted in Fig. 2.4b. AG (agricultural field), GB (grassed border), and WE (wetland edge) soils listed. Each mean and SEM derived from three replicates.

	AG Ap		GB Ap		WE Ap	
	----------Rate of Urea Hydrolysis---------- (mg urea-N kg^{-1} soil h^{-1})					
pH	Mean	SEM	Mean	SEM	Mean	SEM
3.7			†0.169	0.0196		
3.9	0.616	0.04486				
4.2					†0.348	0.0341
4.5			1.29	0.0366		
5.2					1.53	0.0563
5.5	1.99	0.05078				
6.2			1.65	0.0382		
6.7	2.70	0.06134	1.83	0.0485		
6.9	2.67	0.07144				
7.0					1.69	0.0407
7.2					1.69	0.0531

†These values excluded from linear regression plotted in Fig. 2.2b because they are not linear.

Table B.10: Data for rate of urea hydrolysis in pH-manipulated Piedmont B horizon transect soils plotted in Fig. 2.4d. AG (agricultural field), GB (grassed border), and WE (wetland edge) soils listed. Each mean and SEM derived from three replicates.

	AG Bt		GB Bt		WE Bw	
	----------Rate of Urea Hydrolysis----------					
	(mg urea-N kg^{-1} soil h^{-1})					
pH	Mean	SEM	Mean	SEM	Mean	SEM
3.7	0.509	0.01912				
3.9					0.07036	0.0118
4.1			0.200	0.00823		
4.7	0.751	0.0280				
5.5			0.414	0.00750		
5.7					0.357	0.0177
6.1	0.847	0.02543				
7.2			0.533	0.0234	0.457	0.0277
7.3	0.978	0.01289	0.593	0.0115	0.533	0.0165

Appendix C: Additional data and information related to findings in Chapter 3

Table C.1: Data for numbers of Eub 16S genes in pH-manipulated soils presented in Fig. 3.2. AG (agricultural field), GB (grassed border), and RZ (riparian zone) A horizon soils listed.

	AG Ap			GB Ap			RZ A1/A2		
pH	----------Eub 16S gene copies g^{-1} soil----------								
3.3	1.21E+09	1.18E+09	8.02E+08						
3.7							8.67E+09	1.48E+10	1.38E+10
4.0				3.94E+09	6.43E+09	8.78E+09			
5.1							1.65E+10	2.27E+10	2.28E+10
5.4	7.84E+09	1.97E+10	1.14E+10						
5.9				1.57E+10	6.01E+09	1.25E+10			
6.3							3.23E+10	2.80E+10	2.24E+10
6.8	8.36E+09	5.19E+09	9.25E+09						
6.9				1.77E+10	1.28E+10	1.12E+10			

Table C.2: Data for numbers of ITS genes in pH-manipulated soils presented in Fig. 3.3. AG (agricultural field), GB (grassed border), and RZ (riparian zone) A and B horizon soils listed.

	AG Ap			GB Ap			RZ A1/A2		
pH	----------gene copies g^{-1} soil----------								
3.3	1.17E+09	9.94E+08	6.90E+08						
3.7							1.29E+08	2.01E+08	1.42E+08
4.0				1.57E+07	2.93E+07	5.96E+07			
5.1							2.16E+08	1.60E+08	1.77E+08
5.4	4.15E+07	9.20E+07	3.22E+07						
5.9				5.55E+07	4.00E+07	5.48E+07			
6.3							2.59E+08	2.07E+08	1.20E+08
6.8	3.34E+07	1.98E+07	3.53E+07						
6.9				7.42E+07	5.16E+07	4.04E+07			

	AG Bt			GB Bt			RZ BC		
pH	----------gene copies g^{-1} soil----------								
3.1	2.36E+06		4.40E+05						
3.6				2.52E+08	3.31E+08	2.96E+08			
4.1							7.81E+07	1.38E+08	7.14E+07
4.9							5.85E+07	1.31E+08	6.82E+07
5.0				1.51E+07	2.51E+07	2.46E+07			
5.2	5.54E+05	7.82E+05	5.18E+05						
6.6							7.65E+07	1.30E+08	1.09E+08
6.9				2.57E+07	2.70E+07	1.49E+07			
7.1	2.85E+06	3.23E+06	4.58E+06						

Table C.3: Data for numbers of Eub 16S genes in pH-manipulated soils presented in Fig. 3.4. AG (agricultural field), GB (grassed border), and RZ (riparian zone) A horizon soils listed.

	Urea Hydrolysis Rate	----------No urea----------			----------+urea----------		
	(mg urea-N kg^{-1} h^{-1})	----------10^{10} Eub 16S copies g^{-1} soil----------					
CP AG Acid (~3.3)	0.0172	0.0742		0.0678	0.121	0.118	0.0802
CP AG Native (~5.4)	0.807	1.08	1.10	1.09	0.784	1.97	1.14
CP AG CaCO3 #2 (~6.8)	0.974	0.974	1.05	0.901	0.836	0.519	0.925
CP GB Native (4.0)	0.683	1.14	1.19	1.11	0.394	0.643	0.878
CP GB CaCO3#1 (5.9)	0.956	1.23	1.05	1.36	1.57	0.601	1.25
CP GB CaCO3#3 (6.9)	1.15	1.29	1.24	1.26	1.77	1.28	1.12
CP RZ Native (3.7)	0.926	1.70	1.73	1.70	0.867	1.48	1.38
CP RZ CaCO3 #1 (5.1)	1.99	1.40	1.37		1.65	2.27	2.28
CP RZ CaCO3 #3 (6.3)	2.20	1.93	1.92	1.87	3.23	2.80	2.24

Table C.4: Data for numbers of *ureC* genes and rate of urea hydrolysis in pH-manipulated soils presented in Fig. 3.5. AG (agricultural field), GB (grassed border), and RZ (riparian zone) A and B horizon soils listed.

	Urea Hydrolysis Rate (mg urea-N kg^{-1} h^{-1})	No urea			+urea		
		10^8 ureC copies g^{-1} soil					
CPAG Ap Acid (~3.3)	0.0172	0.0127	0.0117	0.0113	0.00238	0.0036	0.0017
CP AG Ap Native (~5.4)	0.807	1.49	1.48	1.47	1.73	2.42	1.31
CP AG Ap CaCO3 #2 (~6.8)	0.974	1.93	1.88	1.81	1.07	0.777	1.43
CP GB Ap Native (4.0)	0.683	1.11	1.13	1.13	0.52	0.654	0.649
CP GB Ap CaCO3#1 (5.9)	0.956	1.48	1.48	1.43	1.64	1.36	1.18
CP GB Ap CaCO3#3 (6.9)	1.15	1.51	1.45	1.40	1.85	1.32	1.23
CP RZ A1/A2 Native (3.7)	0.926	2.44	2.30	2.32	1.83	3.09	2.03
CP RZ A1/A2 CaCO3 #1 (5.1)	1.99	3.21	3.10	3.16	2.89	2.46	2.48
CP RZ A1/A2 CaCO3 #3 (6.3)	2.20	2.74	2.82	2.70	3.73	2.91	3.24
CP AG Bt Acid (3.1)	0.00402	0.00071	0.000674	0.000654	0.00197		0.0004
CP AG Bt Native (5.2)	0.141	0.0874	0.0843	0.0877	0.0385	0.0451	0.0514
CP AG Bt CaCO3 #2 (7.1)	0.198	0.0881	0.0960	0.102	0.157	0.145	0.196
CP GB Bt Acid (~3.6)	0.0136	0.00899	0.00868	0.00882	0.0403	0.0613	0.0319
CP GB Bt Native (5.0)	0.139	0.343	0.352	0.334	0.471	0.535	0.547
CP GB Bt CaCO3#2 (6.9)	0.249	0.388	0.374	0.372	0.849	0.858	0.719
CP RZ BC Native (4.1)	0.163	0.350	0.345	0.334	0.578	0.520	0.518
CP RZ BC CaCO3 #1 (4.9)	0.201	0.392	0.389	0.389	0.329	0.689	0.464
CP RZ BC CaCO3 #3 (6.6)	0.289	0.598	0.599	0.600	0.967	1.15	1.32

Table C.5: Data for the correlation between numbers of ureC and Eub 16S genes in pH-manipulated soils before urea was added. Presented in Fig. 3.6 a and c. AG (agricultural field), GB (grassed border), and RZ (riparian zone) A and B horizon soils listed.

	No urea added					
	------10^8 ureC copies g^{-1} soil------			------10^{10} Eub 16S copies g-1 soil------		
CPAG Ap Acid (~3.3)	0.0127	0.0117	0.0113	0.0742		0.0678
CP AG Ap Native (~5.4)	1.49	1.48	1.47	1.08	1.10	1.09
CP AG Ap CaCO3 #2 (~6.8)	1.93	1.88	1.81	0.974	1.05	0.901
CP GB Ap Native (4.0)	1.11	1.13	1.13	1.14	1.19	1.11
CP GB Ap CaCO3#1 (5.9)	1.48	1.48	1.43	1.23	1.05	1.36
CP GB Ap CaCO3#3 (6.9)	1.51	1.45	1.40	1.29	1.24	1.26
CP RZ A1/A2 Native (3.7)	2.44	2.30	2.32	1.70	1.73	1.70
CP RZ A1/A2 CaCO3 #1 (5.1)	3.21	3.10	3.16	1.40	1.37	
CP RZ A1/A2 CaCO3 #3 (6.3)	2.74	2.82	2.70	1.93	1.92	1.87
CP AG Bt Acid (3.1)	0.000710	0.000674	0.000654	0.000017	0.0000157	0.0000158
CP AG Bt Native (5.2)	0.0874	0.0843	0.0877	0.0459	0.0455	0.0448
CP AG Bt CaCO3 #2 (7.1)	0.0881	0.0960	0.102	0.0524	0.0513	0.0523
CP GB Bt Acid (~3.6)	0.00899	0.00868	0.00882	0.119	0.114	0.118
CP GB Bt Native (5.0)	0.343	0.352	0.334	0.297	0.296	0.299
CP GB Bt CaCO3#2 (6.9)	0.388	0.374	0.372	0.323	0.329	0.315
CP RZ BC Native (4.1)	0.350	0.345	0.334	0.371	0.353	0.351
CP RZ BC CaCO3 #1 (4.9)	0.392	0.389	0.389		0.536	0.514
CP RZ BC CaCO3 #3 (6.6)	0.598	0.599	0.600	0.462	0.452	0.470

Table C.6: Data for the correlation between numbers of ureC and Eub 16S genes in pH-manipulated soils after urea was added. Presented in Fig. 3.6 b and d. AG (agricultural field), GB (grassed border), and RZ (riparian zone) A and B horizon soils listed.

	After urea added					
	-------10^8 ureC copies g^{-1} soil------			---10^{10} Eub 16S copies g^{-1} soil---		
CPAG Ap Acid (~3.3)	0.00238	0.00360	0.00170	0.121	0.118	0.0802
CP AG Ap Native (~5.4)	1.73	2.42	1.31	0.784	1.97	1.14
CP AG Ap CaCO3 #2 (~6.8)	1.07	0.777	1.43	0.836	0.519	0.925
CP GB Ap Native (4.0)	0.52	0.654	0.649	0.394	0.643	0.878
CP GB Ap CaCO3#1 (5.9)	1.64	1.36	1.18	1.57	0.601	1.25
CP GB Ap CaCO3#3 (6.9)	1.85	1.32	1.23	1.77	1.28	1.12
CP RZ A1/A2 Native (3.7)	1.83	3.09	2.03	0.867	1.48	1.38
CP RZ A1/A2 CaCO3 #1 (5.1)	2.89	2.46	2.48	1.65	2.27	2.28
CP RZ A1/A2 CaCO3 #3 (6.3)	3.73	2.91	3.24	3.23	2.80	2.24
CP AG Bt Acid (3.1)	0.00197		0.000400	0.0000703		0.0000347
CP AG Bt Native (5.2)	0.0385	0.0451	0.0514	0.00952	0.0177	0.0132
CP AG Bt CaCO3 #2 (7.1)	0.157	0.145	0.196	0.0667	0.0750	0.119
CP GB Bt Acid (~3.6)	0.0403	0.0613	0.0319	0.196	0.259	0.230
CP GB Bt Native (5.0)	0.471	0.535	0.547	0.429	0.423	0.469
CP GB Bt CaCO3#2 (6.9)	0.849	0.858	0.719	0.699	0.670	0.532
CP RZ BC Native (4.1)	0.578	0.520	0.518	0.508	0.512	0.481
CP RZ BC CaCO3 #1 (4.9)	0.329	0.689	0.464	0.339	0.566	0.437
CP RZ BC CaCO3 #3 (6.6)	0.967	1.15	1.32	0.417	0.636	0.665

Table C.7: ITS gene copy numbers in freshly-sampled vs. stored soils from the agricultural field (AG), grassed field border (GB), and riparian zone (RZ) A and B horizons. Measurements for triplicate samples presented.

	Fresh			Stored		
	ITS gene copies g^{-1} soil					
AG Ap	5.86E+07	6.05E+07	5.99E+07	3.87E+07	3.58E+07	3.80E+07
GB Ap	8.25E+07	7.81E+07	8.40E+07	6.17E+07	6.24E+07	6.00E+07
RZ A1/A2	8.53E+08	9.51E+08	9.64E+08	1.76E+08	1.69E+08	1.55E+08
AG Bt	1.90E+06	1.87E+06	1.85E+06	1.50E+06	1.47E+06	1.40E+06
GB Bt	4.60E+06	4.30E+06	4.30E+06	5.20E+06	5.08E+06	5.14E+06
RZ BC	6.95E+07	7.04E+07	7.00E+07	2.81E+07	2.84E+07	2.47E+07

Appendix D: Additional data and information related to findings in Chapter 4

Table D.1: Chemical characteristics of AG Ap soil treated with different types and concentrations of added C.

Site	Horizon	Organic Acid	C added	†Urea-N	Fe	Mn	Al	pH	Eh	pe
			----------mg kg^{-1} soil----------							
AG	Ap	Ascorbic	0	90.7	0	0	0	5.2	449	7.6
AG	Ap	Ascorbic	100	70.5	0	26	0	5.2	443	7.5
AG	Ap	Ascorbic	400	82.5	2.7	100	8.6	4.9	432	7.3
AG	Ap	Ascorbic	1,000	106	18	160	42	4.1	416	7.0
AG	Ap	Ascorbic	4000‡	72.1	240	210	82	4.0	130	2.2
AG	Ap	Ascorbic	10000‡	38.7	390	230	130	3.5	149	2.5
AG	Ap	Gallic	0	85.3	0	0	0	5.1	482	8.1
AG	Ap	Gallic	100	68.0	0	32	0	5.3	459	7.7
AG	Ap	Gallic	400	69.4	0	73	0	5.2	288	4.9
AG	Ap	Gallic	1,000	87.7	5	110	6.7	4.5	272	4.6
AG	Ap	Gallic	4000‡	74.7	30	170	71	3.7	303	5.1
AG	Ap	Gallic	10000‡	51.0	50	200	140	3.4	319	5.4
AG	Ap	Benzoic	0	91.0	0	0.0	0	5.1	519	8.8
AG	Ap	Benzoic	100	93.0	0	0.4	0	5.1	496	8.4
AG	Ap	Benzoic	400	92.4	0	0.3	0	4.5	536	9.0
AG	Ap	Benzoic	1,000	85.0	0	0.3	0.98	4.1	560	9.5
AG	Ap	Benzoic	4,000	73.8	0	1.5	18	3.5	587	9.9
AG	Ap	Benzoic	10,000	78.1	0	3.3	44	3.2	607	10.3
AG	Ap	Cinnamic	0	96.1	0	0.0	0	5.0	491	8.3
AG	Ap	Cinnamic	100	96.9	0	0.0	0	4.8	478	8.1
AG	Ap	Cinnamic	400	89.6	0	0.2	0	4.6	480	8.1
AG	Ap	Cinnamic	1,000	84.1	0	0.7	0	4.2	517	8.7

†Remaining after 24 hour incubation; 100 mg N kg-1 soil added initially
‡Treatments that resulted in values beneath the FeOOH → Fe2+ redox line on the pe/pH diagram (Fig. 4.3), indicating the presence of reduced Fe.

Table D.2: Chemical characteristics of AG Bt soil treated with different types and concentrations of added C.

Site	Horizon	Organic Acid	C added	†Urea-N	Fe	Mn	Al	pH	Eh	pe
			----------mg kg^{-1} soil----------							
AG	Bt	Ascorbic	0	88.7	0	0	0	5.1	377	6.4
AG	Bt	Ascorbic	100	74.2	0	4.7	0	4.8	389	6.6
AG	Bt	Ascorbic	400	84.0	10	8.7	0	4.5	330	5.6
AG	Bt	Ascorbic	1,000	94.8	51	9.7	2.2	4.3	287	4.9
AG	Bt	Ascorbic	4000‡	77.4	330	13	80	3.4	246	4.2
AG	Bt	Ascorbic	10000‡	38.2	980	18	130	3.4	165	2.8
AG	Bt	Gallic	0	82.2	0	0	0	5.0	503	8.5
AG	Bt	Gallic	100	65.8	0	4.6	0	5.0	499	8.4
AG	Bt	Gallic	400	43.3	0	7.8	0	4.9	484	8.2
AG	Bt	Gallic	1,000	8.79	0.35	10	0	4.8	401	6.8
AG	Bt	Gallic	4000‡	24.7	13	10	41	3.8	283	4.8
AG	Bt	Gallic	10,000	70.3	32	11	87	3.4	320	5.4
AG	Bt	Benzoic	0	89.4	0	0	0	5.3	477	8.1
AG	Bt	Benzoic	100	72.3	0	0.58	0	5.2	482	8.1
AG	Bt	Benzoic	400	59.1	0	1.8	0	5.1	482	8.1
AG	Bt	Benzoic	1,000	99.8	0	0	0	4.1	525	8.9
AG	Bt	Benzoic	4,000	98.6	0	0.22	13	3.6	547	9.2
AG	Bt	Benzoic	10,000	98.2	0	0.75	31	3.2	559	9.4
AG	Bt	Cinnamic	0	95.9	0	0	0	5.3	496	8.4
AG	Bt	Cinnamic	100	85.7	0	0	0	5.2	494	8.4
AG	Bt	Cinnamic	400	55.8	0	0.12	0	5.2	482	8.1
AG	Bt	Cinnamic	1,000	68.3	0	0.15	0	4.6	498	8.4

†Remaining after 24 hour incubation; 100 mg N kg-1 soil added initially
‡Treatments that resulted in values beneath the FeOOH → Fe2+ redox line on the pe/pH diagram (Fig. 4.3), indicating the presence of reduced Fe.

Table D.3: Chemical characteristics of RZ A1/A2 soil treated with different types and concentrations of added C.

Site	Horizon	Organic Acid	C added	†Urea-N	Fe	Mn	Al	pH	Eh	pe
			----------mg kg^{-1} soil----------							
RZ	A1/A2	Ascorbic	0	58.7	1.1	2.8	0	4.2	435	7.3
RZ	A1/A2	Ascorbic	100	44.3	0.34	51	0	4.4	444	7.5
RZ	A1/A2	Ascorbic	400	44.8	3.1	110	12	4.3	447	7.5
RZ	A1/A2	Ascorbic	1,000	58.2	14	140	64	3.6	431	7.3
RZ	A1/A2	Ascorbic	4000‡	47.7	230	150	75	3.5	249	4.2
RZ	A1/A2	Ascorbic	10000‡	21.7	500	160	110	3.5	168	2.8
RZ	A1/A2	Gallic	0	45.0	0	2.4	0	4.2	536	9.1
RZ	A1/A2	Gallic	100	35.8	0	61	0	4.4	523	8.8
RZ	A1/A2	Gallic	400	37.0	0.6	110	0	4.2	448	7.6
RZ	A1/A2	Gallic	1,000	44.5	3.3	120	7.8	4.0	334	5.6
RZ	A1/A2	Gallic	4000‡	29.2	14	140	44	3.5	336	5.7
RZ	A1/A2	Gallic	10000‡	19.9	25	150	84	3.3	346	5.9
RZ	A1/A2	Benzoic	0	50.2	0.08	2.6	0	4.3	525	8.9
RZ	A1/A2	Benzoic	100	46.6	0	9.8	0	4.4	521	8.8
RZ	A1/A2	Benzoic	400	35.5	0	7.8	0	4.3	521	8.8
RZ	A1/A2	Benzoic	1,000	24.4	0	4.2	1.7	4.1	531	9.0
RZ	A1/A2	Benzoic	4,000	0.0	0	6.6	10	3.6	550	9.3
RZ	A1/A2	Benzoic	10,000	27.8	0	11	28	3.3	564	9.5
RZ	A1/A2	Cinnamic	0	52.6	0	2.5	0	4.0	530	8.9
RZ	A1/A2	Cinnamic	100	44.7	0	2.8	0	4.1	528	8.9
RZ	A1/A2	Cinnamic	400	24.6	0	2.7	0	4.1	528	8.9
RZ	A1/A2	Cinnamic	1,000	17.6	0	3.0	0	4.1	534	9.0

†Remaining after 24 hour incubation; 100 mg N kg-1 soil added initially
‡Treatments that resulted in values beneath the FeOOH → Fe2+ redox line on the pe/pH diagram (Fig. 4.3), indicating the presence of reduced Fe.

Table D. 4: Chemical characteristics of RZ BC soil treated with different types and concentrations of added C.

Site	Horizon	Organic Acid	C added	†Urea-N	Fe	Mn	Al	pH	Eh	pe
			----------mg kg^{-1} soil----------							
RZ	BC	Ascorbic	0	87.3	0.61	1.1	10	4.0	447	7.5
RZ	BC	Ascorbic	100	52.5	0.51	40	9.2	4.2	457	7.7
RZ	BC	Ascorbic	400	43.4	3.6	77	11	4.3	455	7.7
RZ	BC	Ascorbic	1,000	37.7	36	91	21	4.3	314	5.3
RZ	BC	Ascorbic	4000‡	18.9	310	99	88	4.0	170	2.9
RZ	BC	Ascorbic	10000‡	28.3	750	110	220	3.2	167	2.8
RZ	BC	Gallic	0	71.7	0	1.0	9.2	4.0	537	9.1
RZ	BC	Gallic	100	54.7	0	46	5.7	4.2	525	8.9
RZ	BC	Gallic	400	51.0	1.0	68	5.0	4.3	454	7.7
RZ	BC	Gallic	1,000	23.2	4.2	76	5.0	4.4	347	5.9
RZ	BC	Gallic	4000‡	41.2	35	88	110	3.6	273	4.6
RZ	BC	Gallic	10000‡	59.7	68	96	170	3.2	325	5.5
RZ	BC	Benzoic	0	78.2	0.20	0.89	11	4.1	537	9.1
RZ	BC	Benzoic	100	61.8	0	2.8	11	4.1	535	9.0
RZ	BC	Benzoic	400	50.2	0	7.2	11	4.2	535	9.0
RZ	BC	Benzoic	1,000	63.9	0	3.8	31	3.7	540	9.1
RZ	BC	Benzoic	4,000	73.3	0.20	2.3	75	3.2	557	9.4
RZ	BC	Benzoic	10,000	89.5	0	3.6	120	3.0	570	9.6
RZ	BC	Cinnamic	0	76.4	0.10	1.0	11	4.1	531	9.0
RZ	BC	Cinnamic	100	72.3	0.20	1.4	11	4.2	527	8.9
RZ	BC	Cinnamic	400	90.6	0	1.3	19	3.9	539	9.1
RZ	BC	Cinnamic	1,000	78.7	0	1.3	24	3.8	548	9.3

†Remaining after 24 hour incubation; 100 mg N kg-1 soil added initially
‡Treatments that resulted in values beneath the FeOOH → Fe2+ redox line on the pe/pH diagram (Fig. 4.3), indicating the presence of reduced Fe.

Appendix E: Additional data and information related to discussion in Chapter 5.

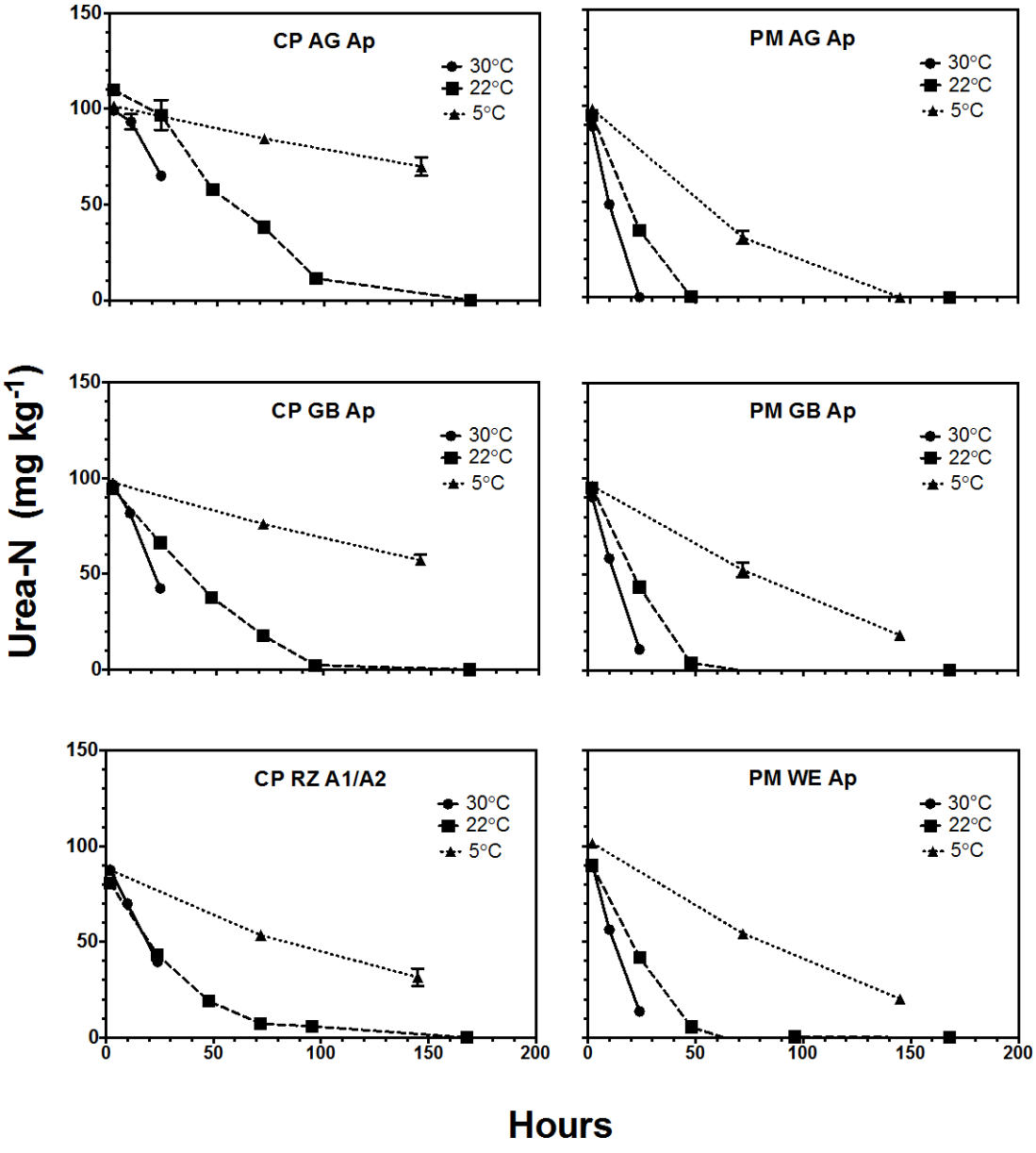


Figure E.1: Temperature effects on urea hydrolysis in Coastal Plain (CP) and Piedmont (PM) A horizon soils. AG (agricultural field), GB (grassed field border), RZ (riparian zone), WE (wetland edge) soils plotted. SEM plotted, error bars may be within symbol area (n=3).

Appendix F: Studies of effects of added P on soil urea hydrolysis

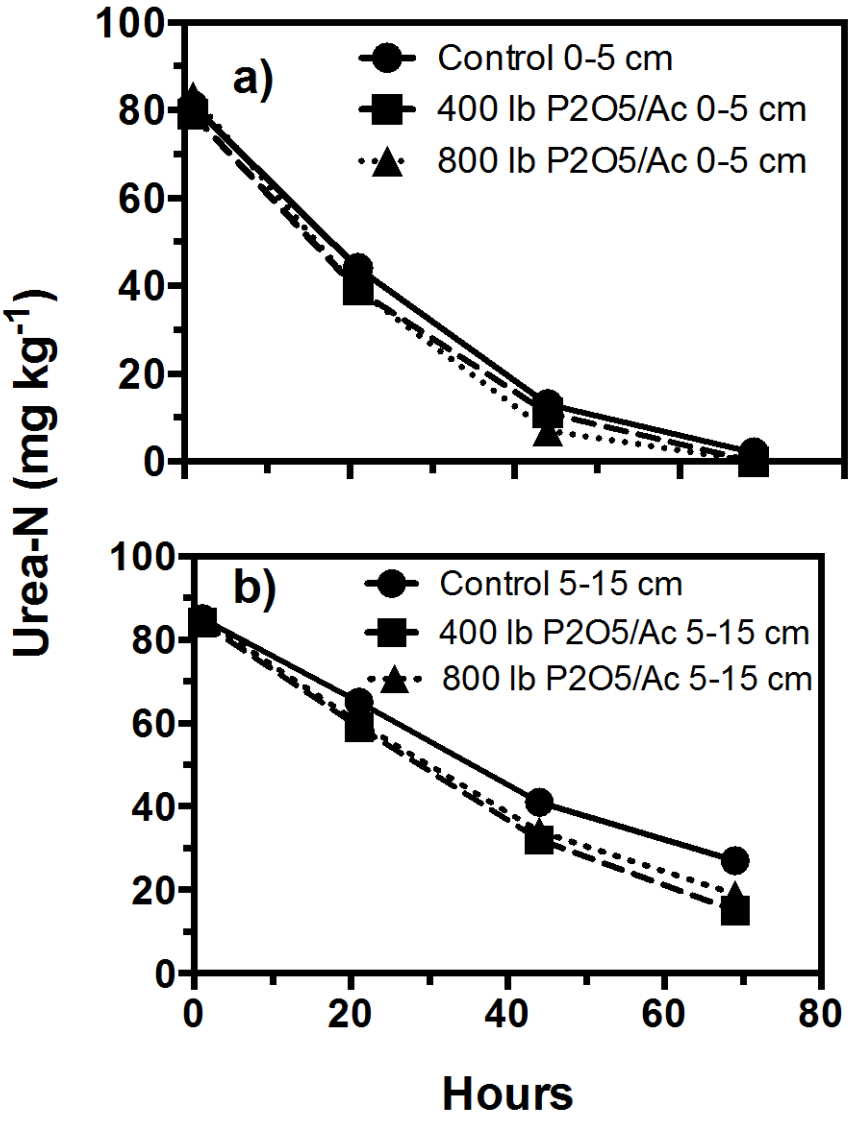


Figure F.1: Urea hydrolysis in soils sampled from the Wye Research and Education Center, which have a history of different levels of P_2O_5 application. Lack of evidence of inhibition of urea hydrolysis by soil P directed research toward soil C (Chap. 4).

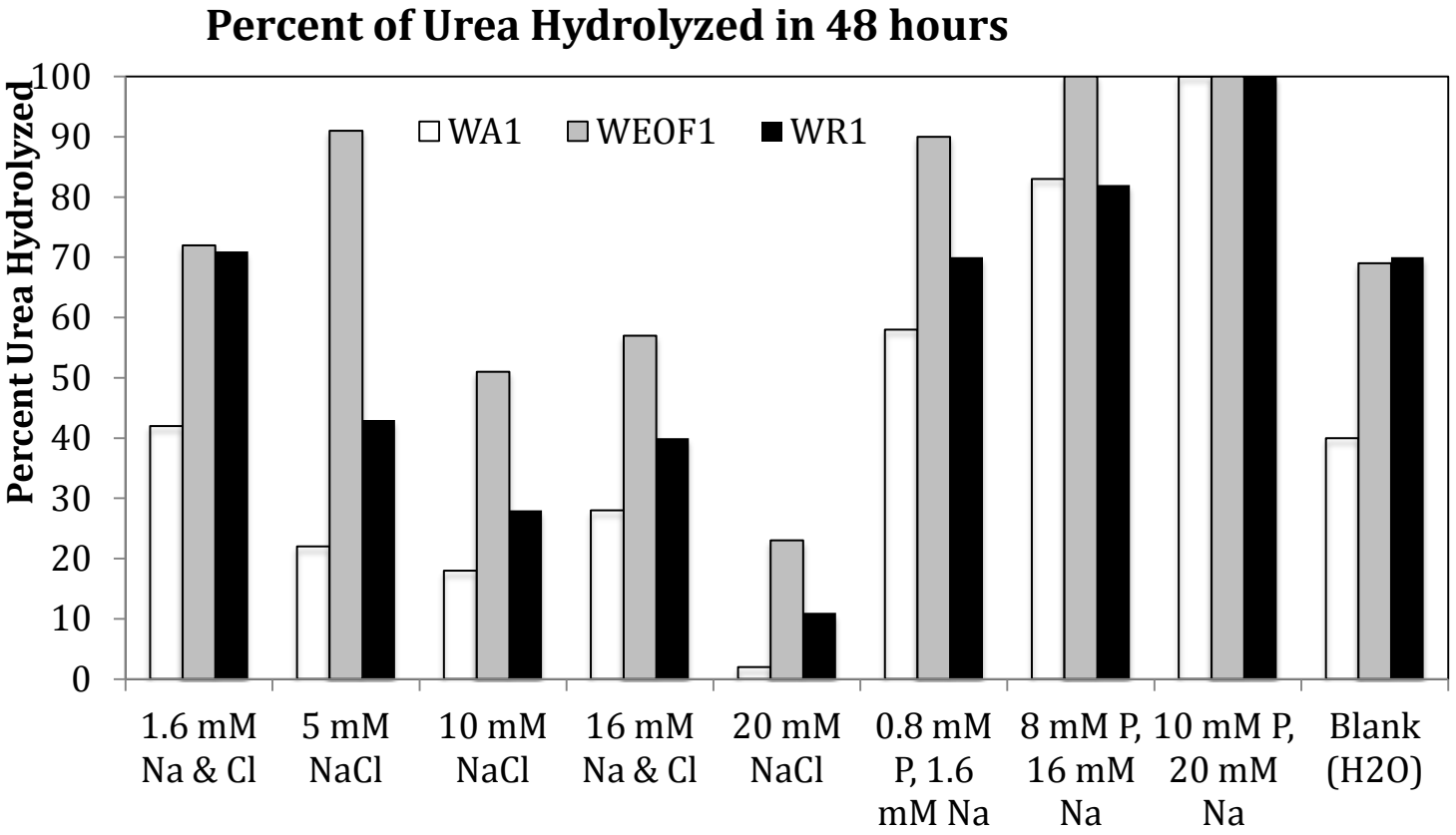


Figure F.2: Urea hydrolysis in soils treated with varying concentrations of NaCl and Na_2HPO_4. Wye (or Coastal Plain) agricultural field, transect 1 (WA1), Wye grassed field border, transect 1 (WEOF1), and Wye riparian zone, transect 1 (WR1) values plotted. Results of this work and that presented in Figure F.1 directed future research toward soluble C effects on soil urea hydrolysis.

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