UNDERSTANDING COMPLEX EARTH SYSTEMS: VOLATILE METABOLITES AS MICROBIAL ECOSYSTEM PROXIES AND STUDENT CONCEPTUAL MODEL DEVELOPMENT OF COASTAL EUTROPHICATION

A Dissertation

by

KAREN SUE MCNEAL

Submitted to the Office of Graduate Studies of
Texas A&M University
in partial fulfillment of the requirements for the degree of

DOCTOR OF PHILOSOPHY

May 2007

Major Subject: Geology

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ABSTRACT

Understanding Complex Earth Systems: Volatile Metabolites as Microbial Ecosystem Proxies and Student Conceptual Model Development of Coastal Eutrophication.

(May 2007)

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Understanding complex Earth systems is challenging for scientists and students alike, because of the characteristics (e.g. bifurcations, self-organization, chaotic response) that are associated with these systems. This research integrates two research strands which contribute to the scientific and pedagogical understanding of complex Earth systems. In the first strand, a method that characterizes volatile organic compounds (VOCs) as ecological proxies of soil microbial ecosystems was validated. Unlike other measures of microbial community structure (e.g. Biolog and FAME), VOCs are advantageous because they are non-destructive and can provide temporal and spatial data. Additionally they are rich sources of information that describe the microbial metabolism, community structure, and organic carbon substrates utilized by soil microorganisms. Statistical results indicate that the detected and identified VOCs were significant ($\rho < 0.05$) indicators of microbial community composition shift in soil

microcosm studies. Geographical information systems (GIS) illustrates that VOCs varied with space and time in south Texas soils.

The second strand focuses on a geoscience education study exploring student conceptual model development of complex Earth systems. The efficacy of multiple representations and inquiry was tested as the pedagogical strategy in upper and lower level undergraduate courses to support students' conceptual model development of complex Earth systems. Comparisons in student performance were based on prior knowledge (low and high) and on exposure to the implemented pedagogy (control and experimental groups). Results indicate that an inquiry-based learning model coupled with the use of multiple representations had significant positive performance impacts on students' conceptual model development and content knowledge.

This dissertation model integrates science and education research and is particularly useful for graduate students who intend to pursue a career in academia and envision teaching as part of their professional duties. It allows for synergy between teaching and research to be achieved where the classroom becomes a laboratory for research. Ultimately, the research conducted in the classroom informs pedagogy and enhances scholarship. Graduates learn to bridge the gap between education and science departments where they become leaders in science who conduct cutting-edge scientific research and also value making a broader impact on society through enhancing public education.

DEDICATION

I would like to dedicate this work to my Lord God because he had given me the talents in science and teaching and has been faithful to His promises to me. I would also like to dedicate this work to my husband as he has been a blessing in my life and a helpful lab, field, and GIS partner, too. I can not wait for many more years of marriage. I love you, Aaron. Further, I would like to dedicate this work to my parents as they have been a wonderful support and a loving Christian example. They have passed on a high regard for teaching and science to me, and I am grateful for all they have taught me. Finally, I would like to thank all of my family and friends for their support, advice, and listening ear over the years.

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Any opinions, findings and conclusions or recommendations expressed in this material are those of the author(s) and do not necessarily reflect the views of the National Science Foundation or the collaborating institutions.

NOMENCLATURE

ANOVA	Analysis of Variance
ATD	Automated Thermal Desorber
C/N	Carbon to Nitrogen Ratio
CSUP	Carbon Substrate Utilization Profile
FAME	Fatty Acid Methyl Ester
GC-MS	Gas Chromatography-Mass Spectrometry
GIS	Geographical Information Systems
IBL	Inquiry Based Learning
IT	Information Technology
LANWR	Laguna Atascosa National Wildlife Refuge
PCA	Principle Component Analysis
PCR	Polymerase Chain Reaction
PLFA	Phospholipid Fatty Acid
STEM	Science, Technology, Engineering and Math
TIC	Total Inorganic Carbon
TOC	Total Organic Carbon
VOC	Volatile Organic Compound

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CHAPTER I

INTRODUCTION

Understanding Complex Earth Systems

The central paradigm of the Earth sciences is the systems concept. An Earth system is a physical system of interrelated phenomena, processes and cycles, where components are characterized by defined boundaries and structures that transfer matter and energy. Complex systems are open systems, usually far from equilibrium, which exhibit non-linear behavior and positive and negative feedback loops where some behaviors emerge as a result of the patterns of relationships or interactions between the elements or components of the system, not through some external agent that imposes order (Herbert, 2006). A complex system may exhibit the appearance of stability through pattern formation (e.g. self-organization or. emergent properties), and bifurcations to new stable states (Bak, 1996; Bar-Yam, 1997; Scheffer et al., 2001; Young and Crawford, 2004) or it may exhibit chaotic dynamics, where the state of the system is sensitive to initial conditions (Becks et al., 2005). Most environmental issues involve complex Earth systems which are defined as near-surface Earth systems that exhibit complex spatial and temporal characteristics and dynamics (Herbert, 2006).

This thesis follows the style of *Geochimica et Cosmochimica Acta.*

As a result of the complex behavior of Earth systems, there are three major challenges associated with understanding and learning about complex Earth systems. These include (i) identifying the interactions between system components, (ii) conceptualizing the changes in the system's state over space and time, and (iii) applying models to predict self-organization and feedback behaviors (Colucci-Gray et al., 2006; Herbert, 2006; Sell et al., 2006). Further challenges may include using interdisciplinary knowledge to understand the relationships between system components and identifying the importance of scale and its influence on a particular system (Sell et al., 2006).

Research Overview

This research aims to enhance understanding of complex systems in the Earth and environmental sciences. Soils are important complex Earth systems and therefore this work is centered about the understanding of soil microbial ecosystems. Current methods that are used to indicate soil ecosystem behavior (e.g. enzymatic approaches) lack the ability to track the spatial and/or temporal dynamics of microbial ecosystems. Therefore, this work aims to validate VOCs as indicators of microbial community compositional changes or "shifts" in soil microcosm studies as a means to understand the behavior of these systems. The hypothesis of this work is that perturbations of a variety of environmental factors during soil microcosm experiments will induce shifts in the associated microbial community structure and in result the subsequent production of VOC metabolites will vary. In addition, this research intends to observe the temporal and spatial dynamics of VOCs in south Texas soils through the use of the validated VOC

method. The goal is to answer the following overarching research questions. Are VOCs valid indicators of microbial community shift in soil microcosm studies? and How does soil VOC production vary over space and time in south Texas soils?

The second goal of this research is to use authentic inquiry and multiple representations as the classroom pedagogy to enhance undergraduate student learning of complex Earth systems. Particularly this work aims to determine how this pedagogical approach will impact both upper and lower division undergraduate classrooms. Specifically, the research will focus on various student groups' conceptual model development of eutrophication, a complex environmental process. The aim is to answer the following over-arching research questions. Can high and low prior knowledge students' conceptual model expressions predict their inquiry performance? and How are students' conceptual model development and content knowledge impacted by the implementation? In order to answer these questions, the research focuses on both high and low prior knowledge students and on the use of experimental and control groups.

Soil Volatile Organic Compound Research

Soils as Complex Earth Systems

This dissertation focuses on the use of laboratory experiments to understand the complexity of soil environments and microbial ecosystems. Soils have been described as the "most diverse ecosystem" and "complex biomaterial" on the planet where complexity is associated with its physical structure and relationship with soil

microorganisms (Young and Crawford, 2004). Soils are fundamental to many activities in which human civilization are dependant including agriculture, forestry, water purification, and biogeochemical cycling. However, they are one of the least understood habitats on Earth (Behan-Pelletier and Newton, 1999). Soil microorganisms play a crucial role in the quality and health (Gil-Stores et al., 2005; Marinari et al., 2006) and diversity of soils (Hackl et al., 2005) where in a single handful of soil, millions of microorganisms representing hundreds of different species, including bacteria, fungi, protoza, and algae (Behan-Pelletier and Newton, 1999). If we consider soils to be an ecosystem, it is likely that they will respond to perturbations as most other ecosystems do. Ecosystems have been described as complex systems in regard to species interactions (Colucci-Gray et al., 2006), catastrophic shifts (Scheffer et al., 2001), and food-web dynamics (Becks et al., 2005); the characteristics of their behavior follows the above description of complex systems.

The Use of Environmental Proxies

Environmental proxies are used by scientists as indicators of ecosystem change in complex Earth systems. Microbial proxies are frequently utilized to determine the microbiological impact on soil ecosystems as well as the response of the microbial community to changing environmental factors (Jones and Bradford, 2001; Fang et al., 2006; Torsvik and Øvreås, 2002). Examples include: (i) functional characterization methods (e.g. enzymes), (ii) taxonomic and community approaches (e.g. PLFA and DNA), (iii) microbial activity measurements (e.g. CO_2 and CH_4), and (iv) carbon and

nutrient pathway studies (e.g. stable isotope labeling). Although the above proxies are all useful for examining soil microbial ecosystems, each has advantages and disadvantages associated with their use. The ability to conduct temporally dynamic studies is the most limited aspect of the above proxies, where only the microbial activity methods (e.g. CO_2) can be utilized. This is due to the non-destructive sampling practices associated with their collection. However, introducing the use of VOC metabolites provides an additional proxy method to study microbial metabolism over various time scales and is certainly worth investigating and validating because of the quality of information that is included with the use of VOCs, such as insights to organic carbon substrates and the microbial communities present. Since microbial community and structure is influenced by soil water content, carbon sources, and nutrient availability (Dahlhoff, 2004; Drenovsky and Richards, 2004; Fierer et al., 2003; Franklin and Mills, 2003; Musslewhite et al., 2003) and these characteristics frequently vary over time (and space), VOC metabolite production likely also varies temporally (and spatially).

VOC Sources and Previous Research

Previous research has shown that VOCs can have many sources in soil including germinating seed and roots (Stotzky and Schenck, 1973) plant rhizophere regions (Moore-Landecker, 1988), foliage and wood (Adebajo et al., 1989; Dean and Svoboda, 1990; Hartill and Suton, 1980), dead plants and residues (Berestetskii and Kravchenko, 1984; Menzies and Gilbert, 1967; Owens et al., 1969), and soil microorganisms (Stahl and Parkin, 1996). VOCs have been measured during direct-culture of microorganisms

and have found to be dependent on both fungal species type and growth mediums (Larsen and Frisvad, 1995a; Sunesson et al., 1995). Other research has shown that certain actinomycetes (Gerber, 1977) and gram-negative bacteria (Schöller et al., 1997) produce specific VOCs. This work suggests that VOCs can be utilized as indicators of microorganism presence where various environmental conditions may produce different volatile organic metabolites (Grametbauer et al., 1988). Stahl and Parkin's (1994, 1996) work is the only to date that has analyzed VOC production in soil microcosms and not direct-culture studies. Their results are promising where the types of VOCs produced vary during shifts in microbial community.

Undergraduate Earth and Environmental Science Education

Environmental issues serve as effective contexts for problem-based and inquiry-based learning in primary and secondary schools, supporting a number of important learning outcomes including knowledge gains in a range of environmentally-related academic disciples, improving student attitudes concerning science, and supporting student predisposition to responsible environmental behavior (Hart and Nolan, 1999; Rickinson, 2001). Though research-based evidence is much more limited, similar learning outcomes are also possible at the undergraduate level, supporting reform of undergraduate science, technology, engineering and mathematics (STEM) education (Herbert, 2006). Improving STEM education has been a focus of higher education for the last decade (NRC, 1996; The Boyer Commission, 1998). This focus is in response to the perceived limitations of STEM education that is dominated by didactic models of

teaching, with their focus on rote memorization of factual knowledge and superficial development of conceptual understanding of knowledge domains (Barab and Luehmann, 2003; Lee and Songer, 2003; Mathewson, 1962; Whitehead, 1929). Didactic modes of teaching can promote a number of learning outcomes of little value including student passivity, naïve views of science, a focus on grades, and limited knowledge transfer and higher-order thinking skill development (Dori and Hersovitz, 1999; Dutch, 1996; Sandoval and Reisner, 2003; Smith, 1955). Moreover, the perceived dullness of the material, a lack of concrete applications, and preconceptions among both students and instructors can make STEM classes difficult for non-science majors and lead to lower retention rates of majors and poor student attitudes about science (Delaughter et al., 1998; Schibeci and Riley, 1986).

The Role of Authentic Inquiry

The design and implementation of authentic inquiry in support of Earth and environmental education at the university level remains an important research thread. Authentic inquiry can be both a pedagogical method and student learning tool that simulates the practices and methods of scientific inquiry within diverse educational settings (Chinn and Malhotra, 2002; Rowell and Ebbers, 2004). Scientific inquiry is defined as the "diverse ways in which scientists study the natural world and propose explanations based on the evidence derived from their work" (NRC, 1996). It requires asking and refining questions, designing and conducting investigations, gathering and analyzing data, making interpretations and conclusions, and reporting findings; it

promotes the development, transformation, and representation of ideas, and it emphasizes depth and not breadth (Krajcik et al., 2000). Central to scientific inquiry is that it be in the context of authentic scientific investigations (AAAS, 1993; NRC, 1996, 2000). Authentic inquiry is described as the "research that scientists actually conduct" (Chinn and Malhotra, 2002), the "ordinary practices of the culture" (Brown et al., 1989), and the activities scientists engage in while conducting research (Dunbar, 1995; Latour and Woolgar, 1986).

Quality authentic inquiry promotes student immersion in scientific inquiry by scaffolding student development of scientific attitudes and epistemologies, their use of authentic tools and techniques, and participation in the type of social interactions characterized by scientific discourse (Edelson, 1997). Potential learning outcomes of authentic inquiry include students becoming active learners, acquiring scientific knowledge in a meaningful context, and developing styles of inquiry and communication that will help them to be effective life-long learners (Edelson, 1997). Moreover, authentic inquiry supports conceptual change because it promotes student confrontation of naive conceptions about the way the world works, allowing learning to become a meaningful process instead of merely a knowledge acquisition (Brown et al., 1989; Hart and Nolan, 1999).

The Role of IT-Supported Inquiry

There is significant debate in the science education literature about the nature of authentic inquiry and how it should be implemented in different educational contexts

(Chinn and Malhotra, 2002; Martin et al., 1990; Minstrell, 2000). Recent analyses show that most K-12 science learning environments do not reflect the characteristics of authentic inquiry (Chinn and Malhotra, 2002). This illustrates both the complex nature of scientific inquiry, with its variation across disciplines in terms of required cognitive and metacognitive processes, epistemology, and methods, and the difficulties in simulating scientific inquiry in the classroom. Information technology (IT) is one of the most important tools to help overcome these barriers, mirroring the importance IT in supporting the scientific enterprise. In the classroom, technology offers opportunities to integrate both content and process while providing the tools to communicate, contextualize knowledge, visualize data, manipulate models, and inform both students and teachers (Adams, 2004; Barab and Luehmann, 2003; Edelson, 1997, 2001). In his summary of IT-based learning, Herbert (2006) maintains that IT supports the development of higher-order cognitive skills and competencies in learners including problem-solving, knowledge transfer, and decision making by limiting barriers to student immersion in authentic scientific tasks in typical classroom contexts.

The Role of Model-Based Activities

Modeling is the central activity that defines the essence of science (Giere, 1988; Giere, 1994) where knowledge is organized around simplified models of nature. Scientists use the predictive power of models that characterize a studied system in order to induce conceptual change among the scientific community where expressed internal models are shared and revised. An internal model is defined as a relatively enduring and

accessible, but limited internal representation of an external natural phenomenon (Doyle and Ford, 1998). Student manipulation of internal models is a central component of authentic inquiry activities that support conceptual change of Earth and environmental systems. Students should be given opportunities to construct, reflect on, and revise their own conceptual models in order to assist their understanding of the nature of scientific inquiry and their ideas about the processes which drive Earth and environmental systems. Student conceptual change is often needed because of the innate complexity associated with the Earth and environmental sciences. It has been widely reported that students prefer simple conceptual model constructions (Coll and Treagust, 2003; Harrison and Treagust, 1998; Groves and Pugh, 2002), and it is likely that they have difficulty in understanding Earth systems of even modest complexity, predicting future system behavior, and reasoning correctly about associated issues (Ekborg, 2003; Forrester, 1994; Kuhn et al., 2000). However, creating conceptual models that accurately depict the components and causal relationships of a system leads to correct mechanistic and predictive power (Kaplan and Black, 2003). Instructional sequences and learning environments that stress model-based teaching and learning may address the above student learning issues and assist in the development of accurate conceptual models (Boulter and Gilbert, 2000).

CHAPTER II

VOC METABOLITES AS INDICATORS OF MICROBIAL COMMUNITY SHIFTS IN SOIL MICROCOSMS

Overview

The dynamics of soil microorganisms have important implications for the response of subsurface soil ecosystems to perturbations. Functional (e.g. enzymes), taxonomic/community-level (e.g. microbial DNA, lipids), microbial activity (e.g. carbon dioxide), and carbon and nutrient pathway (e.g. stable isotope labeling) methods have been used to characterize soil microbial processes and ecological function. These proxies are generally limited in the amount of temporal/spatial information that they can provide (e.g., community level approaches) and/or the amount of specific information they can offer about carbon sources or microbial community processes (e.g. carbon dioxide). This research validates the use of soil volatile organic compound (VOC) emissions as useful indicators of subsurface microbial community compositional changes or "shifts", as a function of changing environmental factors (e.g. substrate availability, water content, and soil type). Results of method validation using laboratory microcosms are presented, where VOC metabolites as characterized by gas chromatography and mass spectrometry (GC-MS), were related to other components including carbon dioxide (CO_2) via infra-red analysis, and microbial community structure as measured by community substrate utilization profiles (CSUPs) and fatty acid methyl ester (FAME) techniques. Results include the identification of seventy-two VOC

metabolites (< 0.01- 50 µg) produced from the soils. Principle component analysis (PCA) and hierarchical cluster analysis show that the VOC results cluster similarly to FAME and Biolog results. Analysis of variance (ANOVA) supports factor and cluster results where significant differences ($\rho < 0.01$) are shown between treatment groups. Regression analysis further shows that VOCs are significant ($\rho < 0.05$) indicators of microbial community shifts. The results are encouraging and characterize VOC production in soils as easy to measure, relatively inexpensive, and useful proxies of subsurface microbial ecosystems.

Introduction

Characterizing the microbiological role in soil ecosystems is important because soil microorganisms significantly impact the C, N, P and S biogeochemical cycles through biochemical processes including organic matter decomposition and mineralization, inorganic nutrient immobilization and assimilation, and organic nutrient accumulation (Naeem, 1997). Therefore, soil microorganisms are vital to the overall health, quality, and function of soils (Dahlhoff, 2004; Gil-Stores et al., 2005; Zelles, 1999). Common techniques to characterize processes of quantitative significance to soil ecosystems usually include the use of microbial proxies or indicators. Microbial proxies are frequently utilized to determine the microbiological impact on soil ecosystems as well as the response of the microbial community to changing environmental factors (Fang et al., 2006; Jones and Bradford, 2001; Torsvik and Øvreås, 2002). In general, these proxies can be placed into one of four methodological categories: (i) functional

characterization methods (e.g. enzymes), (ii) taxonomic and community approaches (e.g. PLFA and DNA), (iii) microbial activity measurements (e.g. CO_2 and CH_4), and (iv) carbon and nutrient pathway studies (e.g. stable isotope labeling). The following brief review provides examples of research using the above proxy methods. Finzi et al. (2006) used nine extracellular enzymes (Cellobiohydrolase, a-1,4-Glucosidase, b-1,4-Glucosidase, b-1,4-Xylosidase, Phenol Oxidase, Peroxidase, b-1,4-*N*-Acetylglucosaminidase, Leucine-aminopeptidase, and Acid phosphatase) responsible for the decomposition of labile and recalcitrant carbon substrates in order to gain insights into the microbial metabolism of soil carbon and nutrients. In addition to measuring enzyme activity, Marschner et al. (2001) studied the relationship between soil bacterial community structure and function to long-term additions of fertilizers using phospholipid fatty acids (PLFA) and polymerase chain reaction-denaturing gel electropheresis (PCR-DGGE) techniques.

Soil microbial community structure has also been studied using PLFA and CSUP analyses (Bossio and Scow, 1998; Buyer and Drinkwater, 1997; Øvreås, 2000; Widmer et al., 2001; Zelles, 1999). Fang et al. (2006) used PLFA methods to determine the microbial diversity and function in sediments of a saline groundwater seep. Metabolites produced from microbial activity have been measured to determine the microbial degradation of organic carbon in both oxic and anoxic systems (Fierer et al., 2003; Sell and Morse, 2006; Wood et al., 1993), while isotopic labeling (Boschker et al., 1998; Fernandez et al., 2003; Padmanabhan et al., 2003; Rochette et al., 1999; Schroll and Kuhn, 2004) has been used to determine the soil microbial degradation of different types

of labile organic carbon. Finally, various organic carbon compounds have also been studied such as organic acids, sugars, and amino acids to determine microbial transformation pathways of organic matter (Hedges and Oades, 1997; van Hess et al., 2005).

Specific advantages and disadvantages are associated with each proxy method, where an information continuum exists. Generally, there are two ways a proxy may be a poor source of information - if there are many processes, sources or events that may affect the proxy or if there are many intermediary steps before the proxy is produced. For example, solely utilizing soil CO_2 emission methods presents a major shortcoming where there is an inability to link specific microbial communities or organic carbon sources with its production because it is a metabolic end-member that can be produced by a variety of organisms. In contrast, genetic markers, such as PCR amplified 16S rDNA patterns, can be used to describe species variability at the genotype level (Zelles, 1999). An ease of measurement trade-off and ability to measure dynamic studies also exists for each proxy method. For example, gaseous CO_2 is relatively easy to measure and does not require sample destruction thereby supporting temporally dynamic studies. However, genetic studies require sample destruction preventing the use of dynamic studies where time intensive laboratory work dedicated to extraction and amplification procedures are also associated with their use.

VOC Metabolites as Indicators of Soil Microbial Activity and Community

The use of volatile metabolites has been suggested as a means to determine activity of microorganisms (Stahl and Parkin, 1994). A metabolite may be described as "volatile" if it is a gas, has a high vapor pressure, and under usual conditions is liberated from a cell (Hutchinson, 1973). Previous research has shown that VOCs can have many sources in soils including germinating seeds and roots (Stotzky and Schenck, 1973), foliage and wood (Adebajo et al., 1989; Dean and Svoboda, 1990; Hartill and Suton, 1980), and dead plants and residues (Berestetskii and Kravchenko, 1984; Owens et al., 1969). Fungi, actinomycetes, and bacteria can also produce VOCs during both aerobic and anaerobic respiration, where VOCs are the by-products of organic matter degradation.

During direct-culture studies, results have been encouraging for the use of VOCs as indicators for microbial activity and microorganism presence through distinct chromatographic profiles where specific microbes under various environmental conditions produce different VOC metabolites (Grametbauer et al., 1988). For instance, several VOCs have been found to be dependent on both fungal species type and growth mediums (Sunesson et al., 1995). Larsen and Frisvad (1995a) studied 47 fungal species from the *Penicillium* taxa and concluded that sesquiterpenes have the most potential to serve as taxonomic markers. Gerber (1977) studied metabolites from an actinomycete (*Steptomyces* spp.) and found three highly odorous VOCs including 2-isopropyl-3-methoxy-pyrazine, methylisoborneol, and geosmin. Schöller et al. (1997) studied gram-negative bacteria (*Pseudomonas* spp., *Serratia* spp., and *Enterobacter* spp.) and found

emissions of various VOCs in the different populations. Common VOCs that have been measured during direct-culture include carboxylic acids, ketones, alcohols, aldehydes, terpenes, esters, ethers, hydrocarbons, and organic sulfur derivatives.

Although, the above work is useful for progress in utilizing VOCs as indicators of microbial activity and community structure, all of these studies have been conducted in simple matrices (e.g. petri dishes) with culturable microorganisms in an environment that does not reflect the complexity of a soil system. However, the variety of the examples prompts consideration of VOCs potential significance as proxies in soils (Linton and Wright, 1993). Stahl and Parkin's (1994, 1996) work is the only to date that has been conducted in soil microcosms and includes the measurement of VOC metabolites. Their work encompassed the use of 2-methylisoborneal and geosmin as indicators of fungal growth. Geosmin and 2-methyl isoborneal are popularly studied because they are attributed to the "earthy smell" typical for soil. Geosmin has been found as a metabolite from many different members of the actinomycetes (Gerber, 1967), by cyanobacteria (Safferman et al., 1967), and by fungus (Karahadian et al., 1985; Matteis and Roberts, 1992). It is responsible for the unpleasant taste of drinking water and off-flavor of beets and beans (Trowitzsch et al., 1981). Stahl and Parkin (1996) also conducted a study that utilized soils treated with fungicides and bacteriocides to shift the community dominance. They found low microbial activity (via CO_2 measurements) also had low VOC production (and visa versa) and treatments with different dominating microbial groups produced different kinds of VOCs. Their results

indicate that measurement of VOC metabolites may be able to aid in the differentiation of microbial groups in soil.

As part of the information continuum, VOCs may be considered as both microbial activity and community proxies. VOCs may provide more information than CO_2 activity studies but less information than genetic studies. This can be attributed to the linking of VOCs to both the organic carbon source and the microbial community structure, but linking to specific microbial species is much more difficult during ecosystem studies. Furthermore, VOC collection does not require sample destruction, thereby supporting temporally dynamic studies. However, sensitive instrumentation (e.g. GC-MS) must be used to quantify and identify VOCs since the recoverable mass is usually measured at the nano- to micro-gram ranges.

Research Objectives

The objectives of this work were to (i) establish a methodology to measure VOC production as an indicator of microbial activity in soil microcosms, (ii) determine if shifts in VOC metabolites correspond with changes in other measures of microbial activity (e.g. CO_2) and community structure (e.g. CSUP and FAME), and (iii) determine if VOCs can be used as predictors of microbial community compositional changes or "shifts" during perturbation in soil microcosm studies. Soils for microcosm studies were collected from Unit 7 of the Laguna Atascosa National Wildlife Refuge (LANWR) in Southern Texas, a former US Army Air base converted to what is currently the largest protected area (263 km^2) of natural habitat left in the Lower Rio Grande Valley. This

area was chosen because it is a coastal interfacial area that represents a nexus for variations in substrate availability, water content, and soil type. Microcosm experiments were conducted with selected LANWR soils where an assortment of environmental factors were varied and included the effects of substrate availability/microbial presence, water content, and soil type on microbial emissions of VOC metabolites. These perturbations were selected because it is well known that environmental conditions affect microbial survival and community structure (Franklin and Mills, 2003; Dahlhoff, 2004; Musslewhite et al., 2003). Additional measurements including CO_2, FAME and CSUP analyses were used to determine the validity of the VOC technique.

This study focused on the measurement of VOCs produced by microorganisms to infer states of perturbations in a soil microcosm. It was hypothesized that the amount of microbially produced VOCs would vary as the environmental factors in a soil microcosm were perturbed and the VOC results would align with results of the additional measures of microbial activity and community structure.

Materials and Methods

Sample Collection Location and Procedures

Soils from three LANWR locations were used in soil microcosm studies: site 3 (fine, smectitic, hyperthermic Udic Haplusterts), site 8 (fine-silty, mixed, active, hyperthermic Typic Aquisalids), and site 11 (fine, mixed, active, calcareous, hyperthermic Typic Halaquepts). Site 11 represented soil collected near the freshwater

(salinity = 7) Laguna Atascosa Lake and is characterized as a seasonal wetland area; site 8 represented soil collected at a greater elevation (~3 m) close to the hyper-saline (salinity = 47) Laguna Madre Estuary and is characterized as an coastal upland area; and site 3 represented soil collected near a Rasaca (dried-up meandering river) from a grassland area.

At each site, surface samples in the A-horizon (< 0.25 m) were collected during August in replicates of three, < 1 m apart, mixed into one homogeneous soil sample, and taken back to the laboratory where they were sieved (2 mm), air-dried, and analyzed for typical soil characteristics as described in the methods and materials section. Since the summer months reach very high temperatures (> 37°C) and soils have low moisture (0.08-0.27) in the region, air-drying of samples likely had little impact on the soil moisture content; further, it is not the intent of this work to describe the field conditions but instead develop and validate the VOC technique and therefore any possible alterations of *in-situ* soil moisture does not impede the objectives of this research. In the field, soil moisture was measured using a Delta-T (model HH2) moisture meter and Theta (model ML2x) moisture probe. Three replicate soil moisture measurements at each surface location were made and results were averaged for each site (Table 2.1). *In-situ* ground conductivity measurements were made at each site using a Geonics Limited (EM31) analyzer, where replicate measurements from each site were averaged (3 m^2; Table 2.1).

Soil Characteristics

The laboratory characterization of the soils from the LANWR sites included citrate dithionate extractable iron following the methods of Leoppert and Inskepp (1996) using a Perkin Elmer flame atomic absorption analyzer; quantification of total organic carbon (TOC), total inorganic carbon (TIC), and total nitrogen using a Vario El II elemental analyzer following the methods of Leoppert and Suarez (1996); quantification of soil protein via extraction following the methods of Raab et al. (1999) and assay measurement following Bradford (1976) using a Hewlett Packard UV-Visible spectrophotometer; soil texture fractionation following the methods of Whitting and Alladrice (1986); and identification of the sand, silt, and clay mineralogy using a Rigaku powder x-ray diffractometer (XRD). Results from these analyses for each soil location are provided in Table 2.1.

Microcosm Experimental Design

The microcosm experiments followed a blocked design where treatments of soil water content, soil type and substrate availability were varied (Table 2.2). Two sets of controls were used in the experiments. One set of controls had no growth amendment added to the soil (Cna). The second set were abiotic controls (Ch) that had growth amendments added but underwent heating at 250˚C (Dunn et al., 1985) for two hours and had a saturated solution of mercuric chloride ($HgCl_2$) added to the soil instead of nanopure water (Tuominen et al., 1994).

Table 2.1
Site characterization for each LANWR soil used in microcosm studies. The latitude and longitude, site description, site elevation, soil texture, total organic carbon (TOC), total inorganic carbon (TIC), carbon-to-nitrogen (C/N) ratio, soil conductivity, soil moisture, reactive iron, protein concentrations, and the sand, silt, and clay soil mineralogy

Soil Characteristics	**LANWR SITE**		
	11	**8**	**3**
Collection Location (Deg. Min. Sec.)	26° 13”24.7’ 99° 21”47.8’	26° 09”38.8’ 104° 18”28.9’	26° 12”54.6’ 107° 23”30.8’
Site Description	Seasonal Wetland	Coastal Upland	Grassland Region
Site Elevation (m)	0	3	0
Soil Texture	Sand Loam	Clay Loam	Silt Loam
Soil TOC (%)	1.15	1.85	1.09
Soil TIC (%)	0.97	0.88	1.44
Soil C/N (Ratio)	9.85	16.98	9.81
Soil Moisture (%)	27.0	10.8	8.0
Soil Conductivity (mmohs)	490.0	180.0	305.0
Soil Reactive-Fe (μmol g^{-1})	27.79	35.69	53.07
Soil Protein (mM g^{-1})	0.062	0.048	0.062
Clay Fraction Minerals	Smectite; Mica; Kaolinite	Smectite; Mica; Kaolinite	Smectite; Mica; Kaolinite
Silt Fraction Minerals	Quartz; Albite	Quartz; Calcite; Cuprite	Quartz
Sand Fraction Minerals	Quartz	Quartz	Quartz; Anorthite

Microcosm experiments utilized 50.00 g (air-dry weight) of sieved (2 mm) surface soils collected from the LANWR. VOC production was quantified as a function of three environmental factors including (1) substrate availability/microorganism presence (clover amended, non-amended, and "killed" controls), (2) water content (5%, 25%, 50%), and (3) soil texture and type (sand loam, silt loam, clay loam). After appropriate additions of growth amendments (1 g of dried ground clover; C/N = 13.24 ± 0.32) and nanopure (18 MΩ) water were added, the microcosm soils were thoroughly mixed and capped. Samples were immediately connected to the micro-oxymax system and experiments took place at room temperature (20˚C ± 1˚) for two weeks. The system was closed with the exception of daily (every 24 hours) atmospheric air refreshments that passed through both a soda lime and drierite column before reaching microcosms to remove atmospheric CO_2 and moisture (Fig. 2.1). Refreshments were conducted in order to prevent CO_2 concentrations from reaching levels that were too high for accurate measurement within the linear range of the instrument.

Preliminary calculations and direct measurements showed low and high CO_2 production rates of 2 - 30 mg day^{-1} where a daily refresh rate would result in O_2 atmospheric amounts of 12.9 - 2.3 mg in the 100 mL jars. We have no direct measurements of the development of aerobic/anaerobic conditions however the presence of particular VOCs (e.g. sulfide and methyl derivatives) suggests that the microcosms may have gone slightly anaerobic during certain regimes (e.g. 50% water additions).

Table 2.2
An outline of the microcosm experiments describing the number of replicates for each of the three environmental factors, treatment levels, and the number of conducted experiments (Superscripted letters represent data supporting Fig. 2.3 – 2.8)

Soil Type	**Water Content**	**Substrate Availability/Microbial Presence**			**Experiment Number** (duration)
		No. of Treatments	No. of Non-Amended Controls	No. of Killed Controls	
Sand Loam	5%	4	3	3	1 (2 weeks)
	25%	4[c]	3	3	2 (2 weeks)
	50%	4	3	3	3 (2 weeks)
Silt Loam	5%	4	3	3	4 (2 weeks)
	25%	4[a,c]	3[a]	3[a]	5 (2 weeks)
	50%	4	3	3	6 (2 weeks)
Clay Loam	5%	4[b]	3	3	7 (2 weeks)
	25%	4[b,c]	3	3	8 (2 weeks)
	50%	4[b]	3	3	9 (2 weeks)

Microbial Respiration Measurements

Microxymax System Measurement of Gaseous CO_2 Measurements

Overlying head-space from each microcosm jar was sampled for CO_2 via the micro-oxymax pump and an infrared sensor and returned to the jar after analysis through a closed system. CO_2 sampling intervals were every four hours for a two-week time period where refreshing of the microcosm headspace occurred once a day or when CO_2 concentrations exceeded the 0-1% threshold of the instrument sensors, which ever occurred first.

GC-MS Measurements of VOCs

VOCs were concentrated on 100 mg of Tenax (Chromosorb©) in a brass sample tube (8 cm x 0.64 cm) and thermally desorbed using a Perkin Elmer ATD 400 and directly injected into a Hewlett- Packard 5890 Series II Plus Gas Chromatograph equipped with a DB-624 fused silicone megabore column (30 m x 0.32 mm) coupled to a Hewlett-Packard 5972 Series mass spectrometer (Fig. 2.1). Before use, Tenax tubes were pre-conditioned for 15 min. at four temperatures (250˚C, 300˚C, 330˚C, 350˚C) and after use, re-conditioned for 10 min. (330˚C). Sample tubes were desorbed with He at 75 mL $min.^{-1}$ for 10 min. at 220˚C and concentrated in a cold trap at -30˚C.

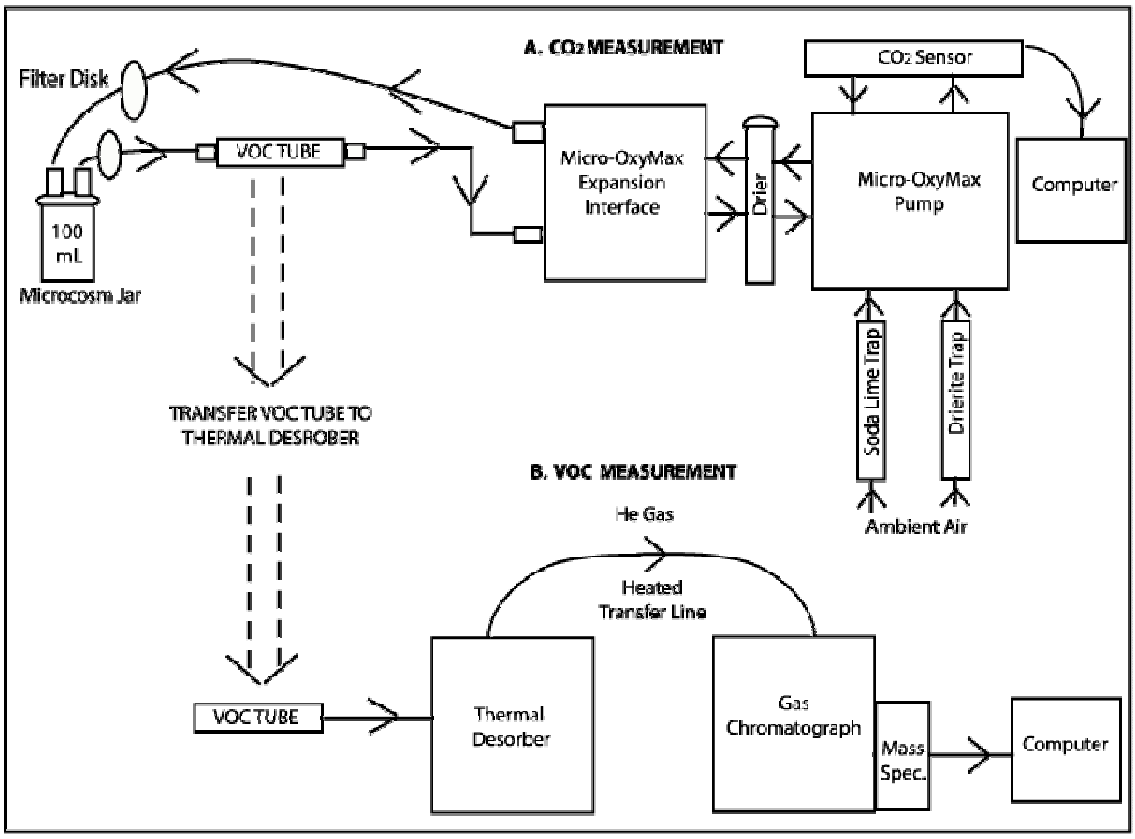


Fig. 2.1. Simplified schematic of the microcosm set-up indicating the microbial metabolism measurements of (A) CO_2 using an infra-red sensor and (B) VOCs using a thermal desorber unit connected to a gas chromatograph and mass spectrometer.

Chromatographic conditions included splitless injection with He carrier gas at 1 mL min.$^{-1}$ constant flow rate. The oven program was held at stages of 35˚C for 2 min. then 6˚C min.$^{-1}$ to 70˚C, then 15˚C min.$^{-1}$ to 145˚C, and isothermal at 145˚C for 17 min. Electron ionization (70eV) GC-MS was conducted in full scan mode for quantitative analysis. Temperature at the source was set at 280°C.

Internal standards (Ultra Scientific; 99.5%) were injected into soil microcosms and included Chlorobenzene-d5, 1,4-dichlorobenzene-d4, and fluorobenzene. An

internal standard recovery of 85% or greater was required. System standards (Ultra Scientific; 99.5%) were injected into sample tubes before analysis on the ATD and included 4-bromofluorobenzene, dibromofluoromethane, and tolune-d8. Surrogate compounds (Absolute Standards; 99.5%) including propanol-2, butanol-1, toluene, xylene, furan, ethyl acetate, geosmin, 2-methylisoborneol, isoprene, 3-methyl-1butanol, 2-pentanone, and 1-undecene were used to quantify compounds identified in the Hewlett-Packard NIST98 library. A 70% library match was required to identify compounds.

Microbial Community Measurements

Carbon Substrate Utilization Profiles

Gram-negative bacterial communities were characterized using community level physiological profiles. The 96 well Biolog (GN-2) plates with various carbon substrates were used to provide information about substrate utilization and functional diversity of aerobic soil bacteria (Goberna et al., 2005; Widmer et al., 2001; Zak et al., 1994). Ten grams of microcosm soil was suspended in 90 mL of sterilized water and 125 µL of the diluted solution was added to the plates and incubated for 96 hours (Widmer et al., 2001). Colormetric changes (595 µm) were detected on a scanner (Labsystems Multiskan MS) every 24 hours for four days and community differences between samples were determined using the Multiskan Transmit Program (revision 1.2).

GC-MS Measurement of Fatty Acid Methyl Esters

FAMEs were analyzed according to Bottomley (2005). Briefly, fatty acids were liberated from soils through heating at 100˚C in a KOH and methanol solution. Conversion to FAME via use of acetic acid and heat was made and extraction was conducted in a hexane solution. Analysis was then possible via gas chromatography (Agilent model 6890). A cross-linked 5% phenyl methyl silicone Hewlett Packard Ultra 2 column (25 m x 0.20 mm) was used with a flame ionization detector (FID) detector. The method lasted a total run time of 36.318 min. at stages of 170˚C for 0 min. then 5˚C $min.^{-1}$ to 300˚C and hold for 12 min. Helium flow rates were 0.6 mL $min.^{-1}$ and the split ratio was 50:1. Peaks were named using Sherlock Eukary program (supplied by MIDI - Microbial ID of Newark, Delaware).

Statistical Procedures

In this study, data points that were not detectable were considered as zero values and low recovery (< 85%) samples were not used in statistical analysis. Statistical results from selected examples are shown in the figures and tables of this paper, however the remaining un-presented data that met the recovery requirements showed similar trends. Multivariate data reduction techniques for large datasets, specifically factor and cluster analyses, were used to identify microbial community shifts during the various experimental treatments and have been frequently utilized in environmental geochemical research (Camdevýren et al., 2005; Kowalkowski et al., 2006; van Helvoort et al., 2005) and soil microbiological research (Gobera et al., 2005; Hackl et al., 2005; Øvreås, 2000;

Widmer et al., 2001). Variables were extracted through the use of PCA with a Varimax rotation and Kaiser normalization. Because PCA is based solely on the eigenanalysis of the correlation or covariance matrix, no constraints such as multivariate normality are imposed on the data (Farnham et al., 2003) and therefore these tests were not required of the dataset. The four factor loading plots represented results from either the concentration of VOCs, the concentration of CO_2, the CSUP (Biolog) average well color development, or the FAME response for each of the ten microcosms. Factor analysis reduced the multivariate dataset into linear combinations of the most relevant variables to facilitate interpretations and enable the recognition of differences and similarities between variables in the dataset. Groups of variables that had loadings of the same sign (i.e. positive or negative) and magnitude on a factor component described similar anomalies or patterns in the data set, while variables with opposite signs did not. Preferred clusters indicated the various treatment groups and replicates. Comparisons between plots were made to determine if loading clusters coincided between methods.

Hierarchical cluster analysis was performed in order to show further relationships between groups of similar and dissimilar data and used to support factor analysis results. The distance of five was used as the threshold value to determine differences between clusters. Additionally a one-way, non-parametric (Kruskal-Wallis) analysis of variance (ANOVA) was conducted on the raw VOC data for each experiment where the various treatment level medians were compared. This was done to determine whether the clustered data in the factor analysis were statistically significantly different. Finally, a step-wise, multiple, linear regression was conducted to indicate the predictability of the

FAME results by VOC concentrations, CSUP (Biolog) average well color development, and CO_2 concentrations.

Results

Soil Characteristics

Soil characterization results for each LANWR soil collection location show that the soils were different in regard to soil texture (Table 2.1) and results indicate that although the soil TOC (1.15-1.85%) and TIC (0.88-1.44%) were similar, the C/N ratio for site 8 was greater (16.98) than sites 11 and 3 (9.81-9.85). The reactive-Fe for the soils was also variable at concentrations of 27.79 µmol g^{-1}, 35.69 µmol g^{-1}, and 53.07 µmol g^{-1} for sites 11, 8, and 3, respectively. Both the soil protein (0.048-0.062 mM g^{-1}) and the soil minerals were similar for the three soils, with the exception of the presence of cuprite (Cu_2O) at site 8 which may have been due to the close proximity (< 1.5 km) to the pre-existing gunnery and remaining bullet casings in the soil of that location. Elevation at site 8 was also greater (3 m) than the other two sites (0 m), but soil conductivity was less (180 mmohs) at site 8 than at sites 11 (490 mmohs) and 3 (305 mmohs).

Detected and Quantified VOCs

Table 2.3 lists the seventy-two detected and quantified VOCs in all soil microcosm treatments. VOCs in the functional group categories including alcohols, aldehydes, aromatics, carboxylic acids, esters, hydrocarbons, ketones, nitriles, sulfides, and terpenes were identified using the NIST98 library, where a 70% match was required for identification, and quantified using functionally and/or structurally appropriate surrogate compounds. Not all soil treatments exhibited each of the seventy-two VOCs, as can be seen in the example chromatograph featuring soil from LANWR site 8 at the 25% water content experiment (Fig. 2.2).

Substrate Availability/Microbial Presence Perturbations

Factor Analysis

Fig. 2.3a-d shows component loadings and preferred clusters of the separate factor analyses for the VOC, FAME, Biolog, and CO_2 methods during substrate availability perturbations. VOC results show that component 1 represented the non-amended control (Cna) treatment and included 34.11% of the total variance, component 2 represented the clover amended treatment (t) and included 31.61% of the total variance, and component 3 represented the "killed" control treatment (Ch) and included 30.77% of the total variance (Table 2.4a).

Table 2.3.
Identification (ID) number, name, and retention time (RT) of the detected and identified VOCs (Asterisks indicate compound detected in previous literature studies)

ID	ALCOHOLS (11)	RT
16	*Butanol-1	9.98
15	*Butanol-2methyl	9.98
5	*Propanol-2	7.56
9	Silanol, trimethyl	8.75
26	2-Butanol	12.39
31	*1-Pentanol, 2-methyl	12.65
48	Ethanol	16.03
52	*1-Octen-3-ol	16.64
56	*3-Octanol	16.98
64	*1-Hexanol, 2-ethyl	18.09
68	Phenol	18.68
ID	**ALDEHYDES (9)**	**RT**
6	Butanal	7.97
11	Butanal, 3 methyl	9.83
18	Pentanal	10.65
27	Hexanal	12.78
36	Furfural	13.99
41	Heptanal	14.73
53	Benzaldehyde	16.62
74	Benzaldehyde, 2-hydroxy	19.55
81	Decanal	26.19
ID	**AROMATICS (15)**	**RT**
10	Furan, tetrahydro	8.93
24	Pyridine	11.72
28	Cyclobutanol, 2-ethyl	12.77
29	Pyridine, 2-methyl	12.96
32	Pyridine, 3-methyl	13.2
35	*p-xylene	13.91
37	Styrene	14.45
42	Pyrazine-2,5 dimethyl	14.7
45	Benzene	15.09
47	Oxime-methyoxy-phenyl	15.48
50	Furan, 2-pentyl	16.29
51	Alpha-methylstyrene	16.43
66	*3-Methoxy-2,5 dimethylpyrazine	18.53
	Cyclohexanol, 5-methyl-2 (1methylethyl)	24.73
79	*Furan	24.82
ID	**CARBOXYLIC ACIDS (2)**	**RT**
13	Acetic Acid	9.83
57	Pentanoic Acid	17.56
ID	**ESTERS (2)**	**RT**
8	*Ethyl Acetate	8.32
84	Propanoic Acid, 2-methyl, butyl ester	29.09
ID	**HYDROCARBONS (13)**	**RT**
30	2,4-Dimethyl-1-heptene	12.65
33	Decane, 2-methyl	13.27
62	Pentane,2,2,3,4-tetramethyl	17.6
63	Heptane,2,6-dimethyl	17.62
65	1-Hexene-3,3,4-trimethyl	18.5
67	*1-Undecene	18.55
70	Heptane, 2,4-dimethyl	18.65
69	2,4 Hexadiene	18.97
71	Decane, 2-methyl	19
72	Undecane,2,4- dimethyl	19
73	Undecane,4- methyl	19
77	Octane 2,7 Dimethyl	23.1
83	Dodecane	28.43
ID	**KETONES (8)**	**RT**
17	*2-Butanone, 3 methyl	10.65
19	*2-Pentanone	10.65
25	*2-Pentanone, 3-ethyl	12.65
39	*3-Heptanone	14.43
38	*2-Hexanone	14.45
40	2-Hexanone, 5-methyl	15.03
55	*3-Octanone	16.4
76	Acetophenone	20.48
ID	**NITRILES (3)**	**RT**
12	Propanenitrile	9.76
21	2,4 Pentadienenitrile	11.72
59	Benzonitrile	17.62
ID	**SULFIDES (2)**	**RT**
20	*Disulfide, dimethyl	11.63
54	*Dimethyl, trisulfide	16.74
ID	**TERPENES (7)**	**RT**
46	*Camphene	15.62
43	*1S-Alpha-pinene	15.04
44	1,6 Octadiene, 3,7 dimethyl	15.4
49	*B-Pinene	16.22
58	*Limonene	17.43
78	Santolina triene	24.38
82	*2-Methylisoborneol	26.1
ID	**INTERNAL STANDARDS (6)**	**RT**
IS	Dibromofluorobenzene	9.26
IS	Fluorobenzene	9.98
IS	Toulene-D8	11.88
IS	Chlorobenzene-d5	13.58
IS	4-bromofluorobenzene	15.22
IS	1,4-Dichlorobenzene-d4	17.52

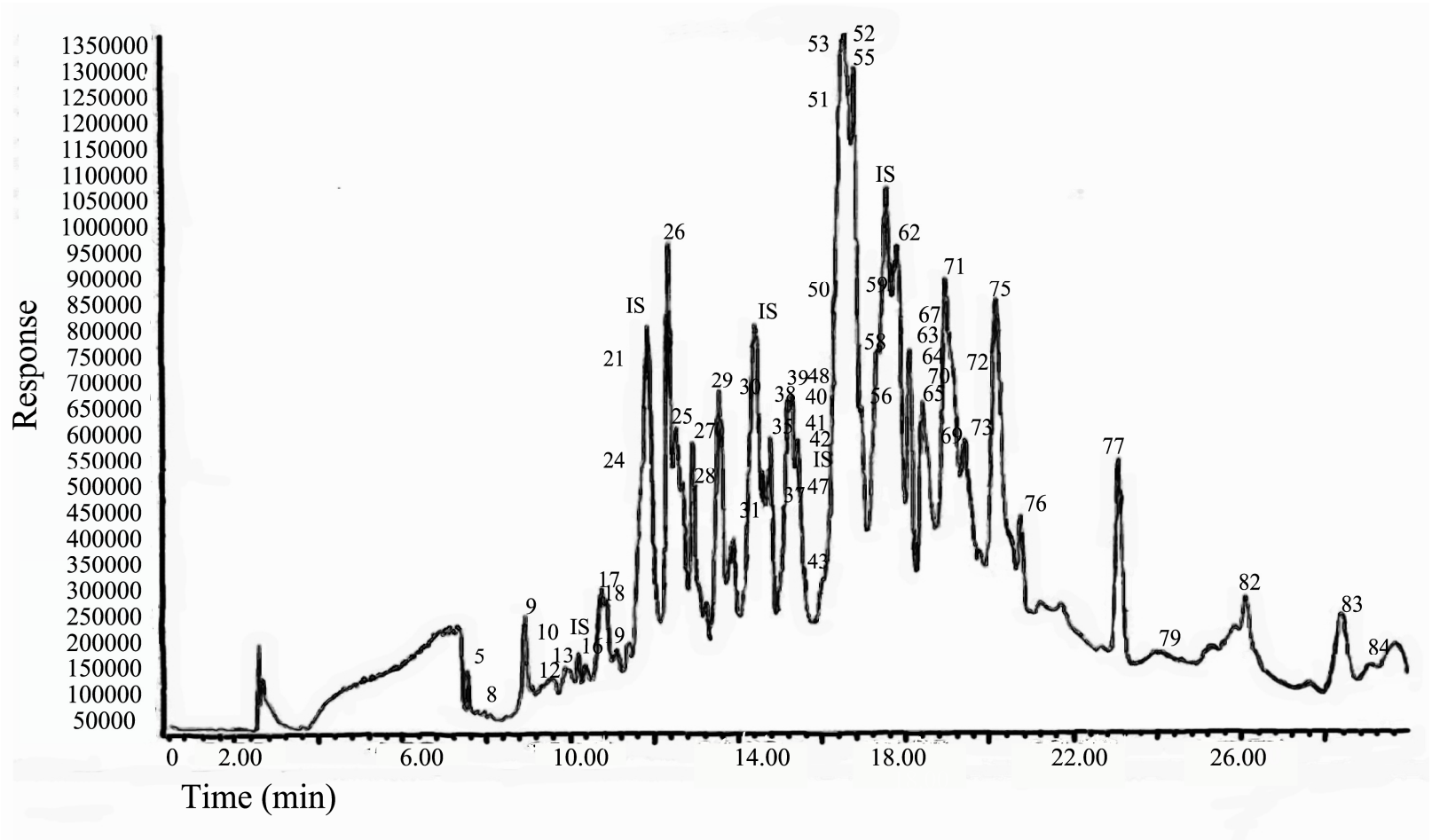


Fig. 2.2. An example chromatograph of identified and quantified VOCs from soil microcosms.

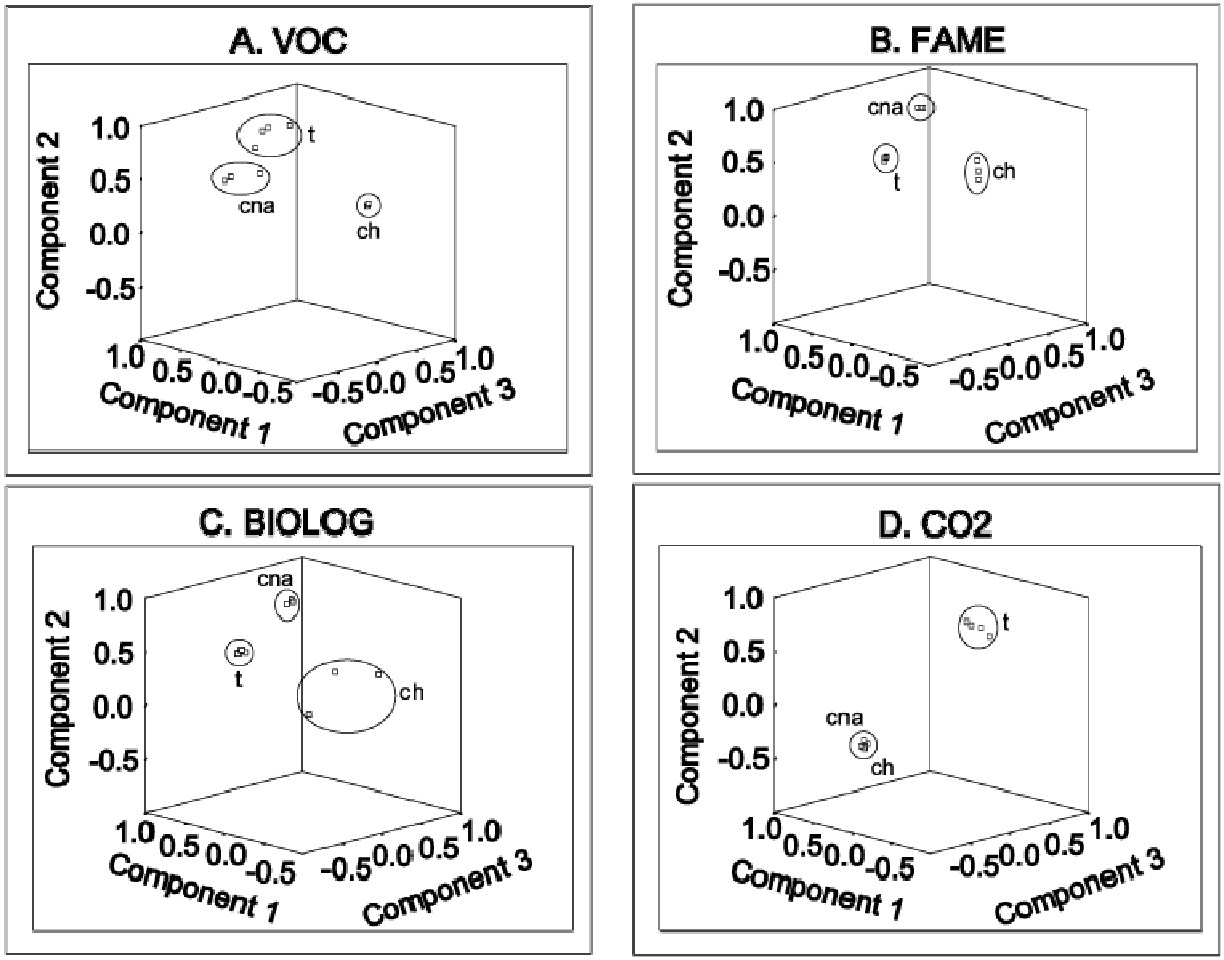


Fig. 2.3. Factor loading plots during substrate perturbations for amended replicates (T, Cna, Ch). Clusters for VOC (A), FAME (B), Biolog (C), and CO_2 (D) methods are represented by a circle. Data symbols overlap in some cases.

Table 2.4.
Substrate availability treatment factor loadings for VOC (A), FAME (B), Biolog (C), and CO_2 (D). Component groupings are indicated in gray. ND = No data

A. VOC

TREATMENT	COMPONENT (% Variance)		
	1 (34.11)	**2 (31.61)**	**3 (30.77)**
T1	0.691	0.636	0.140
T2	0.388	0.911	0.0134
T3	0.505	0.841	0.0479
T4	0.206	0.951	0.105
CNA1	0.849	0.321	0.356
CNA2	0.931	0.302	0.000788
CNA3	0.930	0.330	0.00830
CH1	0.0842	0.0805	0.984
CH2	0.111	0.0503	0.984
CH3	0.111	0.0436	0.987

B. FAME

TREATMENT	COMPONENT (% Variance)		
	1 (42.80)	**2 (32.20)**	**3 (25.00)**
T1	0.880	0.326	0.329
T2	0.902	0.279	0.325
T3	0.896	0.310	0.308
T4	0.892	0.311	0.322
CNA1	0.281	0.932	0.166
CNA2	0.324	0.925	0.181
CNA3	0.268	0.936	0.183
CH1	0.330	0.096	0.938
CH2	0.300	0.287	0.900
CH3	0.307	0.178	0.928

C. BIOLOG

TREATMENT	COMPONENT (% Variance)		
	1 (38.23)	**2 (28.96)**	**3 (15.77)**
T1	0.873	0.305	0.135
T2	0.899	0.328	0.114
T3	0.923	0.287	0.0871
T4	0.906	0.307	0.0178
CNA1	0.293	0.874	0.0792
CNA2	0.177	0.920	0.0479
CNA3	0.272	0.903	0.132
CH1	0.482	-0.259	0.325
CH2	0.388	0.0943	0.789
CH3	-0.767	0.133	0.885

D. CO_2

TREATMENT	COMPONENT (% Variance)		
	1 (55.52)	**2 (44.27)**	**3 (0.17)**
T1	-0.656	0.749	0.903
T2	-0.575	0.816	0.552
T3	-0.523	0.852	-0.139
T4	-0.476	0.878	-0.301
CNA1	0.849	-0.527	0.287
CNA2	0.831	-0.555	-0.0391
CNA3	0.848	-0.529	-0.0418
CH1	0.830	-0.558	-0.0140
CH2	0.842	-0.539	-0.0103
CH3	0.873	-0.486	0.00432

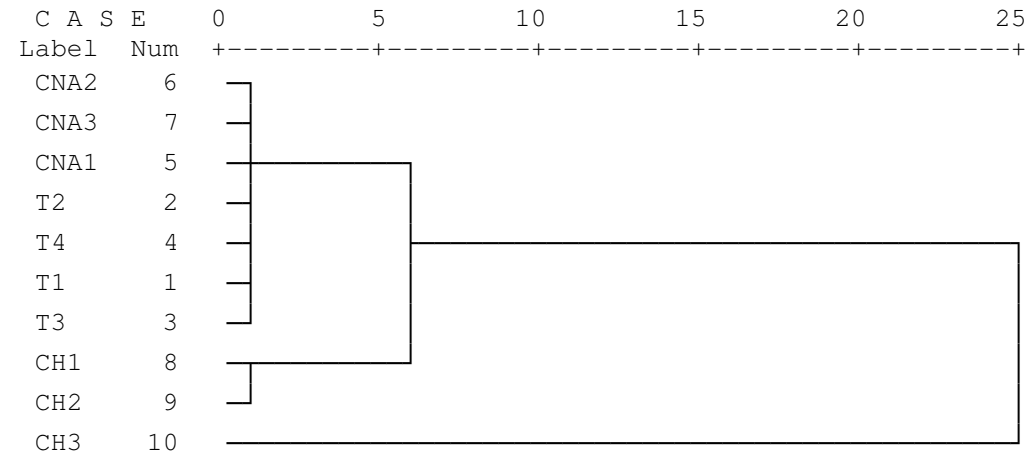
FAME results show that component 1 represented the clover amended treatment (t) and included 42.80% of the total variance, component 2 represented the non-amended control (Cna) treatment and included 32.20% of the total variance, and component 3 represented the "killed" control (Ch) treatment and included 25.00% of the total variance (Table 2.4b). Biolog results show component 1 represented the clover amended treatment (t) and included 38.23% of the total variance, component 2 represented the non-amended control treatment (Cna) and included 28.96% of the total variance, and component 3 represented the "killed" control (Ch) treatment and included 15.77% of the total variance (Table 2.4c). CO_2 results show that only two groups clustered where the component 1 represented both control groups and included 55.52% of the total variance clover, while the amended treatment (t) was represented by component 2 and included 44.27% of the total variance (Table 2.4d).

All methods, but the CO_2 analysis, showed separate clusters in PCA plots for each of the three treatments, where replicates from the same treatment clustered together and those from different treatments clustered farther apart. The CO_2 method did not differentiate between the non-amended and "killed" control groups (Cna and Ch).

Cluster Analysis

Cluster analysis (Fig. 2.4a-d) further illustrates the relationships between treatment groups. Results for the individual methods (VOC, FAME, Biolog, and CO_2) largely support the factor analysis results. Both the FAME and the Biolog analyses have three distinct clusters and the CO_2 analysis only has two clusters.

A. VOC



B. FAME

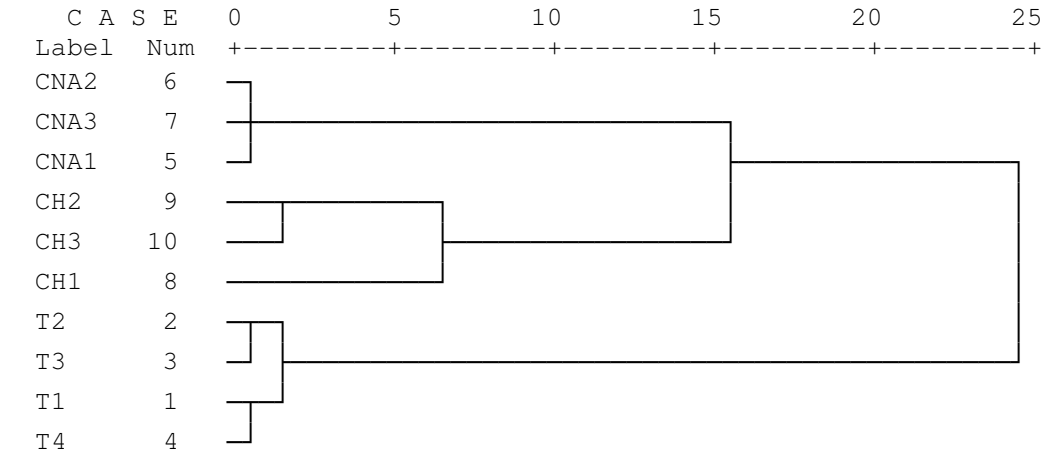
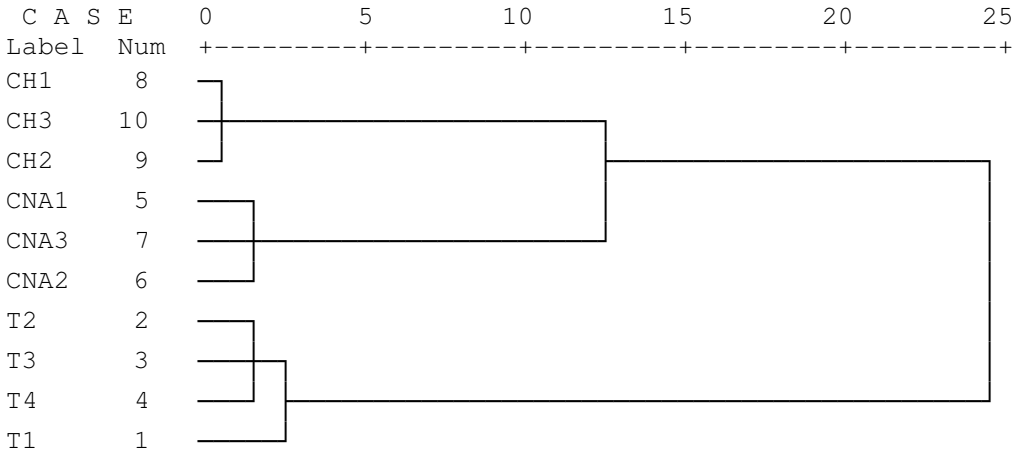


Fig. 2.4. Dendrograms for VOC (A), FAME (B), Biolog (C), and CO_2 (D) methods during substrate perturbations (T, Cna, and Ch).

C. BIOLOG



D. CO_2

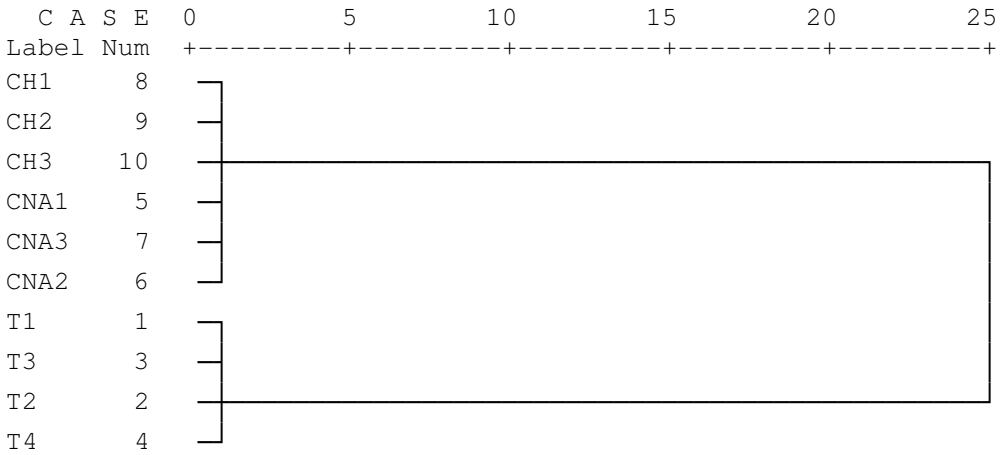


Fig. 2.4. Continued.

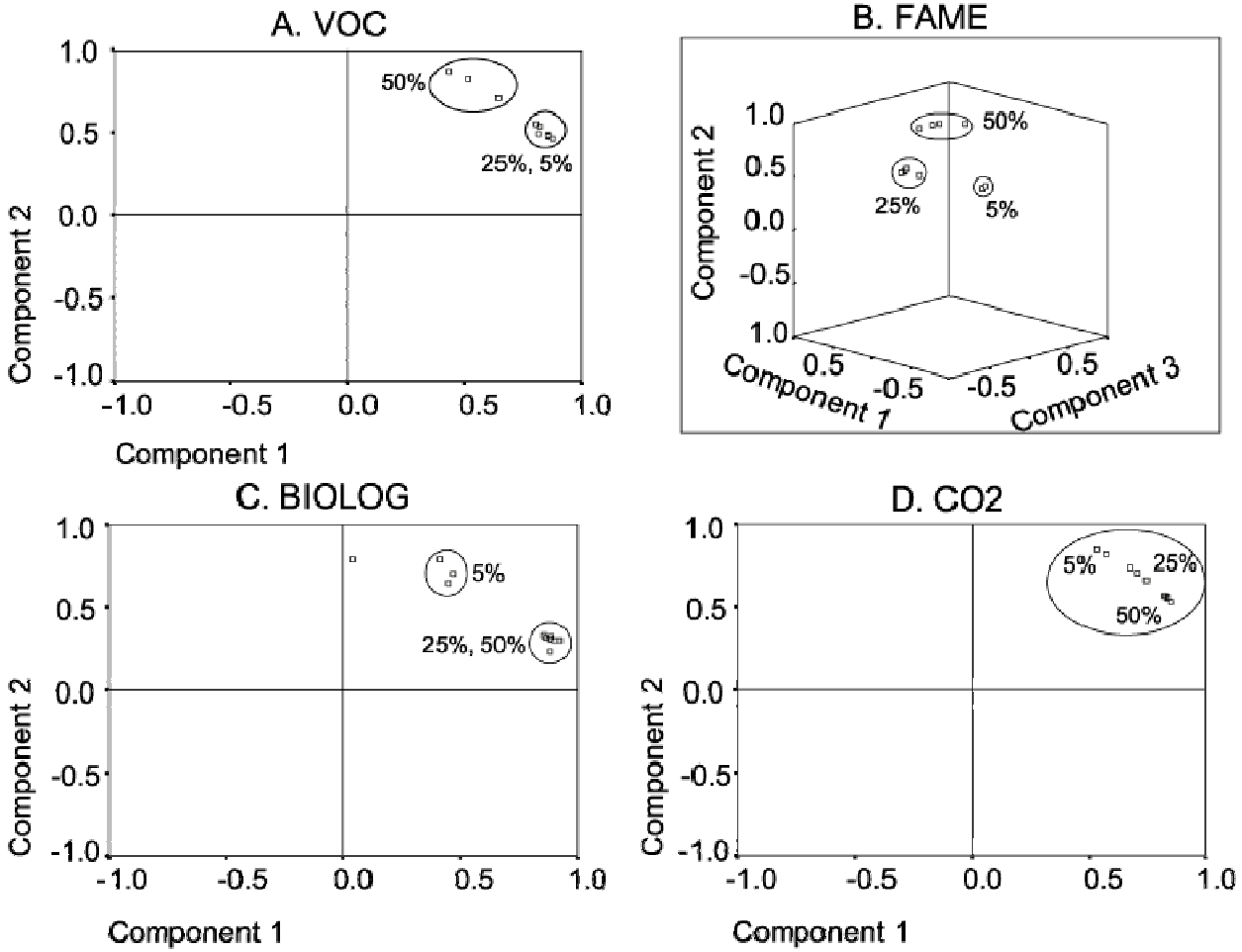


Fig. 2.5. Factor loading plots during water content perturbations (5%, 25%, and 50%). Groups of clusters for VOC (A), FAME (B), Biolog (C), and CO_2 (D) are represented by a circle. Data symbols overlap in some cases.

Table 2.5
Water content treatment factor loadings for VOC (A), FAME (B), Biolog (C), and CO_2 (D). Component groupings are indicated in gray. ND = No data

A. VOC

TREATMENT	COMPONENT (% Variance)	
	1 (56.84)	**2 (38.28)**
5%T1	ND	ND
5%T2	0.821	0.538
5%T3	0.879	0.462
5%T4	0.859	0.479
25%T1	ND	ND
25%T2	ND	ND
25%T3	0.808	0.555
25%T4	0.861	0.487
50%T1	0.431	0.869
50%T2	0.644	0.706
50%T3	0.818	0.493
50%T4	0.514	0.820

B. FAME

TREATMENT	COMPONENT (% Variance)		
	1 (36.13)	**2 (31.46)**	**3 (25.02)**
5%T1	0.0800	0.187	0.946
5%T2	0.425	0.165	0.875
5%T3	0.477	0.140	0.846
5%T4	0.169	0.896	0.340
25%T1	0.846	0.259	0.455
25%T2	0.892	0.309	0.300
25%T3	0.891	0.312	0.276
25%T4	0.881	0.326	0.304
50%T1	0.493	0.835	0.103
50%T2	0.282	0.910	0.143
50%T3	0.620	0.425	0.0534
50%T4	0.322	0.905	0.0994

C. Biolog

TREATMENT	COMPONENT (% Variance)	
	1 (57.90)	**2 (23.98)**
5%T1	0.467	0.703
5%T2	0.0438	0.784
5%T3	0.445	0.644
5%T4	0.412	0.784
25%T1	0.886	0.312
25%T2	0.926	0.300
25%T3	0.906	0.300
25%T4	0.905	0.305
50%T1	0.892	0.324
50%T2	0.884	0.238
50%T3	0.871	0.316
50%T4	0.861	0.328

D. CO_2

TREATMENT	COMPONENT (% Variance)	
	1 (57.92)	**2 (42.00)**
5%T1	ND	ND
5%T2	0.573	0.818
5%T3	0.532	0.846
5%T4	0.531	0.847
25%T1	0.678	0.733
25%T2	0.707	0.705
25%T3	0.747	0.664
25%T4	0.820	0.567
50%T1	0.825	0.564
50%T2	0.832	0.554
50%T3	0.849	0.528
0%T4	0.831	0.556

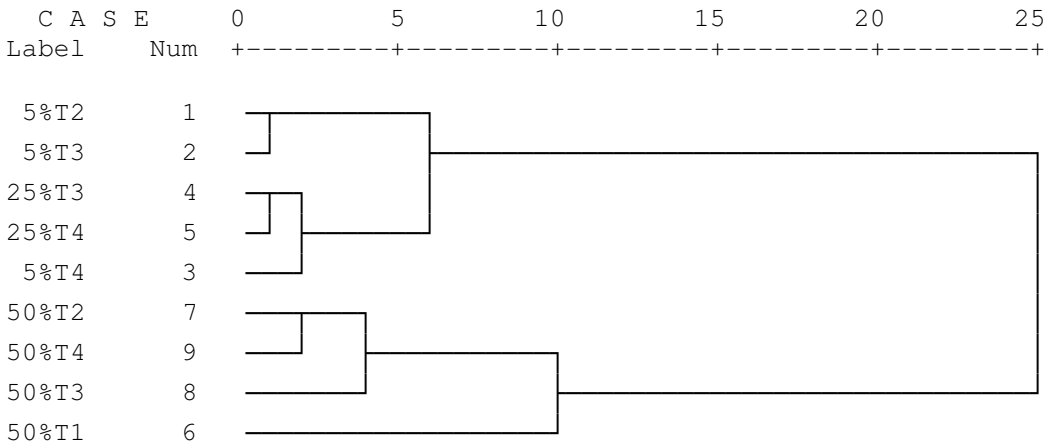
However, the dendrogram for the VOC analysis shows some further insight where the non-amended and amended groups seems to be more closely related than they appear in the factor analysis.

Water Content Perturbations

Factor Analysis

Fig. 2.5a-d shows component loadings and preferred clusters of the separate factor analyses for the VOC, FAME, Biolog, and CO_2 methods during water content treatments. VOC results show component 1 represented the 5% and 25% water content treatments and included 56.84% of the total variance and component 2 represented the 50% water content treatment and included 38.28% of the total variance (Table 2.5a). FAME results show three components where component 1 represented the 25% water content treatment and included 36.13% of the total variance, component 2 represented the 50% water content treatment and included 31.46% of the total variance, and component 3 represented the 5% water content treatment and included 25.02% of the total variance (Table 2.5b). Biolog results show component 1 represented the 25% and 50% water content treatments and included 57.90% of the total variance and component 2 represented the 5% water content treatment and included 23.98% of the total variance (Table 2.5c). CO_2 results show that component 1 represented all of the treatment groups and 99.93% of the total variance (Table 2.5d).

A. VOC



B. FAME

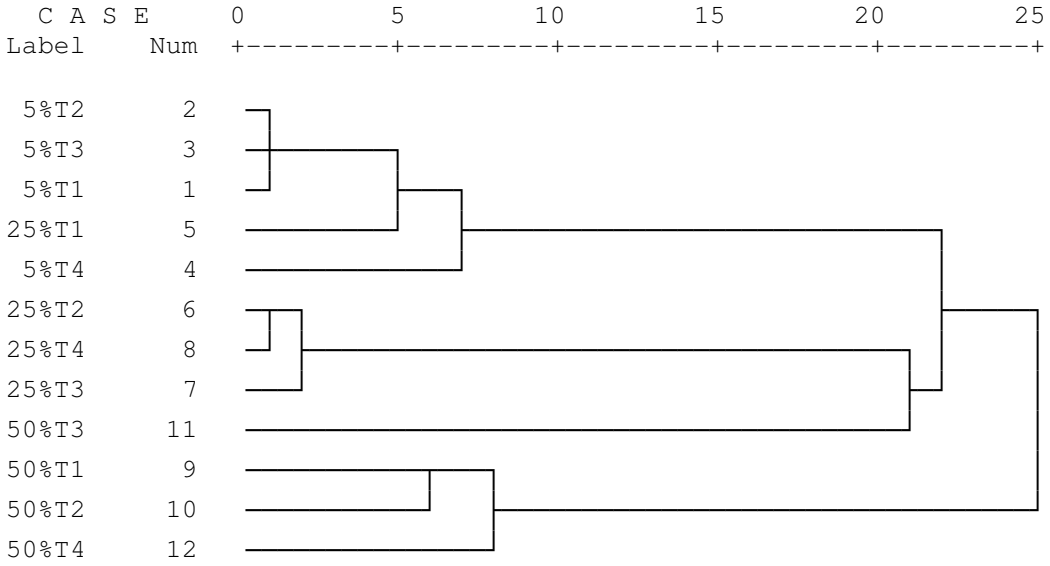
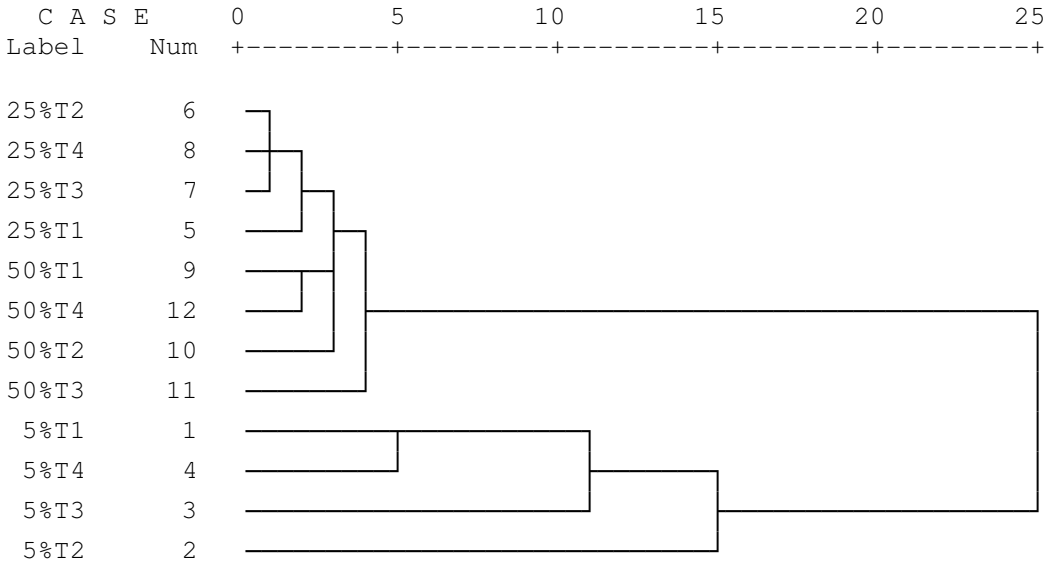


Fig.2.6. Dendrograms for VOC (A), FAME (B), Biolog (C), and CO_2 (D) methods during water content perturbations (5%, 25%, and 50%).

C. BIOLOG



D. CO_2

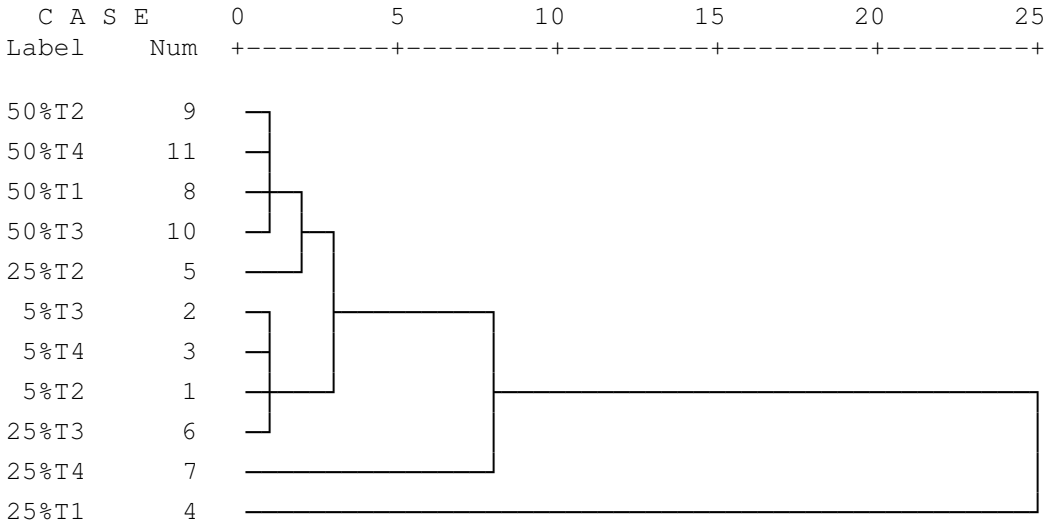


Fig. 2.6. Continued.

Overall, the VOC and Biolog methods detected two separate clusters between the water content treatments in PCA plots, whereas the FAME method was able to distinguish all three groups in separate clusters, but the CO_2 analysis could not detect any differences between the treatments.

Cluster Analysis

Cluster analysis (Fig. 2.6a-d) further illustrates the relationships between treatment groups. Results for the individual methods (VOC, FAME, Biolog, and CO_2) support the factor analysis results. The FAME dendrogram illustrates three distinct clusters, both the VOCs and the Biolog have two distinct clusters, and the CO_2 analysis only has one cluster.

Soil Type Perturbations

Factor Analysis

Fig. 2.7a-d shows component loadings and preferred clusters of the separate factor analyses for the VOC, FAME, Biolog, and CO_2 methods during soil type treatments. VOC results show component 1 represented site 11 (S11) and included 61.08% of the total variance and component 2 represented site 8 (S8) and 3 (S3) and included 33.17% of the total variance (Table 2.6a). FAME results show component 1 represented site 11 and included 42.01% of the total variance and component 2 represented sites 8 and 3 and included 40.65% of the total variance (Table 2.6b). Biolog

results show component 1 represented sites 8 and 3 and included 64.37% of the total variance and component 2 represented site 11 and included 44.16% of the total variance (Table 2.6c). CO_2 results show component 1 represented sites 8 and 3 and included 57.79% of the total variance and component 2 represented site 11 and included 41.97% of the total variance (Table 2.6d). All methods detected two separate clusters between the soil type treatments in PCA plots, where site 11 clustered differently than sites 8 and 3. Site 11 represented the sand loam located near the freshwater Laguna Atascosa Lake which was a seasonal wetland. The differential clustering of this location may be due to the likely presence of anaerobic microorganisms as a result of the environmental attributes of this soil location.

Cluster Analysis

Cluster analysis (Fig. 2.8a-d) further illustrates the relationships between treatment groups. Results support the factor analysis results, where the VOC, FAME, Biolog, and CO_2 dendrograms all illustrate two distinct clusters where the site 11 soils are distinguished from sites 8 and 3.

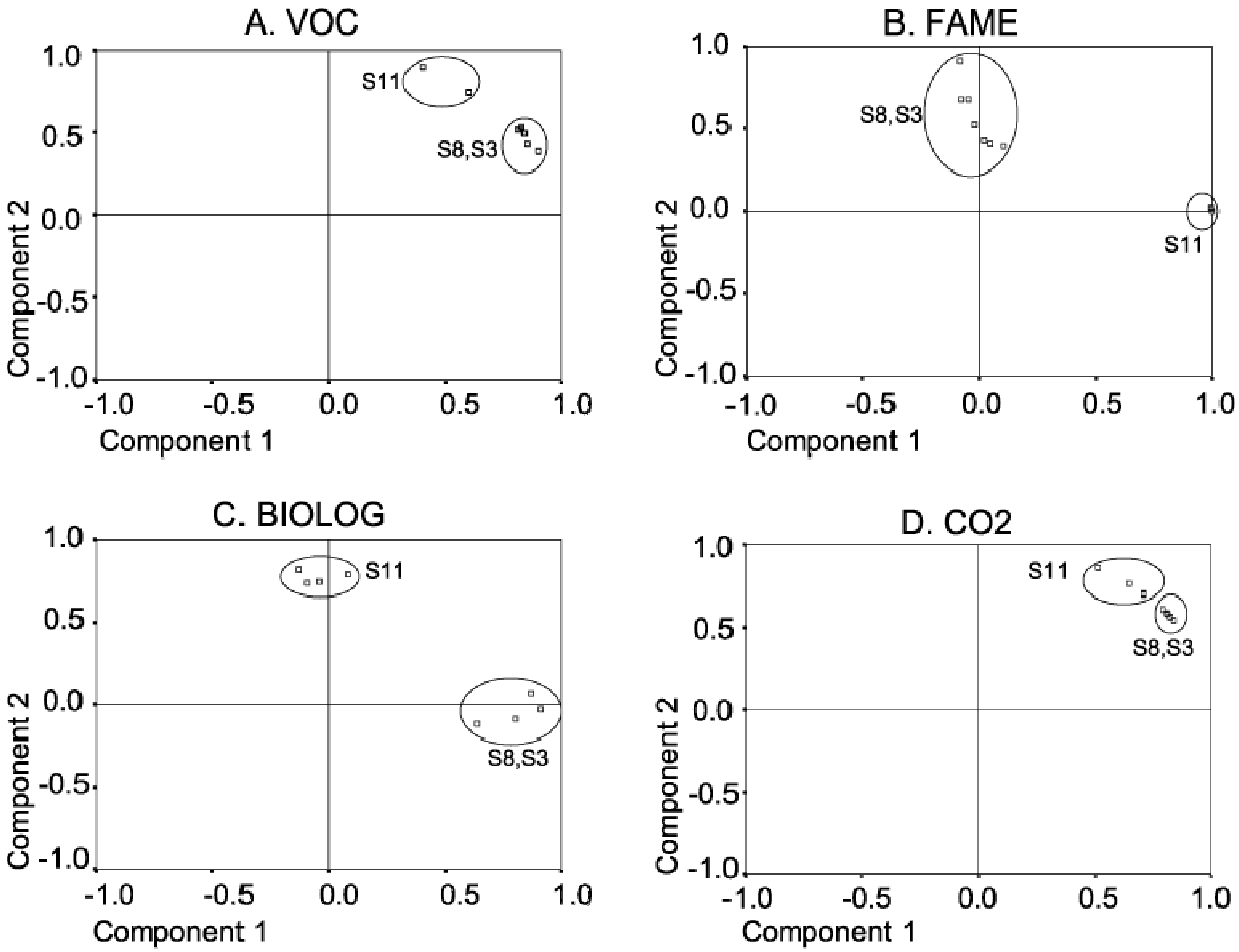


Fig. 2.7. Factor loading plots during soil type perturbations for experimental replicates. Groups of clusters for VOC (A), FAME (B), Biolog (C), and CO_2 (D) are represented by a circle. Data symbols overlap in some cases.

Table 2.6
Soil type treatment factor loadings for VOC (A), FAME (B), Biolog (C), and CO_2 (D). Component groupings are indicated in gray. ND = No data

A. VOC

TREATMENT	COMPONENT (% Variance)	
	1 (61.08)	**2 (33.17)**
S11T1	ND	ND
S11T2	0.400	0.898
S11T3	0.598	0.745
S11T4	ND	ND
S8T1	ND	ND
S8T2	0.823	0.517
S8T3	0.838	0.493
S8T4	0.899	0.382
S3T1	0.812	0.514
S3T2	0.843	0.492
S3T3	0.857	0.426
S3T4	0.829	0.530

B. FAME

TREATMENT	COMPONENT (% Variance)	
	1 (40.65)	**2 (42.07)**
S11T1	0.997	0.018
S11T2	ND	ND
S11T3	0.992	0.022
S11T4	0.998	-0.002
S8T1	0.107	0.389
S8T2	0.020	0.423
S8T3	0.020	0.426
S8T4	-0.045	0.680
S3T1	0.048	0.405
S3T2	-0.019	0.524
S3T3	-0.079	0.674
S3T4	-0.085	0.908

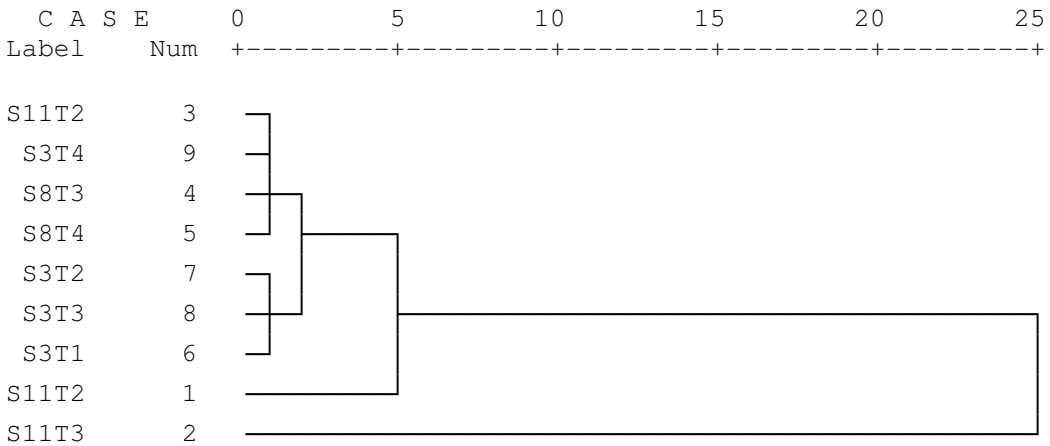
C. Biolog

TREATMENT	COMPONENT (% Variance)	
	1 (44.16)	**2 (64.37)**
S11T1	-0.000612	0.994
S11T2	ND	ND
S11T3	0.00578	0.990
S11T4	-0.0389	0.993
S8T1	0.822	0.152
S8T2	0.865	0.0619
S8T3	0.832	0.0584
S8T4	0.688	-0.0564
S3T1	0.694	0.0487
S3T2	0.821	-0.0231
S3T3	0.681	-0.111
S3T4	0.385	-0.124

D. CO_2

TREATMENT	COMPONENT (% Variance)	
	1 (57.79)	**2 (41.97)**
S11T1	0.0847	0.784
S11T2	-0.131	0.815
S11T3	-0.0401	0.742
S11T4	-0.0959	0.737
S8T1	0.871	0.0664
S8T2	0.635	-0.114
S8T3	0.803	-0.0899
S8T4	0.909	-0.0273
S3T1	0.871	0.0664
S3T2	0.635	-0.114
S3T3	0.803	-0.0899
S3T4	0.909	-0.0273

A. VOC



B. FAME

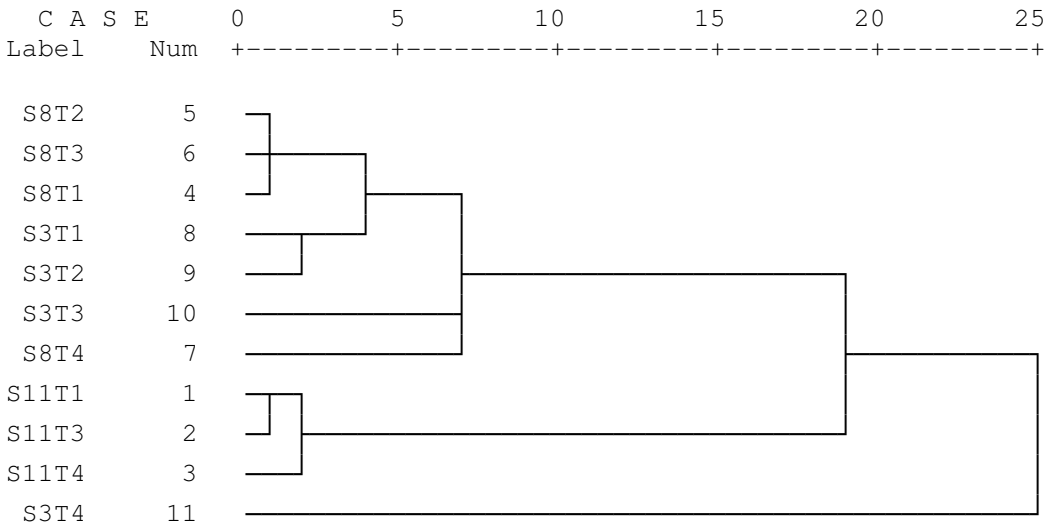
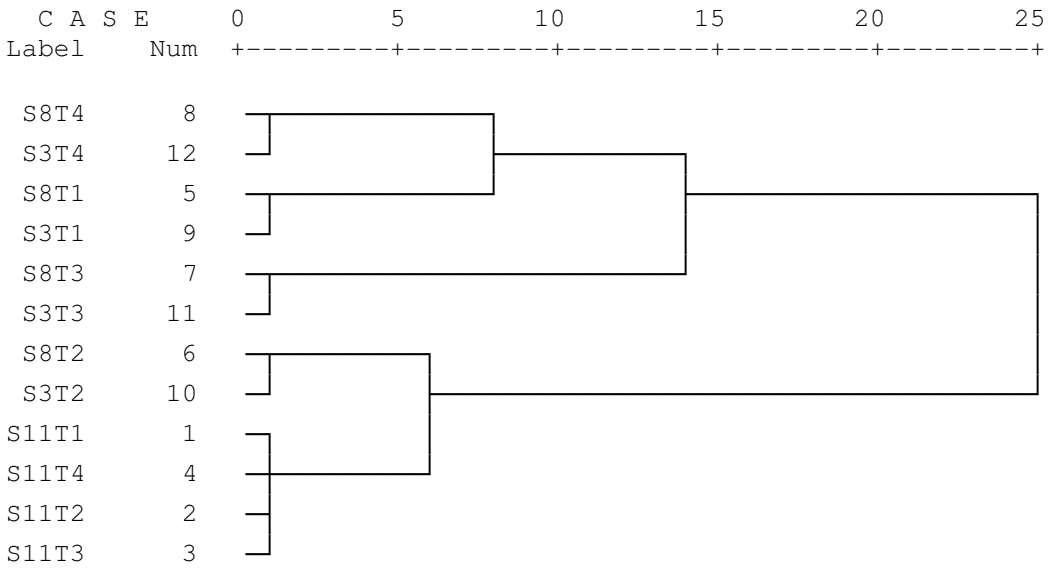


Fig. 2.8**.** Dendrograms for VOC (A), FAME (B), Biolog (C), and CO_2 (D) methods during soil type perturbations (S11, S8, and S3).

C. BIOLOG



D. CO_2

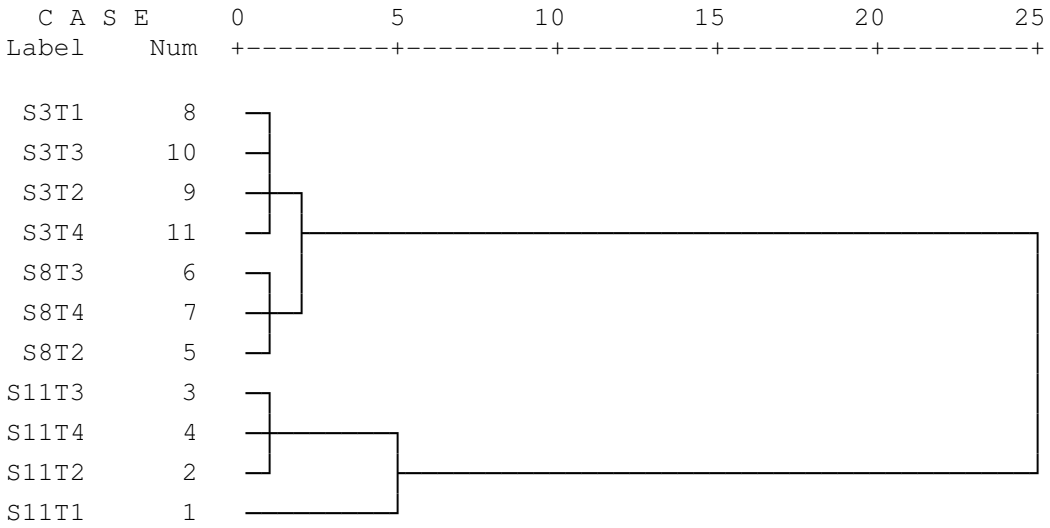


Fig. 2.8**.** Continued.

Multiple Regression Analysis and Non-Parametric ANOVA Results

A step-wise, linear, multiple regression was conducted using averaged data from the water content perturbation experiment for the four methods (Table 2.7). FAME results were considered to be the most accurate measurements of microbial community composition shifts and therefore the ability for the other three methods to predict FAME results was tested. The regression results gave a moderate linear fit (r^2) value of 0.601, an acceptable fit for biological data, for each of the three methods (Table 2.7). Both the VOC and the Biolog methods were significant ($\rho < 0.05$) predictors of changes in the FAME response during water perturbation, however the CO_2 method was not a significant ($\rho > 0.05$) predictor.

A one-way, non-parametric (Kruskal-Wallis), ANOVA was conducted on the raw VOC data for each experiment where median differences between treatment levels were determined. Results supported factor and cluster analysis where significant differences ($\rho < 0.01$) between the treatment levels of substrate availability, soil type, and water content were found (Table 2.8).

Table 2.7
The prediction of FAME concentration via VOC, Biolog, and CO_2 methods using a step-wise linear regression (Asterisks represent statistical significance)

Variable	Indicator	N	Mean	Beta	r^2	Sig. ($\rho < 0.05$)
Dependent	FAME	12	4758.00 (Peak Ht.)	NA	NA	NA
Independent	VOC	12	8.28 (µg)	-1.568	0.601	0.023*
Independent	BIOLOG	12	0.09 (ABS)	-0.858		0.042*
Independent	CO_2	12	26.24 (mg)	-0.627		0.194

Table 2.8
The non-parametric (Kruskal-Wallis) one-way ANOVA results for each VOC experiment (Asterisks represent statistical significance)

Environmental Factor	Treatment Level (N)	Mean VOC mass (µg)	Mean Rank	Significance ($\rho < 0.01$)
Substrate	T (864)	48.71	411.31	0.000*
	Cna (648)		355.65	
	Ch (648)		498.21	
Soil Type	S11 (288)	40.55	324.55	0.010*
	S8 (288)		294.32	
	S3 (288)		274.59	
Water Content	5% (864)	24.25	418.90	0.000*
	25% (864)		393.38	
	50% (864)		340.76	

Discussion

Microbial Activity Proxies – Comparison of the VOC and CO_2 Methods

Both CO_2 and VOCs have been used in previous research to determine soil microbial activity (Kuzyakov, 2006; Schlesinger and Andrews, 2000; Stahl and Parkin, 1996; Wood et al., 1993). Stahl and Parkin (1996) found that when concentrations in VOCs increased or decreased in soil microcosms so did concentrations of CO_2. The current research extends the former authors' work and provides additional insight about the behavior and relatedness of CO_2 and VOC metabolites where both methods were used to determine microbial metabolism shifts during environmental factor perturbation in soil microcosm experiments. Results showed that the VOCs were able to provide more data clusters for both the substrate and water content perturbations than the CO_2 method during factor analysis (Fig. 2.3 and 2.5). CO_2 analysis was not able to differentially cluster data between the two control groups during substrate perturbations (Fig. 2.3d) or between any of the treatments during water perturbations (Fig. 2.5d). This indicates that the CO_2 method is not as sensitive as the VOC method for detecting small differences between soils that produce low metabolite concentrations (e.g. $CO_2 < 20$ mg). Inorganic carbon was detected at the LANWR soil locations at approximately 32-57% of the total measured soil carbon (Table 2.1). This could be responsible for the lower sensitivity of the CO_2 method due to the release of CO_2 via inorganic reactions (e.g. $CaCO_3$ dissolution) in the soil, which may have interfered with the detection of changes in the biotic production of CO_2. Another possibility for lower sensitivity is the

production of biogenic CO_2 as a metabolic end-member during both anaerobic and aerobic respiration, which may have prevented the CO_2 method from detecting any activity differences under low oxygen conditions. This may have been especially important during the 50% water treatments where anaerobic conditions were most likely to exist. However since the VOC method was able to detect differences between microcosms undergoing the same conditions, our method is supported where VOCs are useful for not only the measurement of microbial activity but also community composition shift, a characteristic that CO_2 methods do not hold. Additionally, since the VOC method results were similar to the FAME and Biolog methods during perturbations and significant ($\rho < 0.01$) differences in VOC mass during each treatment level were found, it can be concluded that the VOCs are accurate indicators for microbial community compositional shifts during such changes. In addition, during linear regression analysis (Table 2.7) only the VOC and Biolog methods were significant ($\rho < 0.05$) predictors of the FAME results, further supporting that VOCs are reliable and useful biomarkers to track microbial community compositional shifts during environmental factor perturbations. The invaluable advantages that characterize VOCs over CO_2 methods are the ability to provide information not only about the microbial activity but also about both the organic carbon sources and the microbial community structure; in contrast, the CO_2 method is only able to provide information about the microbial activity. These characteristics of the VOC method are useful because there is a current need for a proxy that captures the behavior and response of soil microbial ecosystems to external perturbations over spatial and temporal scales.

Microbial Community Proxies – Comparison of the FAME and Biolog Methods

Both FAME and CSUPs (Biolog) have been used in previous literature to determine soil microbial community structure (Buyer and Drinkwater, 1997; Øvreås, 2000; Widmer et al., 2001). In the current research, the ability for both methods to determine shifts in the microbial community composition during environmental factor perturbation was studied in soil microcosm experiments. Results show that both the FAME and Biolog methods proved useful as indicators of microbial community shifts. Both methods provided three cluster groups during substrate perturbations in factor analysis (Fig. 2.3). Both methods provided two cluster groups during soil type perturbations, where data from site 11 (the sandy loam) clustered apart from the other two sites (Fig. 2.7). Since these changes were observable in both methods, it can be concluded that both are equally able to determine shifts in soil community composition during such perturbations. However, during water content perturbations, the FAME analysis was able to determine differences between all three treatments levels, whereas the Biolog could only determine differences between two of the three treatments (Fig. 2.5), indicating that the FAME method is more sensitive during variations in water content. Or another possible explanation is that the two methods may not be clearly related because they show different structural and functional responses (Buyer and Drinkwater, 1997; Widmer et al., 2001) due to the fact that the Biolog method has a culture bias for fast-growing, culturable microorganisms, and in this research only aerobic gram-negative bacteria were measured; whereas FAME methods consider the entire community during analysis. Nevertheless, the Biolog method was able to significantly

($\rho < 0.05$) predict changes in the FAME results during regression analysis, suggesting that Biolog analysis is a good measure of microbial community composition shift. This finding supports prior literature results (Buyer and Drinkwater, 1997; Widmer et al., 2001) where CSUP and FAME methods both detected changes in the soil microbial community structure and allowed for different soils to be distinguished.

VOCs as Indicators of Microbial Community Shifts and Labile Carbon Sources

During the experiments conducted in this study, VOC results aligned well with both the FAME and Biolog methods. In all cases, the Biolog and the VOC methods clustered data similarly during factor analysis (Fig. 2.3, 2.5 and 2.7). In all but the water content treatments, the FAME and the VOC methods clustered data similarly (Fig. 2.3, 2.5 and 2.7) during factor analysis. Slight dissimilarities in data clusters between the three methods can be attributed to different microbial functions, processes, and communities measured by each method. Both the VOC and Biolog analyses were significant ($\rho < 0.05$) predictors of the FAMEs, indicating that these two methods should be highly considered for use in microbial community studies.

Taking a closer look at both the VOC and FAME data collected from this research, it is apparent that microorganisms were present and responsible for VOC production in the soils used for microcosm studies. Straight-chained (e.g. 12:0, 14:0, 16:0, 17:0, 18:0), branched (e.g. 14:0 ISO, 15:0 ISO, 16:0 ISO, 17:0 ISO), and double bonded (e.g. 16:1, 17:1, 18:1) fatty acids were detected in soils used in this work. Some of these included the presence of β–OH (~ 4-5%) and branched fatty acids (~ 4-5%) suggesting that gram-

negative and gram-positive bacteria were present in the system (Bossio and Scow, 1998; Zelles, 1999). While the detection of long-chained fatty acids (~3%; e.g. 20:0 to 24:0) and linoleic acid (~ 10%; 18:2ω6) suggests the presence of fungi (Bossio and Scow, 1998; Zelles, 1999). Sitosterol was detected and has been linked to higher order plants (Fang et al., 2006) but its presence was likely due to the use of soils that were collected from the natural environment where higher order plants were common and additions of clover to soils to enhance microbial growth were made. Since sitosterol accounts for a small amount (< 0.5%) of the total FAMEs in this research, the influence of higher-order plants is considered negligible.

In addition to the FAMEs, over seventy-two VOCs were detected during microcosm experiments (Table 2.3). Of these, twenty-five or 35% of the compounds were detected in prior studies describing the microbial production of VOC metabolites (Gerber, 1977; Larsen and Frisvad, 1995a, 1995b; Stahl and Parkin, 1996; Schöller et al., 1997; Sunneson et al., 1995). Detected VOCs such as 1-undecene, 2-pentanone, 3-methyl-1-butanol, and 2-heptanone indicated the presence of gram negative bacteria (Schöller et al., 1997); while others such as α-pinene, camphene, limonene, 2-butanone, and 3-octanone indicated the presence of fungi (Larsen and Frisvad, 1995a, 1995b). This finding, coupled with the microbial origins of the FAME compounds found in the measured soils (and the growth of gram-negative bacteria on the CSUPs), indicates that the detected VOCs were likely produced by soil microorganisms where shifts in microbial community compositions produced shifts in VOC types and amounts.

Furthermore, VOCs provide information about the type of labile carbon compounds that have been degraded and released as volatiles by soil microbes. In this research several major groups of VOC metabolites were emitted including alcohols, aldehydes, aromatics, carboxylic acids, esters, hydrocarbons, ketones, nitriles, sulfides, and terpenes (Table 2.3). Such information can assist researchers in determining whether the degraded organic substrate is a pollutant (e.g. benzene) or a naturally present compound in the environment under study.

Conclusions

This study is the first to measure the production of VOC metabolites in soil microcosms, while simultaneously measuring CO_2 emissions and microbial community composition shifts via FAME and CSUP methods. This work has used multivariate statistics including factor, cluster, and ANOVA analysis to show that soil VOC metabolites are valid proxies for measuring shifts in microbial activity and community structure during perturbations of environmental factors. General agreement in statistical results between traditional methods and VOC methods introduced in this paper supports our interpretations. Furthermore, statistically significant ($\rho < 0.05$) results during regression analysis and ANOVA provide further confidence in our validation of the VOC method. This research has shown that VOCs are easy to measure, relatively inexpensive, and informative proxies of soil microbial ecosystems that undergo environmental perturbation. Since VOC proxies are non-destructive, they fill a current research need to monitor ecosystem behavior over spatial and temporal dynamics and

future work focused on such environmental aspects should consider incorporating their use.

CHAPTER III

USING SOIL MICROCOSMS TO UNDERSTAND THE TEMPORAL AND SPATIAL DYNAMICS OF MICROBIAL VOC METABOLITES

Overview

Traditional proxies that describe the functional (e.g. enzymes), taxonomic/community-level (e.g. microbial DNA, lipids), microbial activity (e.g. carbon dioxide), and carbon and nutrient pathways (e.g. stable isotope labeling) are generally limited in the amount of temporal/spatial information that they can provide (e.g., community level approaches) and/or the amount of specific information they can offer about carbon sources or microbial community function (e.g. carbon dioxide). This research illustrates soil volatile organic compound (VOC) emissions as useful indicators of subsurface microbial community composition as a function of both time and space. Results from laboratory monitoring studies of collected field samples of south Texas soils are presented, where VOC metabolites as characterized by gas chromatography and mass spectrometry (GC-MS), were related to other soil components including carbon dioxide (CO_2) via infra-red analysis, and microbial community structure as measured by community substrate utilization profiles (CSUPs) and fatty acid methyl ester (FAME) techniques. Results include the identification of seventy-two VOC metabolites (< 0.01-80 µg) produced from the soils. Analysis of variance (ANOVA) results show that twenty-eight of the seventy-two detected VOCs significantly ($\rho < 0.01$) differed over either time or space. Statistical significance ($\rho < 0.05$) in microbial activity and

community structure measures (e.g. FAME, Biolog, CO_2) during microcosm studies suggests differences between LANWR soil locations. Temporal evidence shows VOCs increase over time. Further, Geographical Information Systems (GIS) plots show spatial relationships among the measured soil characteristics (e.g. soil moisture, TOC, conductivity, and reactive-Fe), FAME, Biolog, CO_2 and VOC concentrations. Spatial and temporal variability is also observed with detected VOC functional groups. The results are encouraging and characterize VOC production in soils as useful proxies of subsurface microbial ecosystems during spatial and temporal dynamics.

Introduction

VOCs as Indicators of Temporal and Spatial Dynamics

Volatile organic compounds (VOCs) have been measured to be produced by fungi, bacteria, and actinomycetes in both direct-culture (Larsen and Frisvad, 1995a; Gerber, 1977; Schöller et al.,1997; Stotzky and Schenck, 1973; Sunesson et al., 1995) and soil microcosm studies (Stahl and Parkin 1994, 1996) Results have indicated VOCs may be able to aid in the differentiation of microbial groups (Grametbauer et al., 1988; Larsen and Frisvad, 1995a; Linton and Wright, 1993; Stahl and Parkin, 1996). In the previous chapter of this research, VOCs are shown as valid proxies of microbial community compositional shift during perturbation (e.g. water content, substrate availability, and soil type) in soil microcosm studies, where statistical comparisons with

traditional microbial community structure methods (e.g. FAME and Biolog) substantiate our results.

Little research has been conducted, however, on the temporal and spatial dynamics of VOC production in soils. Temporally and spatially dynamic studies in soils are important to increase the understanding of ecosystem change and the environmental controls on soil carbon dynamics. It is necessary to gain this understanding because of the central role soil carbon plays in ecosystem sustainability, nutrient availability, and the production of greenhouse gasses (Waldrup and Firestone, 2004). Microbial activity, in part, controls the release of soil carbon to the atmosphere. The release of gaseous CO_2 contributes to greenhouse gas concentrations (Schlesinger and Andrews, 2000) and the release of certain VOCs can contribute to air pollution (Levis et al., 2003). Furthermore, the composition and activity of soil microbial communities largely impacts the biogeochemical cycling, organic matter types and quantities, and the fertility and quality of soils (Zelles, 1999). Therefore the use of ecological proxies that can capture both the soil carbon and microbial community compositional shifts, such as VOC metabolites, need to be applied over spatial and temporal dynamics in order to determine the impact of natural and anthropogenic influences on soil microbial ecosystems.

Traditional proxies that describe the functional (e.g. enzymes), taxonomic/community-level (e.g. microbial DNA, lipids), microbial activity (e.g. carbon dioxide), and carbon and nutrient pathways (e.g. stable isotope labeling) are generally limited in the amount of temporal/spatial information that they can provide (e.g., community level approaches) and/or the amount of specific information they can offer

about carbon sources or microbial community function (e.g. carbon dioxide). Unlike other measures of microbial community compositional shift (e.g. FAME and Biolog), VOCs are not destructive and can therefore provide information during temporally dynamic studies, much like gaseous CO_2 measurements. CO_2 studies have been frequently utilized to measure the temporal and/or spatial dynamics in the soil metabolism (Fierer et al., 2003; Padmanabhan et al., 2003; Waldrup and Firestone, 2004; Wood et al., 1993; van Hees et al., 2005). Previous experiments conducted by Stahl and Parkin (1996) to validate VOCs as proxies of microbial activity indicated that low microbial activity as measured via CO_2 also had low VOC production (and visa versa) during microcosm studies, thus establishing that VOCs behave similarly to gaseous CO_2 production. However unlike CO_2, an end-member metabolite, VOCs are information rich data sources because they are intermediate bi-products of the microbial mineralization of carbon. Therefore they provide information about both the microbial community structure and carbon substrate source, making them an extremely useful microbial ecosystem proxy. Since microbial community and structure is influenced by soil water content, carbon sources, and nutrient availability (Fierer et al., 2003; Franklin and Mills, 2003; Dahlhoff, 2004; Drenovsky and Richards, 2004; Musslewhite et al., 2003) and these characteristics frequently vary over time and space, VOC metabolite production likely also varies temporally and spatially. This is especially likely since CO_2 production has been documented to be influenced by soil water content (Fierer et al., 2003), carbon sources (Padmanabhan et al., 2003; Waldrup and Firestone, 2004; van Hees, 2005), and nutrient availability (Fierer et al., 2003).

Research Objectives

The objectives of this work were to (i) measure the VOC temporal and spatial dynamics during microcosm studies of south Texas soils, (ii) compare temporal VOC results to traditional indicators of microbial activity (e.g. CO_2) and, (iii) determine the spatial relationships between the various soil characteristics (e.g. soil moisture, TOC, conductivity, and reactive-Fe), microbial community structure (e.g. FAME and Biolog), and activity (CO_2) results with VOC production. Soils for microcosm studies were collected from Unit 7 of the Laguna Atascosa National Wildlife Refuge (LANWR) in Southern Texas, a former US Army Air base converted to what is currently the largest protected area (263 km^2) of natural habitat left in the Lower Rio Grande Valley. The LANWR location was chosen as the study area because it is a coastal margin interface region and a likely nexus for changes in carbon substrate, water content, and nutrient availability. Microcosm experiments were conducted with selected LANWR soils where microbial emissions of VOC metabolites were monitored over time. Additional measurements including CO_2, FAME and CSUP analyses were used to support and interpret VOC results. The guiding hypothesis included that soil VOC emissions in microcosm experiments would increase over time and be variable over space where VOC production would correspond with measured soil characteristics, and traditional microbial community (e.g. FAME and Biolog) and activity measurements (e.g. CO_2).

Materials and Methods

Sample Collection Location

Soils from three LANWR locations (Fig. 3.1) were used in soil microcosm and field studies: site 3 (fine, smectitic, hyperthermic Udic Haplusterts), site 8 (fine-silty, mixed, active, hyperthermic Typic Aquisalids), and site 11 (fine, mixed, active, calcareous, hyperthermic Typic Halaquepts). Site 11 represented soil collected near the freshwater (salinity = 7) Laguna Atascosa Lake and is characterized as a seasonal wetland area; site 8 represented soil collected at a greater elevation (~3 m) close to the hyper-saline (salinity = 47) Laguna Madre Estuary and is characterized as an coastal upland area; and site 3 represented soil collected near a Rasaca (dried-up meandering river) from a grassland area.

Soil Characteristics

At each site, surface samples in the A-horizon (< 0.25 m) were collected in replicates of three, < 1 m apart, mixed into one homogeneous soil sample, and taken back to the laboratory where they were sieved (2 mm), air-dried, and analyzed for typical soil characteristics.

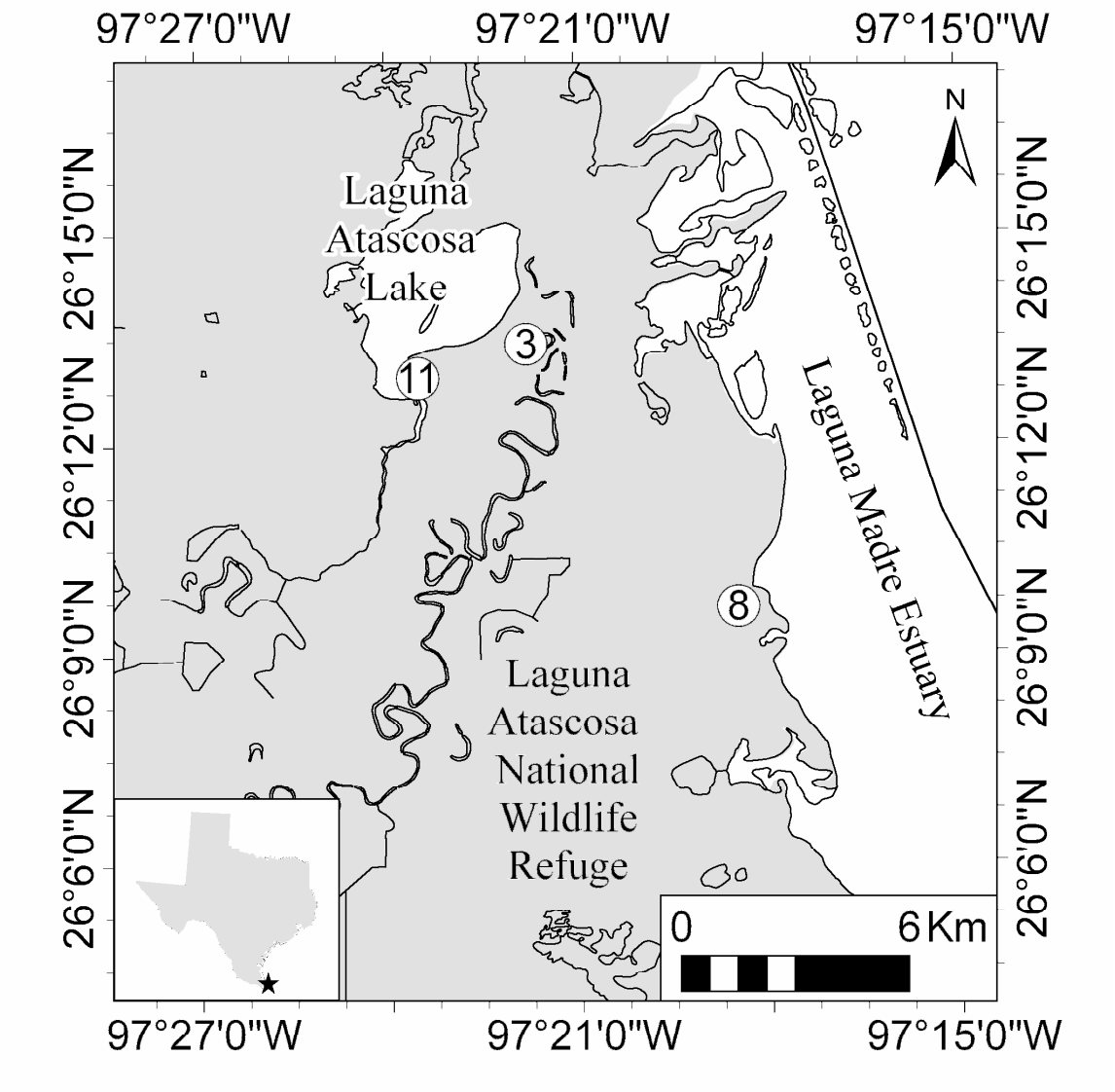


Fig. 3.1. A map of the LANWR study location and selected sites.

The laboratory characterization of the soils from the LANWR sites included citrate dithionate extractable iron following the methods of Leoppert and Inskepp (1996) using a Perkin Elmer flame atomic absorption analyzer; quantification of total organic carbon (TOC) and total nitrogen using a Vario El II elemental analyzer following the methods

of Leoppert and Suarez (1996); quantification of soil protein via Bradford (1976), extraction following the methods of Raab et al. (1999), and assay measurement following using a Hewlett Packard UV-Visible spectrophotometer; soil texture fractionation following the methods of Whitting and Alladrice (1986); and identification of the sand, silt, and clay mineralogy using a Rigaku powder x-ray diffractometer (XRD). Results from these analyses for each soil location are provided in Table 3.1.

Table 3.1
Site characterization for each LANWR soil used in microcosms studies. The latitude and longitude, site description, site elevation, soil texture, total organic carbon (TOC), carbon-to-nitrogen (C/N) ratio, soil conductivity, soil moisture, reactive iron, protein concentrations, and the sand, silt, and clay soil mineralogy

Soil Characteristics	**LANWR SITE**		
	11	**8**	**3**
Collection Location (Deg. Min. Sec.)	26° 13”24.7’ 99° 21”47.8’	26° 09”38.8’ 104° 18”28.9’	26° 12”54.6’ 107° 23”30.8’
Site Description	Seasonal Wetland	Coastal Upland	Grassland Region
Site Elevation (m)	0	3	0
Soil Texture	Sand Loam	Clay Loam	Silt Loam
Soil TOC (%)	1.15	1.85	1.09
Soil C/N (Ratio)	9.85	16.98	9.81
Soil Moisture (%)	0.322	0.137	0.191
Soil Conductivity (mmohs)	490.0	180.0	305.0
Soil Reactive-Fe (μmol g^{-1})	27.79	35.69	53.07
Soil Protein (mM g^{-1})	0.062	0.048	0.062
Clay Fraction Minerals	Smectite; Mica; Kaolinite	Smectite; Mica; Kaolinite	Smectite; Mica; Kaolinite
Silt Fraction Minerals	Quartz; Albite	Quartz; Calcite; Cuprite	Quartz
Sand Fraction Minerals	Quartz	Quartz	Quartz; Anorthite

In the field, soil moisture was measured using a Delta-T (model HH2) moisture meter and Theta (model ML2x) moisture probe. Three replicate soil moisture measurements at each surface location were made and results were averaged for each site (Table 3.1). *In-situ* ground conductivity measurements were made at each site using a Geonics Limited (EM31) analyzer, where replicate measurements from each site were averaged (3 m^2; Table 3.1).

The Laboratory Measurements

The Microcosm Experimental Design

At each site, surface samples in the A-horizon (< 0.25 m) were collected during March in replicates of three, < 1 m apart, and immediately frozen and taken back to the laboratory where they were stored at -80°C until used for microcosm experiments. Microcosm experiments utilized 50.00 g (wet-weight) of surface soils collected from the LANWR. VOC production was quantified as a function of time in three different soil types. After addition of internal standards into microcosms, samples were immediately connected to the micro-oxymax system and experiments took place at room temperature (20˚C ± 1˚) for 15 days. The triplicate soil samples were utilized from each soil location and head-space VOC emissions were collected on a Tenax sampling tube. Every three days the VOC sampling tube was replaced. The system was closed with the exception of daily (every 24 hours) atmospheric air refreshments that passed through both a soda lime and drierite column before reaching microcosms to remove atmospheric CO_2 and

moisture (Fig. 2.1). Refreshments were conducted in order to prevent CO_2 concentrations from reaching levels that were too high for accurate measurement within the linear range of the instrument. Preliminary calculations and direct measurements showed low and high CO_2 production rates of 1.6 – 2.6 mg day^{-1} where a daily refresh rate would result in O_2 atmospheric amounts of 13.3 – 12.3 mg in the 100 mL jars. We have no direct measurements of the development of aerobic/anaerobic conditions however the presence of particular VOCs (e.g. methyl derivatives) suggests that the microcosms may have gone slightly anaerobic. At the end of experimentation, microcosm soils were immediately frozen and stored at -80°C until laboratory microbial community analyses were conducted.

Microxymax System Measurement of Gaseous CO_2 Measurements

Overlying head-space from each microcosm jar was sampled for CO_2 via the micro-oxymax pump and an infrared sensor and returned to the jar after analysis through a closed system. CO_2 sampling intervals were every four hours for a 15 day time period where refreshing of the microcosm headspace occurred once a day or when CO_2 concentrations exceeded the 0-1% threshold of the instrument sensors, which ever occurred first.

Analytical Methods

GC-MS Measurement of Gaseous VOC Measurements

VOCs were concentrated on 100 mg of Tenax (Chromosorb©) in a brass sample tube (8 cm x 0.64 cm) and thermally desorbed using a Perkin Elmer ATD 400 and directly injected into a Hewlett - Packard 5890 Series II Plus Gas Chromatograph equipped with a DB-624 fused silicone megabore column (30 m x 0.32 mm) coupled to a Hewlett-Packard 5972 Series mass spectrometer (Fig. 2.1). Before use, Tenax tubes were pre-conditioned for 15 min. at four temperatures (250˚C, 300˚C, 330˚C, 350˚C) and after use, re-conditioned for 10 min. (330˚C). Sample tubes were desorbed with He at 75 mL $min.^{-1}$ for 10 min. at 220˚C and concentrated in a cold trap at -30˚C. Chromatographic conditions included splitless injection with He carrier gas at 1 mL $min.^{-1}$ constant flow rate. The oven program was held at stages of 35˚C for 2 min. then 6˚C $min.^{-1}$ to 70˚C, then 15˚C $min.^{-1}$ to 145˚C, and isothermal at 145˚C for 17 min. Electron ionization (70eV) GC-MS was conducted in full scan mode for quantitative analysis. Temperature at the source was set at 280°C.

Internal standards (Ultra Scientific; 99.5%) were injected into soil microcosms and included Chlorobenzene-d5, 1,4-dichlorobenzene-d4, and fluorobenzene. An internal standard recovery of 85% or greater was required. System standards (Ultra Scientific; 99.5%) were injected into sample tubes before analysis on the ATD and included 4-bromofluorobenzene, dibromofluoromethane, and tolune-d8. Surrogate compounds (Absolute Standards; 99.5%) including propanol-2, butanol-1, toluene,

xylene, furan, ethyl acetate, geosmin, 2-methylisoborneol, isoprene, 3-methyl-1butanol, 2-pentanone, and 1-undecene were used to quantify compounds identified in the Hewlett-Packard NIST98 library. A 70% library match was required to identify compounds.

Carbon Substrate Utilization Profiles

Gram-negative bacterial communities were characterized using community level physiological profiles. The 96 well Biolog (GN-2) plates with various carbon substrates were used to provide information about substrate utilization and functional diversity of aerobic soil bacteria (Goberna et al., 2005; Widmer et al., 2001; Zak et al., 1994). Ten grams of microcosm soil was suspended in 90 mL of sterilized water and 125 µL of the diluted solution was added to the plates and incubated for 96 hours (Widmer et al., 2001). Colormetric changes (595µm) were detected on a scanner (Labsystems Multiskan MS) every 24 hours for four days and community differences between samples were determined using the Multiskan Transmit Program (revision 1.2).

GC-MS Measurement of Fatty Acid Methyl Esters

FAMEs were analyzed according to Bottomley (2005). Briefly, fatty acids were liberated from soils through heating at 100˚C in a KOH and methanol solution. Conversion to FAME via use of acetic acid and heat was made and extraction was conducted in a hexane solution. Analysis was then possible via gas chromatography (Agilent model 6890). A cross-linked 5% phenyl methyl silicone Hewlett Packard Ultra

2 column (25 m x 0.20 mm) was used with a flame ionization detector (FID) detector. The method lasted a total run time of 36.318 min. at stages of 170˚C for 0 min. then 5˚C $min.^{-1}$ to 300˚C and hold for 12 min. Helium flow rates were 0.6 mL $min.^{-1}$ and the split ratio was 50:1. Peaks were named using Sherlock Eukary program (supplied by MIDI - Microbial ID of Newark, Delaware).

Statistical Procedures

In this study, data points that were not detectable were considered as zero values and low recovery (< 85%) samples were not used in statistical analysis. A one-way, non-parametric (Kruskal-Wallis) ANOVA was conducted on the raw data where means were compared in order to determine significant differences ($\rho < 0.05$) between methods and to determine significant differences ($\rho < 0.01$) in VOCs with time and space. Time series plots for VOC and CO_2 analysis were made from averaged triplicate data for each LANWR location. Geographical Information Systems (GIS; ESRI® ArcGIS™ version 9.2) was used to plot soil characteristics (TOC, Fe, conductivity, and soil moisture), microbial community (FAME and Biolog) and microbial metabolism (CO_2 and VOC) variability over space. The ArcGIS™ Spatial Analyst (version 9.2) was used to derive, reclassify, weight and combine data. Equal intervals from 1-10 were applied to each dataset and equal weighting (normalization to 1) was used for combined datasets. The geostatistical method of kriging was applied to linearly interpolate between data points. The purpose of using the Spatial Analyst tool was to identify potential hotspot regions based on the abiotic and biotic parameters measured in this study and not to necessarily

determine existing conditions between the three study locations. Since the number of sampling locations is low and this may introduce error in the linear extrapolation used during the kringing method, the interpretation of these maps focused on the detection of a hotspot region at one of the three measured locations and not on the conditions between the locations.

Results

Soil Characteristics

Soil characterization results for each LANWR soil collection location show that the soils were different in regard to soil texture (Table 3.1). Furthermore, results indicate that although the soil TOC was similar (1.15-1.85%), the C/N ratio for site 8 was greater (16.98) than sites 11 and 3 (9.81-9.85), and the reactive-Fe for the soils was also variable at concentrations of 27.79 µmol g^{-1}, 35.69 µmol g^{-1}, and 53.07 µmol g^{-1} for sites 11, 8, and 3, respectively. Both the soil protein (0.048-0.062 mM g^{-1}) and the soil minerals were similar for the three soils, with the exception of the presence of cuprite (Cu_2O) at site 8 which may have been due to the close proximity (< 1.5 km) to the pre-existing gunnery and remaining bullet casings in the soil of that location. Elevation at site 8 was also greater (3 m) than the other two sites (0 m), but soil conductivity was less (180 mmohs) at site 8 than at sites 11 (490 mmohs) and 3 (305 mmohs).

Table 3.2
The non-parametric (Kruskal-Wallis) one-way ANOVA results for VOCs that were statistically significant ($\rho < 0.01$) during time and/or soil location in laboratory studies

Retention Time	VOC Name	Time	Location
		Significance ($\rho < 0.01$)	Significance ($\rho < 0.01$)
7.56	Propanol-2	0.01	X
8.75	Silanol, trimethyl	0.01	X
8.93	Furan, tetrahydro	0.00	X
9.98	Butanol-2methyl	0.01	X
9.98	Butanol-1	0.01	X
10.65	Pentanal	0.00	X
10.65	2-Pentanone	0.00	X
11.72	2,4 Pentadienenitrile	0.01	X
11.72	Pyridine	0.01	X
12.77	Cyclobutanol, 2-ethyl	X	0.00
12.65	2,4-Dimethyl-1-heptene	X	0.00
12.65	1-Pentanol, 2-methyl	X	0.00
13.91	p-xylene	0.00	X
14.45	Styrene	0.00	X
15.48	Oxime-methyoxy-phenyl	X	0.01
16.22	B-pinene	X	0.00
16.29	Furan, 2-pentyl	0.00	X
16.43	Alpha-methylstyrene	0.00	X
16.64	1-Octen-3-ol	0.01	X
17.43	Limonene	0.00	X
17.62	Benzonitrile	X	0.01
17.62	Heptane,2,6-dimethyl	0.01	X
18.50	1-Hexene-3,3,4-trimethyl	0.01	X
18.65	Heptane 2,4-dimethyl	0.00	X
19.00	Decane, 2-methyl	0.01	X
19.00	Undecane, 2,4-dimethyl	0.01	X
19.00	Undecane, 4-methyl	0.00	X
29.09	Propanoic Acid, 2 methyl, butyl ester	0.00	X

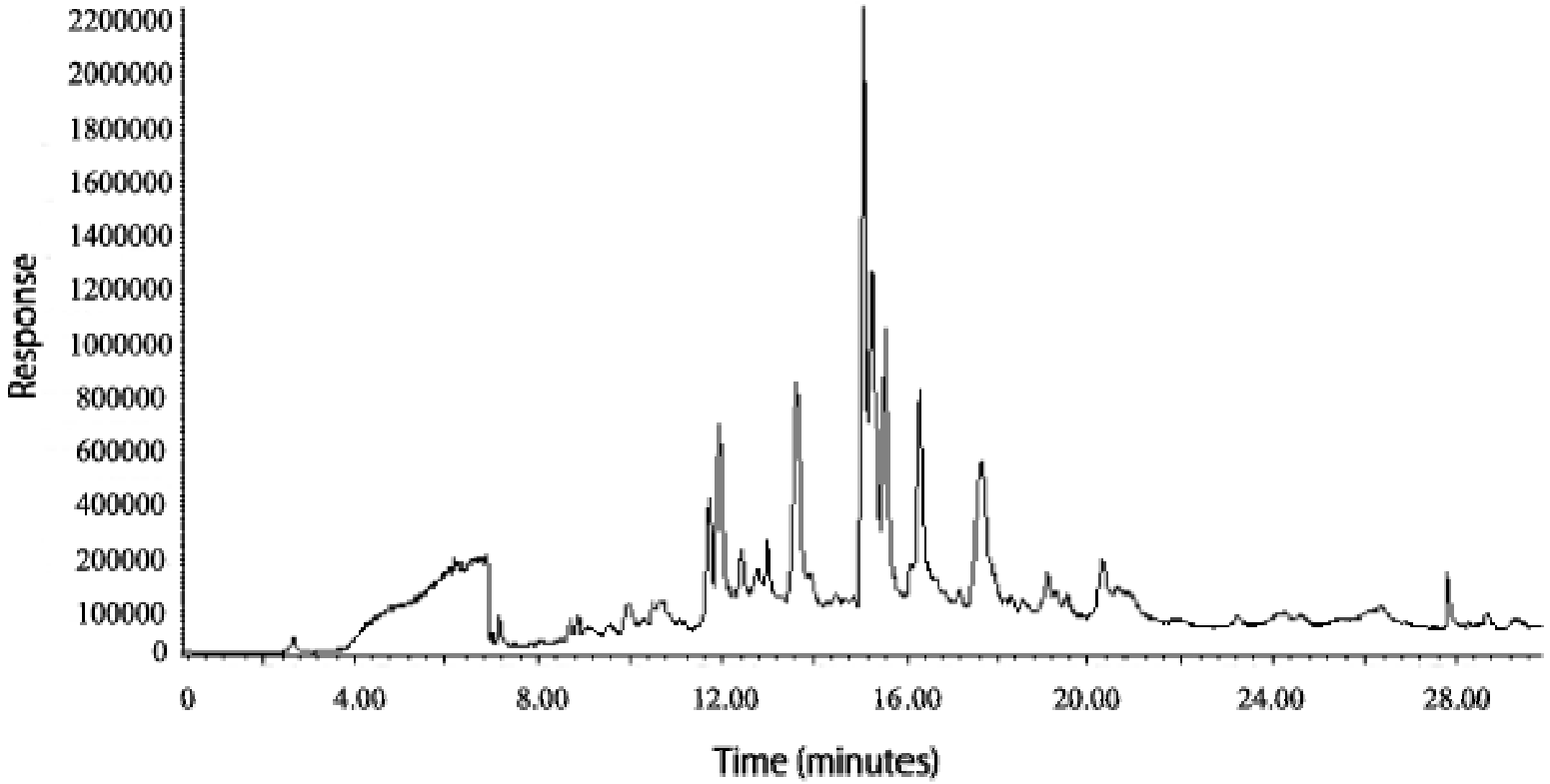


Fig. 3.2. An example VOC chromatograph.

Detected and Quantified Volatile Organic Compounds (VOCs)

Table 3.2 lists twenty-eight of the seventy-two detected and quantified VOCs that were statistically significant ($\rho < 0.01$) with space or time in soil microcosm experiments. VOCs in the functional group categories including alcohols, aldehydes, aromatics, esters, hydrocarbons, ketones, nitriles, and terpenes were identified using the NIST98 library, a 70% match was required for identification, and quantified using functionally and/or structurally appropriate surrogate compounds. Fig. 3.2 illustrates an example chromatogram from LANWR site 11 soils during microcosm studies.

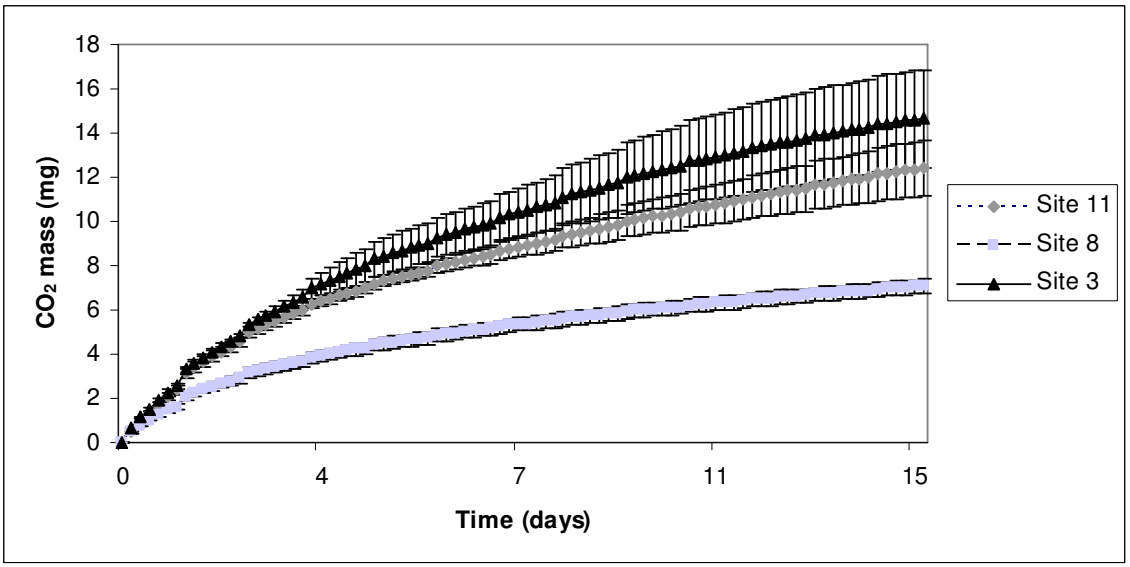
Laboratory Microcosm Results

ANOVA results indicate that there were significant ($\rho < 0.05$) differences between the three LANWR soils in regard to both CO_2 emissions and microbial community structure as measured via FAME and Biolog methods during microcosm experiments (Table 3.3). Furthermore, 28 of the 72 identified VOC means were statistically significant ($\rho < 0.01$) over space and time (Table 3.2) during ANOVA. Additionally, VOC emissions increased over time as did the CO_2 emissions (Fig. 3.3a-b). Where, of these, the VOCs in the alcohol and aldehyde functional groups showed the most concentration build-up over time at each of the LANWR soil locations (Fig. 3.4a-c).

Table 3.3
The non-parametric (Kruskal-Wallis) one-way ANOVA results for FAME, Biolog and CO_2 at each soil location (Asterisks represent statistical significance)

	Location	N	Mean	Rank	Significance ($\rho < 0.05$)
FAME	3	216	7109767 (Peak Ht.)	293.89	0.006*
	8	216		345.37	
	11	216		334.24	
Biolog	3	288	0.093 (ABS)	462.59	0.026*
	8	288		412.88	
	11	288		422.04	
CO_2	3	90	17.62 (mg)	178.49	0.000*
	8	90		75.84	
	11	90		152.16	

A.



B.

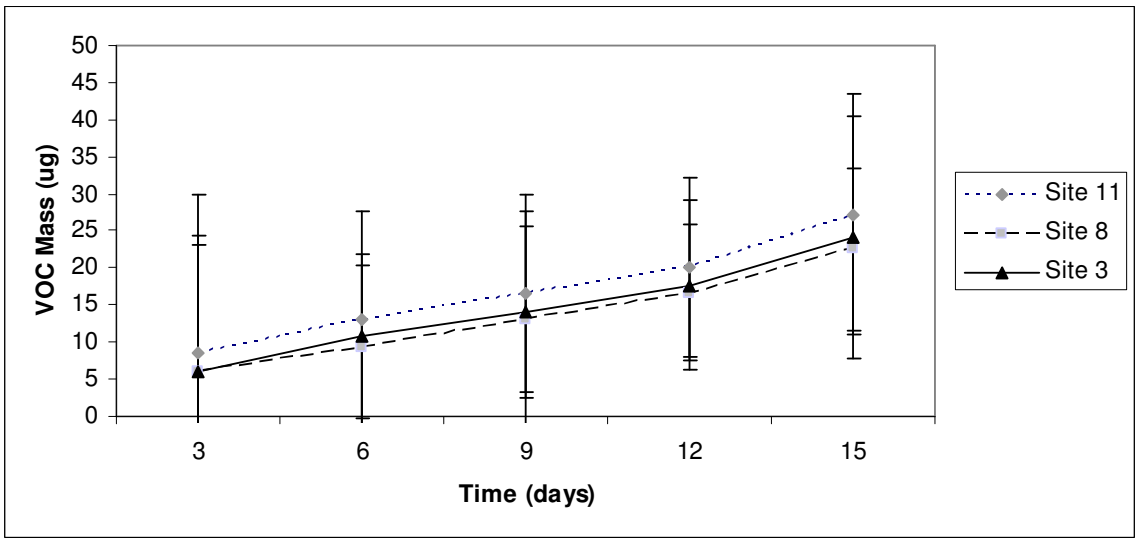


Fig. 3.3. Cumulative time series plots of total soil CO_2 (A) and VOC (B) emissions in microcosm experiments. Unobserved error bars are within the symbols.

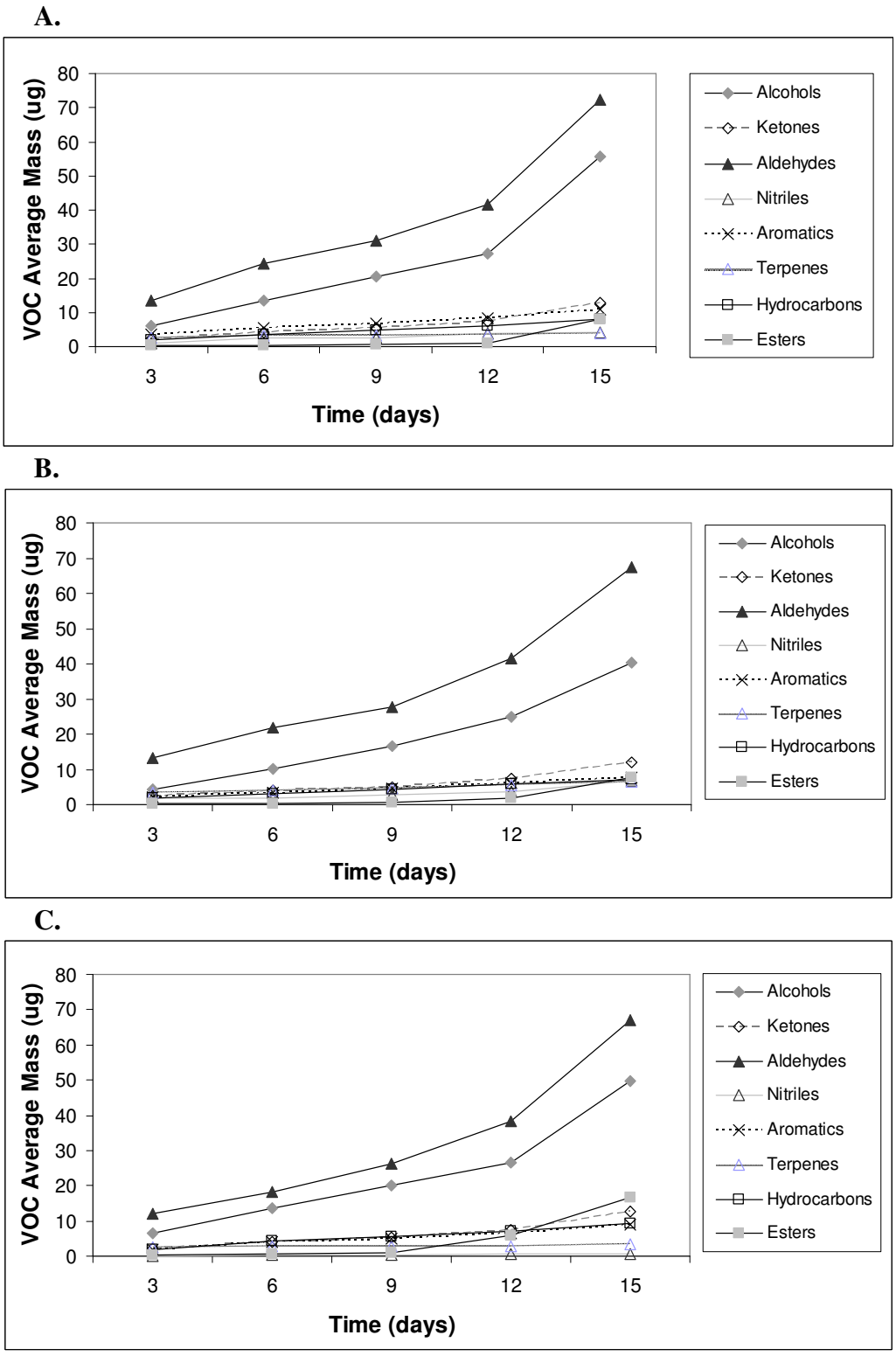


Fig. 3.4. Cumulative time series plots of VOCs by functional group for site 11 (A), site 8 (B), and site 3 (C).

Analysis of VOC data using GIS showed that VOC amounts varied over space in accordance with landscape position as did the microbial community structure (e.g. Biolog and FAME), microbial respiration (e.g. CO_2) and soil characteristics (e.g. TOC, soil moisture, soil conductivity, and reactive Fe; Fig. 3.5). Highest VOC emissions were measured in soils from site 11 and lowest were measured in soils from site 8 (Fig. 3.5a). Soil conductivity and soil moisture trends also followed this pattern (Fig. 3.5c and Fig. 3.5d). Soil CO_2 concentrations indicate site 3 had highest emissions and site 8 had lowest emissions (Fig. 3.5b). Microbial community structure measured by Biolog CSUPs, FAME, soil reactive iron showed similar trends were site 3 had highest responses and site 8 had the lowest (Fig. 3.5f - h). The total organic carbon results were the only results that were inversed, where high amounts were at site 8 and low amounts were at site 3 (Fig. 3.5e). After the autocorrelation of all factors using the GIS spatial analyst tool, the combined datasets indicated that site 3 was the hotspot for microbial productivity as according to VOC and CO_2 emissions, microbial community, and soil conductivity, moisture, reactive iron, and organic carbon measurements.

The amount of VOCs in regard to identified functional groups also varied spatially (Fig. 3.6). The aldehydes, alcohols, aromatics, and ketones all showed highest emissions at site 11 and lowest at site 8 (Fig. 3.6a-c, and Fig. 6f). The esters and hydrocarbons both showed highest emissions at site 3 and lowest at site 8 (Fig. 3.6d-e).

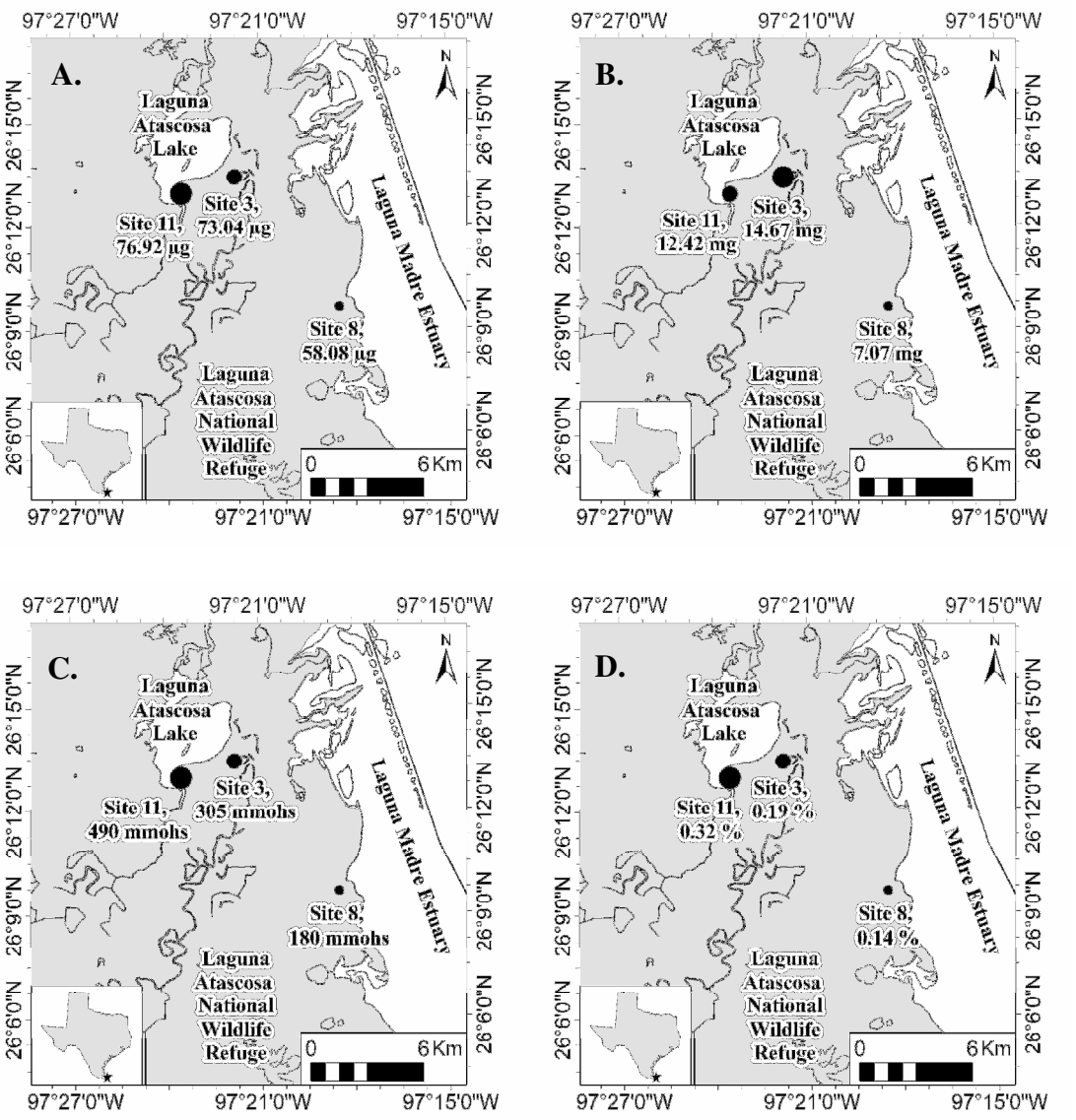


Fig. 3.5. Spatial plots of soil VOCs (A), CO_2 (B), conductivity (C), moisture (D), TOC (E), Biolog (F), reactive iron (G), FAME (H) and the combined data (I).

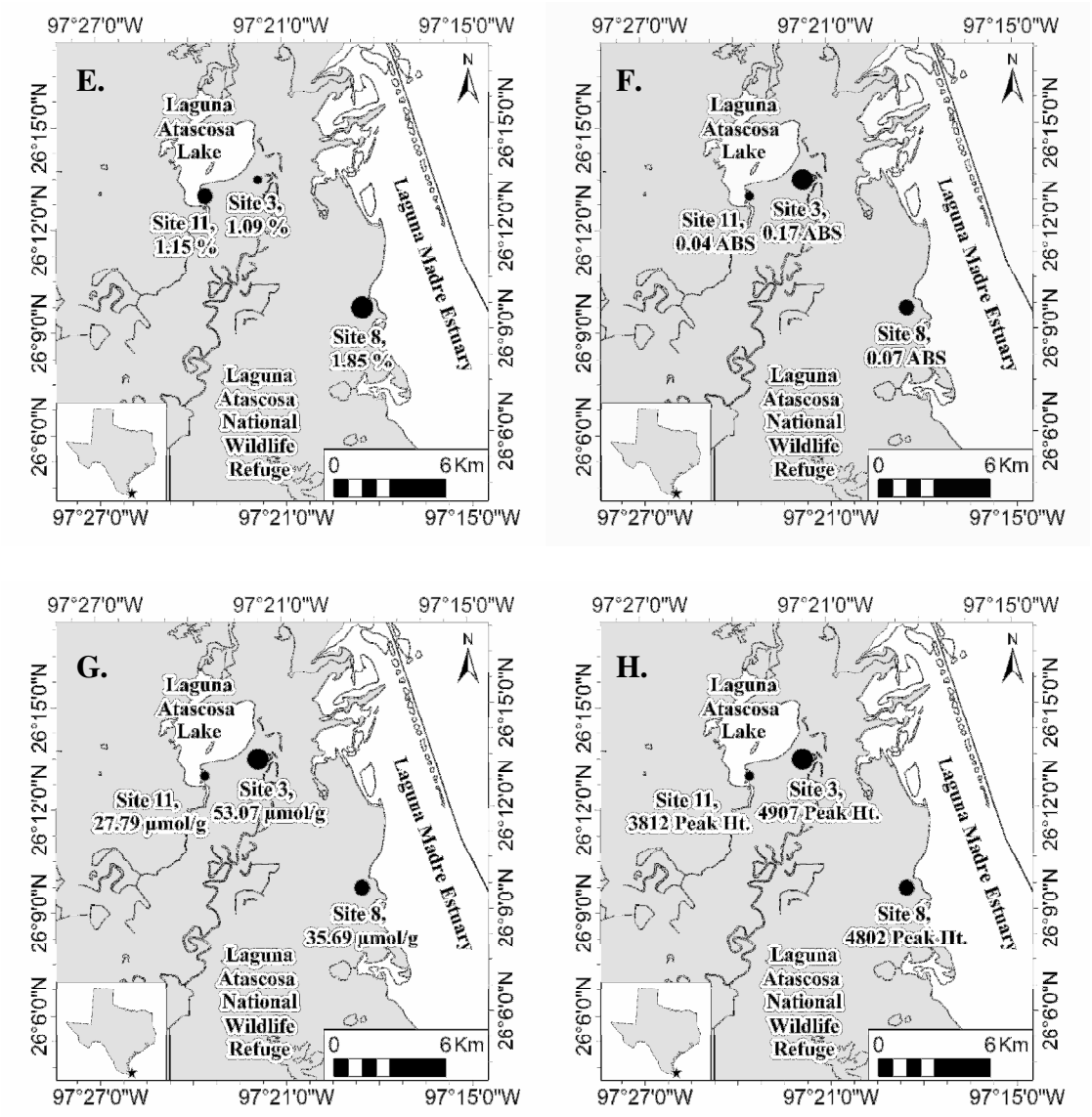
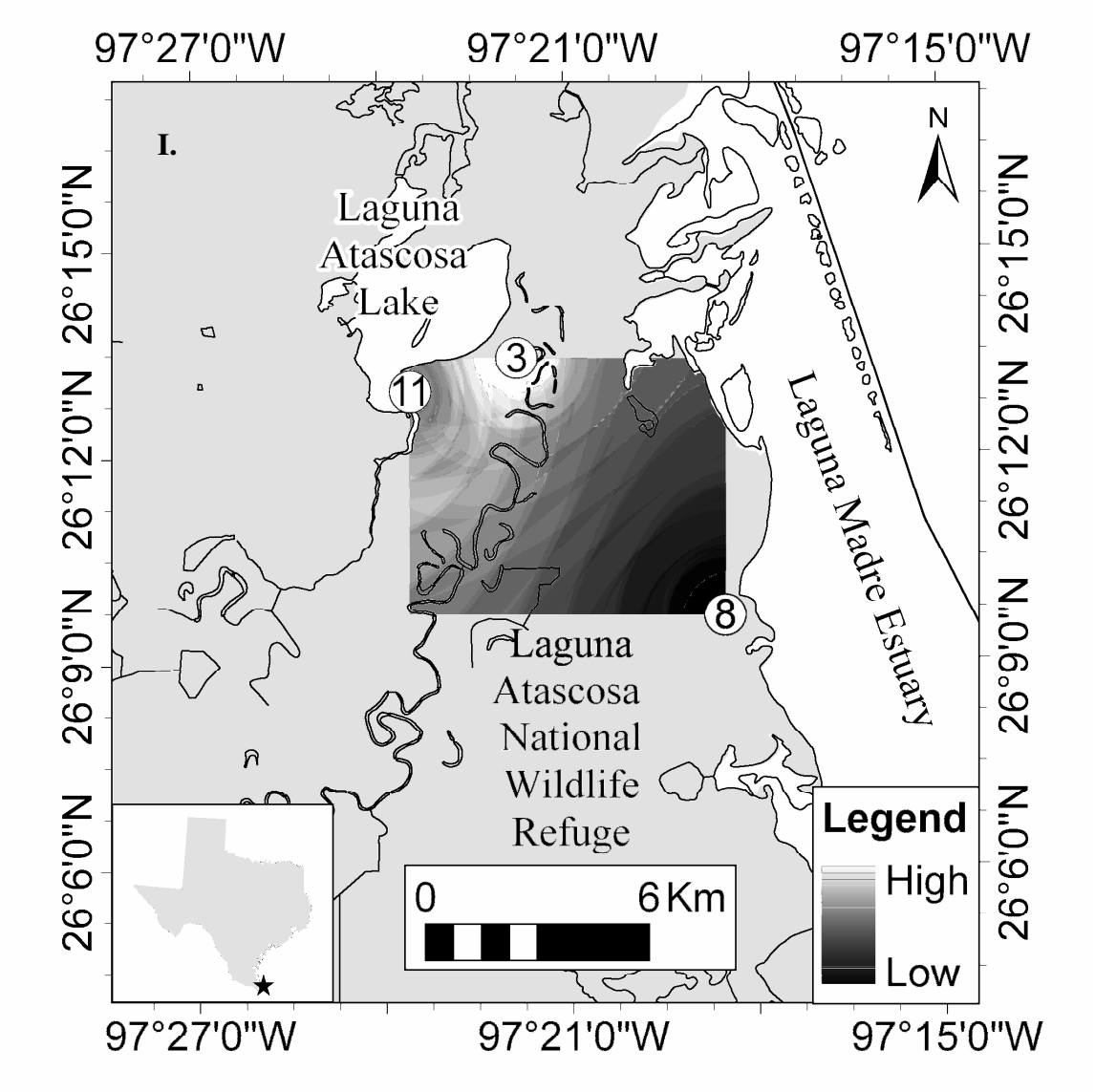


Fig. 3.5. Continued.



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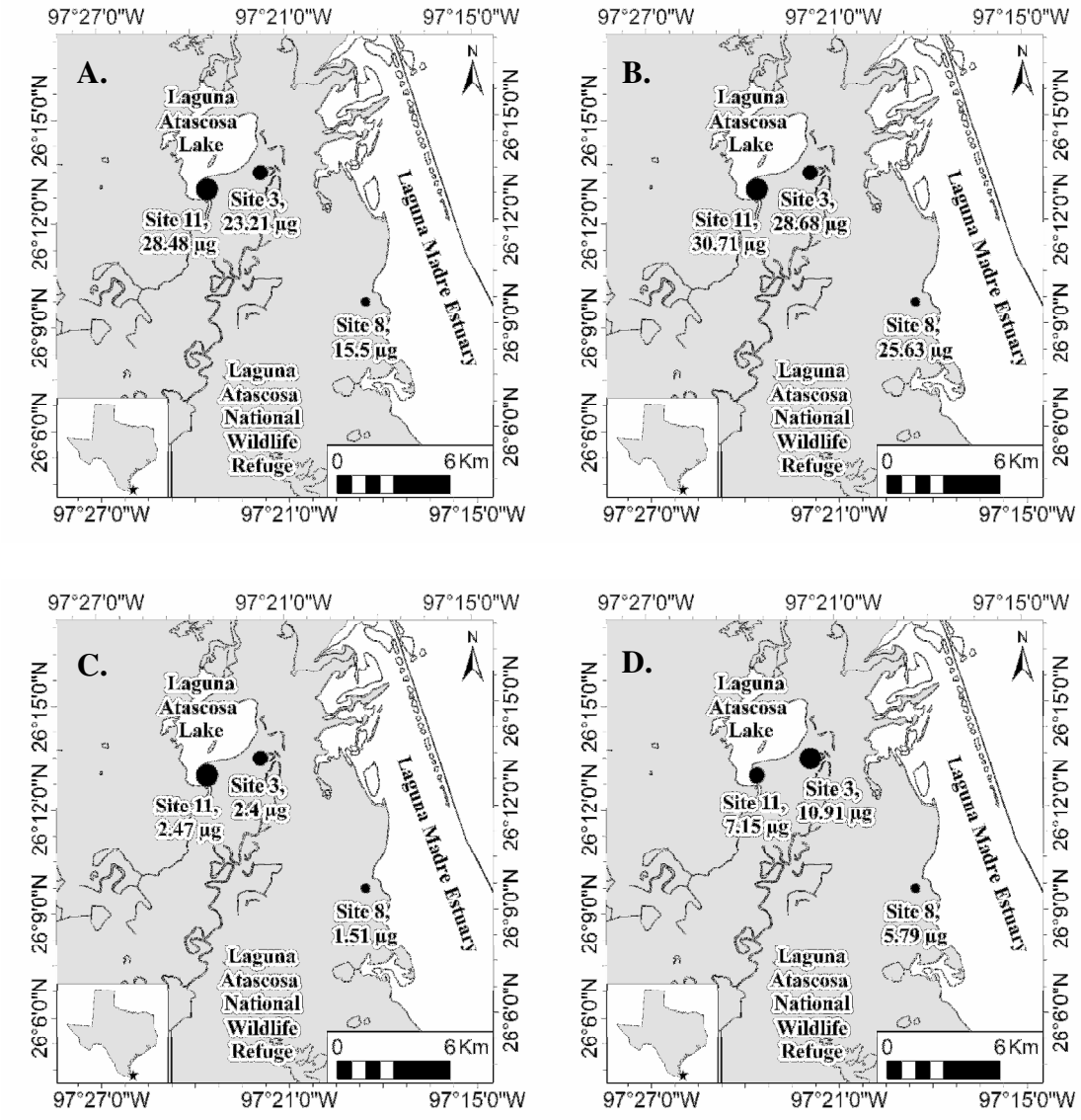

Fig. 3.6. Spatial plots of VOC concentrations by functional group: alcohols (A), aldehydes (B), aromatics (C), esters (D), hydrocarbons (E), ketones (F), nitriles (G), terpenes (H), and the combined data (I).

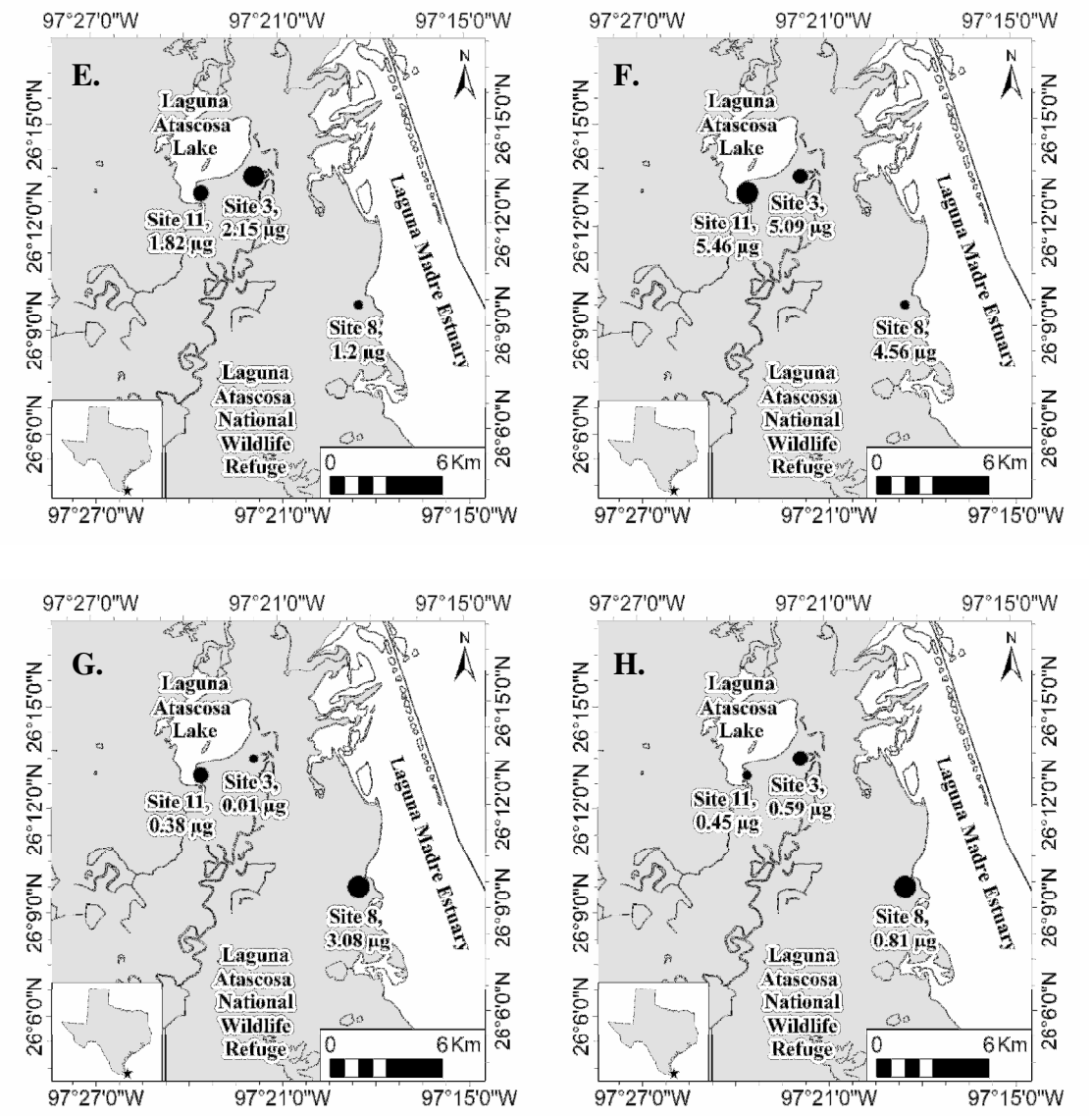

Fig. 3.6. Continued.

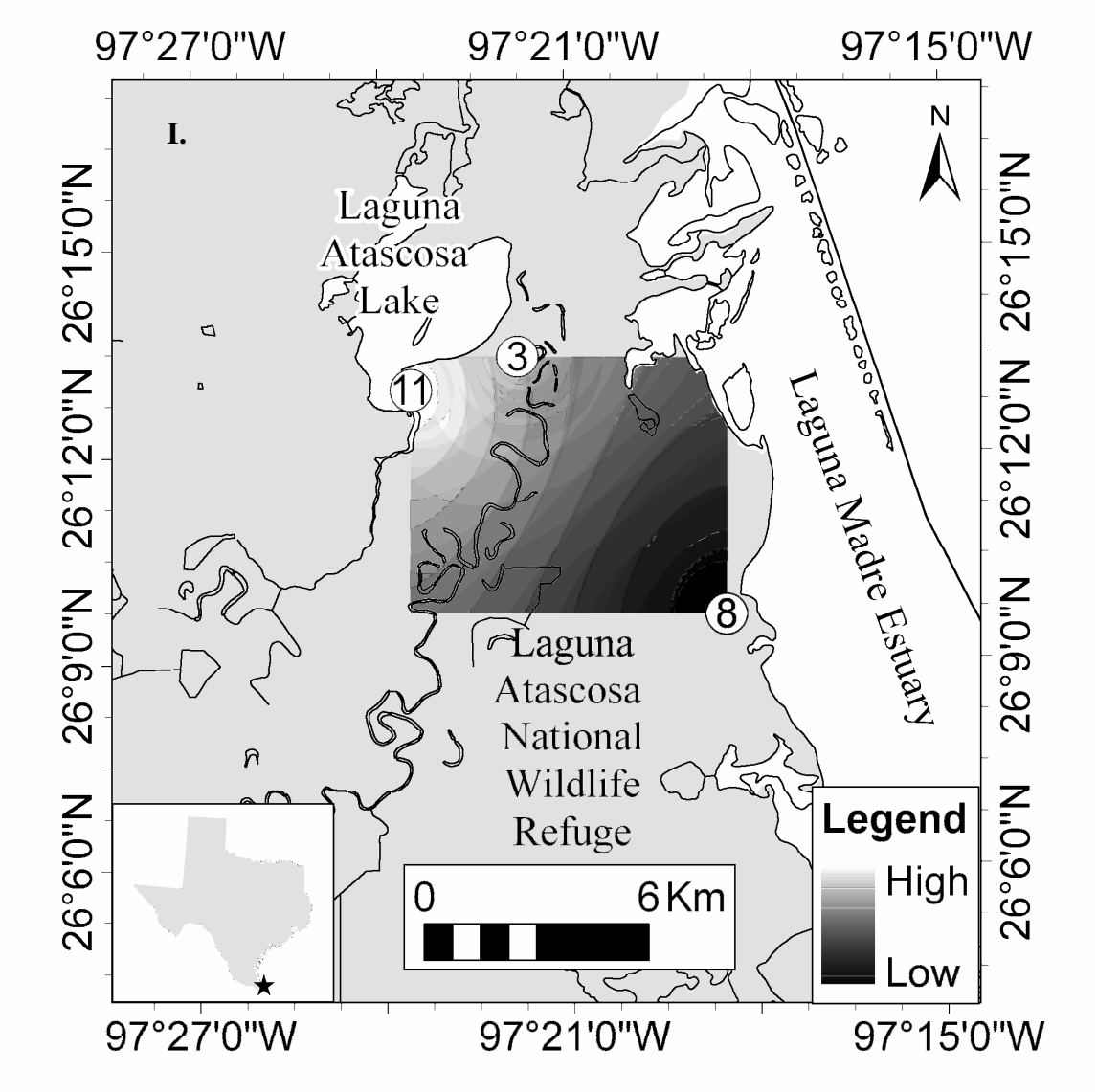

Fig. 3.6. Continued.

The nitriles showed greatest emissions at site 11 but lowest emissions at sites 3 (Fig. 3.6g) and the terpenes were most concentrated at site 8 and least concentrated at site 11 (Fig. 3.6h). The combined data indicated site 11 as a hotspot for VOC production (Fig. 3.6i).

Discussion

Temporal Dynamics of Soil VOCs

Both the VOC and CO_2 emissions increased with time indicating that the VOCs are linked to similar microbial metabolism processes as CO_2 production (Fig. 3.3). This result supports Stahl and Parkin's (1996) work where similar trends in VOC and CO_2 emissions were found over time. Furthermore the VOC measurements (range: < 0.01-80 µg) compare with previous measures recorded in chapter II (range: < 0.01- 50 µg). The results also compare with Stahl and Parkin's (1994) VOC measurements (range: 0.02-9.16 µg) and Sunesson et al. (1995) measurements (range: 0.01 – 360 µg). To gain further insight about the variability in the production of various VOCs different functional groups were examined. The analysis shows differences in functional group production with time (Fig. 3.4). This result aligns with Sunesson et al. (1995) work where differences in individual VOC production were measured over time. In the current work, the alcohols and aldehydes were produced in the greatest amounts and increased the most over time (Fig. 3.4). This is likely due to the small structure and the light mass of these compounds where they may have been derived from the most labile

soil organic carbon sources. The production of these volatiles was likely also dependent on the microbial communities present in our soil samples (Grametbauer et al., 1988; Linton and Wright, 1993; Larsen and Frisvad 1995a; Stahl and Parkin, 1996). According to Schöller et al. (1997) gram-negative soil bacteria produce alcohols and aldehydes. The identification of β–OH and branched fatty acids indicate that gram-negative and gram-positive bacteria were present in our system (Bossio and Scow, 1998; Zelles, 1999) and accounted for ~ 8-10% of the total FAMEs in our soils. Coupling these results to the gram-negative Biolog plate observations where colorimetric changes occurred, we can conclude that gram-negative bacteria were present and speculate that they were possibly dominating the LANWR soils and therefore likely responsible for the production of the alcohol and aldehyde VOCs.

Additionally, 40% (28 of 72) of the identified and quantified VOCs were determined to be significantly different (Table 3.3) in regard to either study location (space) or time. Only 8% (6 of 72) of these VOCs were determined to be significant indicators for community composition shifts in location (or space) whereas 32% (22 of 72) were significant indicators for temporal changes. This result suggests that VOCs were more sensitive to changes in time than space. It is our recommendation that future studies focusing on the temporal dynamics of soil microbial ecosystems consider making use of the VOCs identified in Table 3.3.

Spatial Dynamics of Soil VOCs

CO_2, FAME and Biolog methods all showed significant differences between LANWR locations indicating that there were microbial community composition and activity differences between collected soils (Table 3.2). Furthermore, six VOCs showed significant differences with location (cyclobutanol, 2-ethyl; 2,4-dimethyl-1-heptene; 1-pentanol, 2-methyl; oxime-methyoxy-phenol; and benzonitrile) and may be useful indicators for future studies that examine VOC production over large spatial landscapes.

LANWR Site Location Trends

The spatial trends in soil characteristics (e.g. soil moisture, conductivity, reactive iron, and organic carbon), microbial community structure as measured via FAME and Biolog, and microbial activity using VOC and CO_2 emissions in three distinct soil types (e.g. sand, silt, clay) and spatial landscapes (e.g. coastal upland, seasonal wetland, and grassland) were examined in this research. The GIS analysis indicated that all of these factors varied with space and the Spatial Analyst autocorrelation of the data indicated that site 3 was a potential hotspot for soil microbial processes at the LANWR (Fig. 3.5i). This result may have been due to the grassland landscape indicative of site 3, which likely provided a rich supply of quality carbon from the surrounding vegetation (e.g. root exudates), and the presence of iron oxide minerals. A supporting result of this outcome was the TOC loads which inversely varied from the other measured components where site 8 had the greatest concentrations and site 3 had the lowest concentrations (Fig. 3.5e). Therefore, organic substrate types and quality were likely more important than organic

carbon amounts in indicating the microbial activity and community structure at LANWR site 3 (Gu et al., 2004; Padmanabhan et al., 2003; Waldrop and Firestone, 2004). Furthermore, reactive iron (Fig. 3.5g) trends correlated well with the microbial community measures (Fig. 3.5f and 3.5h) at the LANWR where site 3 had the greatest amounts of each. This was likely due to changing redox conditions in microzones of the sampled soils at site 3, where microorganisms used iron-oxides as terminal electron acceptors (TEAs) during anaerobic respiration (Sell and Morse, 2006; Thamdrup, 2000). It has been well documented that microorganisms couple the oxidation of organic carbon to iron reduction under anaerobic conditions (Crosby et al., 2005; Lovley et al., 2004; Luu and Ramsey, 2003; Weber et al., 2006) and examples of organisms that have been evidenced to participate in dissimilatory iron-reduction reactions include *Psuedemonas* species, *Shewanella putrefaciens*, *Geobacter metallireducens* (GS-15), and *Desulfuromonas acetoxidans* (Nealson and Saffarini, 1994). The citrate dithionate extraction of iron minerals utilized in this research tends to remove the most reactive iron oxides such as lepidochrosite, hematite, goethite, and ferrihydrite (Morse et al., 2002) and therefore it is likely that these minerals served as the iron-oxides available for microbial reduction at site 3.

The results also indicated that site 11 was the location with the greatest soil moisture and conductivity, while site 8 was the driest with lowest conductivity (Fig. 3.5c-d). One would expect this result since site 11 was nearest to the freshwater Laguna Atascosa Lake and typically underwent seasonally wet and dry periods, whereas site 8 was located at a greater elevation (3 m) where the collection of water in soil would be

minimal. The VOC emissions closely paralleled the soil moisture indicating solubility plays a role in the quantification of naturally produced VOCs, where under drier conditions it is possible that volatiles are adsorbed to soil particles and not easily released to the soil atmosphere. In support of this outcome, many researchers have highlighted the importance of soil water content in the volatilization of organic compounds (Site, 2000; Kim et al., 2005; Kobayashi et al., 2004). Where these authors have found that VOC sorption by the soil (and soil organics) is likely to be the most rate-limiting volatilization process but the presence of water may strongly reduce these adsorbent capacities.

VOC Functional Group Trends

Soil VOC functional group production also varied over space at the LANWR (Fig. 3.6). The spatial variability of certain VOC functional groups may be explained by shifts in microbial community composition, organic carbon availability, and soil moisture. This observation is supported by the similarities between the various site measurements (Fig. 3.5) and the VOC functional groups (Fig. 3.6). Hydrocarbons (Fig. 3.6e) and esters (Fig. 3.6d) were at the greatest concentrations at site 3. Site 3 was identified as the hotspot for soil microbial processes (Fig. 3.5i) and therefore hydrocarbons and esters were also likely influenced by the environmental and microbial factors in high concentration at this location. Specifically, hydrocarbons and esters mimicked reactive iron, Biolog, and FAME results (Fig. 3.5g and 3.5f) where concentrations were produced in the greatest amounts at site 3. This indicates the

production of these functional groups were possibly dependent on the presence of iron-reducing microorganisms.

The alcohols, aldehydes, aromatics, and ketones were produced in greatest amounts at site 11 (Fig. 3.6-c and Fig. 3.6f) which also correlated with greater soil moisture and conductivity (Fig. 3.5c-d) and indicated that the release of these VOC functional groups was influenced by water solubility. This is also evidenced in the GIS Spatial Analysis where site 11 was indicated as the hotspot for VOC production. Although Fig. 3.5i indicates LANWR site 3 as the hotspot for microbial processes, site 11 is the likely location for VOC release because of the influence of water content at this location. This conundrum illustrates that although VOCs are very useful indicators of microbial community composition shifts (as evidenced in Chapter II), the abiotic factors affecting their release must also be considered during field investigations.

Finally, nitriles and terpenes (Fig. 3.6g-h) were produced in the greatest amounts at site 8 and mimicked TOC amounts (Fig. 3.5e) which indicated that the release of these compounds were not influenced by organic carbon content during low soil moisture conditions in clay-dominated soils (Fig 3.5d). This finding may seem contrary to the common belief that organic content, clay texture, and low-water content retard VOC release (Mulligan et al., 2001) however the chemical and structural characteristics of terpenes and nitriles may prevent influence by these factors.

Implications for Soil VOCs as Biogenic Sources of Atmospheric Carbon

The natural or biogenic VOC pool is an important component in cycling carbon from the biosphere to the atmosphere. Researchers have estimated global rates of biogenic VOC emissions from vegetation to be over 10^{15} g C yr^{-1} (Levis et al., 2003). The majority of the work to date that analyzes biogenic VOC production does not consider the soil microbial production of VOCs and largely instead focuses on the plant VOC production (Di Carlo et al., 2004; Fukui and Doskey, 1998; Klinger et al., 2002; Levis et al., 2003; Warneke et al., 2002). This research, however, suggests that the emission of microbially produced VOCs from soils to the atmosphere during the mineralization of soil organic carbon may significantly add to this biogenic VOC pool. Biogenic VOCs react with ozone and other oxidants (e.g. OH and NO_x) in the atmosphere to cause longer trophospheric lifetimes of methane and form secondary aerosols (Di Carlo et al., 2004; Levis et al., 2003). Many of the volatiles we have identified in this research to be emitted from soils (e.g. terpenes, alcohols, aldehydes, and aromatics) have been identified as compounds that participate in such reactions (Di Carlo et al., 2004; Martien et al., 1998). This phenomenon may have significant impacts on the densely populated Texas coastal areas (e.g. Houston) that already have VOC concerns from other biogenic, motor vehicle exhaust, and chemical manufacturer sources (Zhao et al., 2004). We suggest that future research focusing on the production and troposphere reactivity of biogenic VOCs should also consider soil VOCs produced during microbial metabolic processes in soil.

Conclusions

This work characterizes VOC production over time and space in south Texas soils while simultaneously measuring traditional proxies of microbial community structure (e.g. FAME and Biolog) and activity (e.g. CO_2). The results not only support the prior work in this dissertation where VOCs were determined to be valid indicators of microbial community composition shifts, they also support Stahl and Parkin's (1996) research that showed VOC production varies similarly to CO_2 production with time. Further, GIS analysis indicates that VOCs and the associated functional groups varied over the LANWR spatial landscape. This evidences that VOCs can be used as microbial community composition proxies over spatial scales. However, this work also illustrates that VOC release is influenced by abiotic environmental factors such as soil water content, soil texture, and organic content and these variables should be monitored during field studies. Finally, this work shows that soils may be significant sources of biogenic VOCs and may contribute to local air pollution, an outcome that requires future attention and research. Overall, this research has aided in determining the potential usefulness of VOC indicators in monitoring spatio-temporal dynamics in soil microbial ecosystems.

CHAPTER IV

SUPPORTING STUDENT CONCEPTUAL MODEL DEVELOPMENT OF COMPLEX EARTH SYSTEMS THROUGH THE USE OF MULTIPLE REPRESENTATIONS AND INQUIRY*

Overview

Students organize scientific knowledge and reason about issues in the Earth sciences by manipulating internally-constructed mental models and socially-constructed, expressed, conceptual models. The Earth sciences, which focus on the study of complex, dynamic, Earth systems, may present unique cognitive difficulties to students in their development of authentic, accurate expressed conceptual models of these systems. This pilot study came about as we were seeking to construct inquiry modules to assist undergraduate students as they developed an understanding of eutrophication along the coastal margin, a good example of a complex, dynamic, environmental process. The modules we developed coupled the use of physical models and information technology (IT)-based multiple representations with an inquiry-based learning environment that allowed our students to develop and test their conceptual models based on available evidence and to solve authentic, complex, and ill-constrained problems.

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The hypothesis was that the quality of students' conceptual models would predict their performance on inquiry modules, and that students' prior knowledge (measured by number of previous courses in geology) would mediate the strength of the relationship between students' model expression and their inquiry performance. Statistical results of this study indicated such a relationship existed only among students in the high prior knowledge group. In the light of the findings, recommendations for pedagogical accommodations to improve all undergraduates' abilities to understand complex, dynamic, environmental systems, with a particular emphasis on students who have lower levels of prior knowledge are made.

Introduction

Student Conceptual Model Development

Cognitive scientists and science education researchers have used the term *mental model* to describe internal representations of external, natural phenomena (diSessa, 1993; Doyle and Ford, 1998; Gentner and Stevens, 1983; Johnson-Laird, 1983; Johnson-Laird and Byrne, 1991). A mental model becomes expressed once it is represented or communicated through drawings, symbols, objects or words. Recently in Earth science education, the term *conceptual model* has been used to describe students' expressed mental models, defined as an accurate, reasonable representation of natural phenomena that is adopted by groups and indicates a level of expertise (Greca and Moreira, 2000; Libarkin et al., 2003). Scientific models, a type of c*onceptual model*, are used by

scientists as cognitive tools to aid in experimental design, develop understanding of complex systems through comparisons with observations, and to make qualitative and quantitative predictions concerning system behaviors under specified conditions. Engagement in authentic practices, which are similar to the activities and tasks performed by scientists, is recommend as a way to support conceptual change among students (Carey, 1985; She, 2004; Vosniadou and Brewer, 1987).

Understanding and manipulating conceptual models of complex Earth systems can present significant learning difficulties for many students (Herbert, 2006). Common learning issues include limited meaningful conceptual understanding of associated knowledge domains and the use of naïve models that guide explanations (Coll and Treagust, 2003; Harrison and Treagust, 1998; Guisasola et al., 2004; Sanger and Greenbowe, 1997). Specific learning issues surrounding student understanding of complex Earth systems include student conceptualization of dynamic Earth systems in static disjointed terms and identification of a single causal factor to explain complex natural phenomena (Raia, 2005).

The Role of Prior Knowledge in the Development of Conceptual Models

It is well established that prior knowledge influences learning (Mintzes and Wandersee, 1998; She, 2004). A common view of learning is that students construct new knowledge and understandings based on what they already know (Bransford et al., 1999); this view is often considered a *constructivist* view. It is based on the premise that the learner selects and transforms information from past and current knowledge into new

constructs and decisions. Since students come to courses in the Earth sciences with a range of prior knowledge, skills, beliefs, and misconceptions, their abilities to remember, reason, solve problems, and acquire new information is affected (Bransford et al, 1999). If the instructor pays attention to the prior knowledge of their students, it has been evidenced that learning is enhanced (Bransford et al., 1999). Although there may be a number of other variables that may affect student learning of complex Earth systems (e.g. student science attitudes/motivations, instructor pedagogical content knowledge, gender differences, and student visualization/spatial ability), we felt that student prior knowledge was the most important variable to base our study because of the range of knowledge backgrounds that frequently characterize students enrolled in Earth science courses.

Complex Earth Systems

The central paradigm of the Earth sciences is the systems concept where Earth systems are physical systems of interrelated phenomenon, processes, and cycles. It is likely that many students have difficulty in understanding systems of even modest complexity, predicting future system behavior, and reasoning correctly about associated environmental issues (Ekborg, 2003; Forrester, 1994) because they often lack the cognitive skills required to understand causality of multivariate systems (Grotzer, 1993; Kuhn et al., 2000). In this context, we have identified five fundamental challenges in studying complex Earth systems that may present significant cognitive difficulty to students during the development of conceptual models. The first challenge is the

conceptualization of natural Earth environments as systems with accurate definition of boundaries and the nature of interactions among the elements of the system (Herbert, 2006). Understanding the transfer and manipulation of matter and energy within systems and across system boundaries as well as relations between systems are also part of this first learning challenge. The second challenge is the characterization and explanation of the complex nature of Earth systems through a description of the system's state over space and time, self-organization, or emergence of structure or patterns (Herbert, 2006). A system's state encompasses a description of the all the important variables of the system and how they change under both steady state and non-equilibrium conditions. Most Earth systems are regulated through positive and negative feedbacks that result in responses to perturbations. The third major challenge is the application of conceptual and scientific models of Earth systems to support predictions (Herbert, 2006). The fourth challenge is that most Earth problems are interdisciplinary, requiring student transfer of content knowledge from a variety of traditional academic fields. Finally, Earth systems encompass phenomena on a multitude of scales, including the nano-, micro-, macro-, local-, regional- and global-scales requiring students to identify the importance of scale and its relationship to a particular problem.

Complexity at the Coastal Margin Interface

Eutrophication, a major research focus over the last decade (Sell and Morse, 2006), characterizes the impact of nutrient enrichments in coastal ecosystems (Cloern, 2001). It is the process in which excess nutrients (nitrogen and phosphorous) stimulate

the growth of phytoplankton and indirectly the bacteria thriving on the seafloor that feed upon the sinking phytoplankton and other sources of particulate organic material (e.g. phytoplankton, detritus, fecal pellets, etc.). Eutrophication ultimately causes hypoxia in bottom waters as the bacterial metabolism depletes oxygen levels (Nixon, 1995; Oviatt et al., 1986), especially when stratified conditions persist from limited mixing or from salinity/density gradients. Benthic-pelagic coupling is frequently important during hypoxic conditions because the anaerobic benthic metabolism quickly dominates as the primary mode of respiration where the production of reduced dissolved species (e.g. H_2S and Fe^{2+}) in sediment porewaters can either penetrate into the overlying waters, further contributing to the hypoxic event, or precipitate to form a variety of minerals (e.g. Fe-Sulfides). Hypoxia, traditionally defined when dissolved oxygen is < 2 mg O_2 L^{-1} in the water column, has been reported in approximately fifty places in the world. It commonly occurs in aquatic, marine, and riverine systems that are stressed by anthropogenic nutrient inputs which are controlled by land-use patterns, production and application of fertilizers, discharge of human waste, animal production, and combustion of fossil fuels (Nixon, 1995). Eutrophication and hypoxia have been observed in Chesapeake Bay (Sagasti et al., 2001), the Northern Gulf of Mexico (Harper et al., 1981; Rabalais et al., 1994; Turner and Rabalais, 1991), the Baltic Sea (Gamenick et al., 1996), the Black Sea (Tolmazin, 1985), Corpus Christi Bay, TX (Sell and Morse, 2006), and the Adriatic Sea (Justic et al., 1987).

We used eutrophication along the coastal margin as the scientific concept addressed in this research because it is a good example of a complex, dynamic,

environmental process that is "made of many highly interconnected parts on many scales" (Vicsek, 2002; Fig. 4.1). It is likely students may experience many of the learning difficulties outlined in the previous section in understanding eutrophication, since it indeed fits the criteria of a complex process within Earth systems.

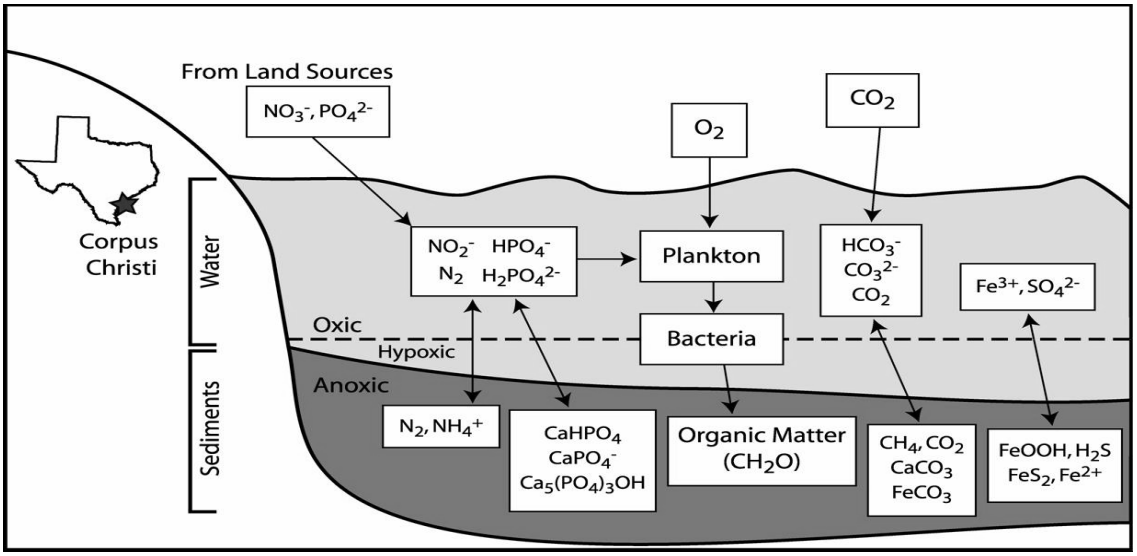


Fig. 4.1. A conceptual model of coastal eutrophication.

For instance, to satisfy the first identified learning challenge, system boundaries must be crossed. During the onset of eutrophication, matter and energy from riverine systems are transferred to pelagic estuarine systems and, with time, then transferred to benthic, sedimentary systems (as outlined in the detailed description of eutrophication above). A student must be able to understand this transfer of matter and energy in order to

understand eutrophication and its impact on Earth systems. In another example, to satisfy a portion of the second learning challenge, there must be spatial or temporal patterns in the process under study. As described above, eutrophication does not exist in all bodies of water nor does it exhibit a year-round occurrence in the water-bodies it does exist in. Therefore, students are challenged to recognize the causes of seasonal eutrophication and to seek explanations for the spatial variability. The other learning challenges of complex systems can be addressed similarly in regard to understanding eutrophication, as made explicit in the above description. Finally, eutrophication serves as the overarching inquiry-based learning module topic because of its consistent occurrence in the Gulf of Mexico which is located less than 100 miles from the university, causing it to be a relevant topic for many of the students residing in Texas.

Supporting Student Learning through Inquiry and Multiple Representations

Instructional sequences and learning environments that stress the use of inquiry and multiple representations (symbols, objects, equations, and pictures) can enhance students' understanding of the nature of science and the development of cognitive and metacognitive skills (Boulter and Gilbert, 2000; Buckley and Boulter, 2000; Herbert, 2006; Stuessy et al., 2005) while simultaneously improving students' abilities to connect their conceptual models to real world phenomena in the Earth sciences (Fig. 4.2).

Inquiry can be defined as the "diverse ways in which scientists study the natural world and propose explanations based on the evidence derived from their work" (NRC, 1996). It requires asking and refining questions, designing and conducting

investigations, gathering and analyzing data, making interpretations and conclusions, and reporting findings; it promotes the development, transformation, and representation of ideas; and it emphasizes depth and not breadth (Krajcik et al., 2000). Central to inquiry is that it be in the context of authentic scientific investigations (AAAS, 1993; NRC, 1996, 2000). Authentic inquiry is described as the "research that scientists actually conduct" (Chinn and Malhotra, 2002), the "ordinary practices of the culture" (Brown et al., 1989), and the activities scientists engage in while conducting research (Dunbar, 2000; Latour and Woolgar, 1986). Inquiry is essential to the teaching and learning of science because it is one of the practices that characterize science (Rowell and Ebbers, 2004) and it has been shown to increase students' depth of knowledge (Bransford et al., 1999; NRC, 1996). Potential benefits of authentic inquiry include students becoming active learners, acquiring scientific knowledge in a meaningful context, developing communication skills, becoming life-long learners, and confronting their own ideas about how the world works (Brown et al., 1989; Edelson, 1997; Hart and Nolan, 1999).

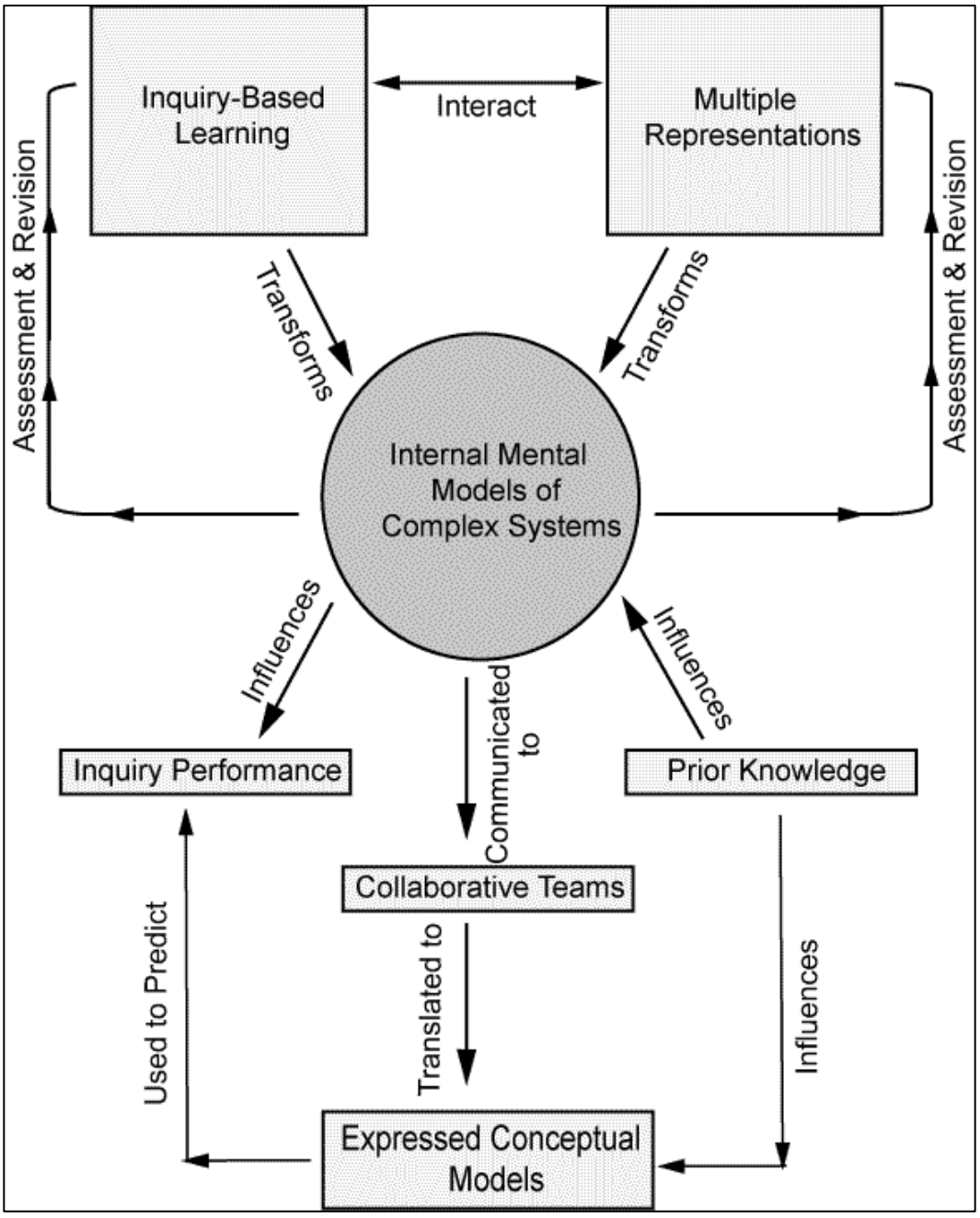


Fig. 4.2. A concept map describing internal models of complex systems, their transformation by instruction that uses inquiry and multiple representations, and their translation to expressed conceptual models, where revision of internal models occurs through continued exposure to the instruction.

Further, authentic inquiry is important for learners because it enables them to act meaningfully and purposefully (Brown et al., 1989) and assists in development of knowledge that is connected to its circumstance of use, rather than existing as a set of disembodied facts (Goldman et al., 1999). We use the acronym IBL to describe the *inquiry-based learning* modules that were implemented in the classroom where we have aligned our framework to the above description of inquiry.

In conjunction with inquiry-based learning, student manipulation of multiple representations can support the development of higher quality conceptual models (Fig. 2). Multiple representations include symbols, objects, pictures, and mathematical equations. They can range from software packages, such as ChemSense© where students can manipulate and build chemical structures on a computer; to well-known formulas such as $E=MC^2$ where students can mathematically solve for E, energy, by substituting numbers for the known variables, M and C; to using the popular "black box" project, a pre-constructed box in which students try to solve the inner-workings of an unknown mechanical process within the box through trial and error as a way of understanding the nature of science. Specific examples appropriate to our study include physical models (e.g. Winogradsky columns) and IT-based representations (e.g. GIS) such as simulations, distributed networks of scientific and social science knowledge, visualization of complex datasets which builds on the strong human ability to comprehend patterns, and the extension of human senses by collecting data and observations at spatial and temporal scales. Successful applications of physical models have been recorded and results indicate that students can develop rich understandings of science (Penner et al., 1998).

In the classroom, learning technologies offer students opportunities to integrate both content and process learning while providing the tools to communicate, contextualize, visualize, manipulate, and inform both students and teachers (Barab and Luehmann, 2003; Edelson, 1997; Edelson, 2001). Furthermore, it expands the range of questions that can be investigated, the type of information that can be collected, the kinds of data representations that can be displayed, and the products that can be created (Krajcik et al., 2000).

Research Goals and Objectives

Undergraduate students need to understand complex, dynamic, near-surface Earth systems and processes, such as those which occur during eutrophication along the coastal margin. GEOL 420 is a course taken by undergraduate students with varying amounts of background knowledge in Earth systems, and our past experience in teaching the course had indicated student difficulties in developing an understanding of eutrophication. In an attempt to assist students in their understanding of this complex Earth process, we coupled the use of physical models and IT-based multiple representations with an inquiry-based learning environment. Overall, the purpose of this pilot study was to assess the effectiveness of these modules in terms of student outcomes associated with their development of accurate conceptual models of eutrophication and in their abilities to think like scientists in an authentic scientific inquiry-based environment.

Educational research methodologies were employed to answer two questions related to our students' learning: (1) What is the role of prior knowledge in mediating students' abilities to think like scientists and develop conceptual models about complex Earth systems in an inquiry-based learning environment? (2) Do imperfect conceptions about complex Earth systems persist, even within a learning environment specifically designed to incorporate scientific inquiry and enrichment through the use of physical models and IT-based multiple representations?

In this pilot study, we attempted to address the above questions by characterizing student conceptual model expressions and inquiry performance of two groups of students characterized by their level of prior knowledge, as measured by previous geology courses completed. We hypothesized that students' conceptual model expressions of complex systems would be predictors of their performance on inquiry modules focused in the Earth sciences, regardless of their prior knowledge.

Materials and Methods

Participants and Context

The upper-level environmental geology course included 15 (6 male, 9 female) juniors and seniors enrolled at Texas A&M University. Membership of the class was predominantly geology majors (93.3%) with diverse course background/content knowledge (Table 4.1). Eight of the learners were considered to have low-prior knowledge (completed < 3 relevant courses) backgrounds and seven learners were

considered to have high prior knowledge backgrounds (completed > 3 relevant courses). All students achieved grades of C or higher in all of their relevant prior courses, indicating at least moderate mastery of the content. Even though there were eight learners classified in the low prior knowledge groups, one was omitted during statistical analysis because of incomplete assignments.

We designed the course to facilitate IBL, specifically, student-directed inquiry as defined by Bonnstetter (1998) where the students were responsible for determining their own research questions, deciding on which materials to use, formatting their own experimental design, and collecting/analyzing their own data/results. In the IBL setting of the course, we encouraged students to work in groups to complete assignments, to explore outside resources aside from their given text, and to use the scientific method during problem solving. Modules focused on coastal eutrophication where sub-topics included water quality; sedimentary biogeochemistry; estuarine and freshwater carbon diagenesis; nutrient sources, sinks, cycles, spatial and temporal trends; and land-use impacts on watersheds. The IT tools used by students included Excel©, ESRI ArcView© GIS, and the World Wide Web. Classroom tutorials during lab exercises prior to the implemented modules provided the scaffolding for the use of the technologies. We used PowerPoint© lectures in association with laboratory exercises when needed, as well as background readings and one-on-one small group help through Socratic inquiry methods.

Table 4.1
Student course backgrounds and prior knowledge rank (L=low, H=high)

Student	Relevant Courses Taken							Total #	Prior Knowledge Rank
	Geo-morph.	Geo-chem.	Hydro-Geology	Intro Chem.	Intro Geo.	GIS	Sed/ Strat		
1				✓	✓			**2**	**L**
2							✓	**1**	**L**
3	✓		✓	✓		✓	✓	**5**	**H**
4				✓				**1**	**L**
5					✓			**1**	**L**
6				✓			✓	**2**	**L**
7		✓	✓	✓			✓	**4**	**H**
8				✓	✓		✓	**3**	**H**
9		✓		✓			✓	**3**	**H**
10				✓				**1**	**L**
11				✓			✓	**2**	**L**
12				✓	✓			**2**	**L**
13			✓	✓	✓		✓	**4**	**H**
14		✓	✓	✓	✓	✓	✓	**6**	**H**
15	✓	✓	✓	✓			✓	**5**	**H**

We implemented three IBL modules and one final project consisting of three related eutrophication topics where students manipulated these multiple representations (Table 4.2): (1) sediment biogeochemistry through use of a physical model, (2) spatial and temporal water quality trends of estuarine systems through visualization and analysis of large datasets, and (3) the impact of land-use on riverine systems and their associated

drainage basins through visualization and analysis of large datasets. In the first module, the students used the physical model; and in the next two, students engaged in IT-based learning. In the final activity, students delivered group presentations describing an animated conceptual model expression.

Table 4.2
Implemented inquiry module topics, studied systems, represented scales, modes of representation, and the produced student learning products

Module	**Topic**	**System**	**Scale**	**Representations**	**Learning Products**
1	Sediment Biogeochemistry	Wetland & Estuarine Sediments	Local Scale (m)	Physical Model: Winogradsky Columns	Written Reports
2	Water Quality	Texas Coastal Margins	Spatial Variability: Regional Scales (km)	Visualization of Complex Data (IT: Excel, GIS, Internet)	Written Reports
3	Land Use & Water Quality	South Platte, CO	Spatial Variability: Regional Scales (km)	Visualization of Complex Data (IT: Excel, GIS, Internet)	Written Reports
Final Project	Eutrophication	Texas Coastal Margins	Temporal Variability: Seasonal Scales (months)	Animation of Conceptual Model Expression	Presentation of Computer Animation

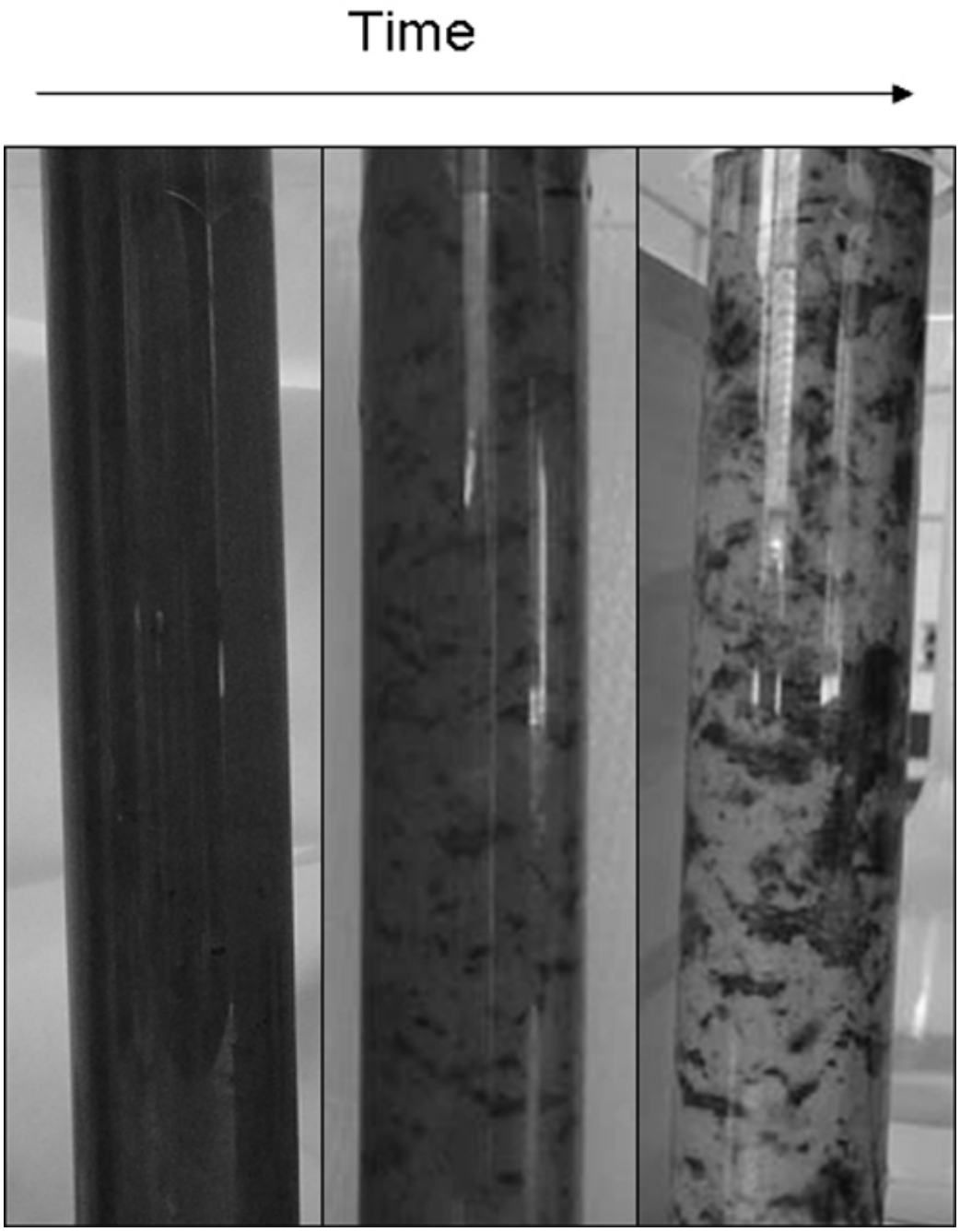


Fig. 4.3. Photographs capturing the spatio-temporal characteristics of a Winogradsky column.

Instructional Sequence

Student-directed inquiry using the physical model included the building of a Winogradsky column, which is a clear tube of sediment that was allowed to go anaerobic through the utilization of O_2 during microbial respiration (Fig. 4.3; Deacon, 2004). Since hypoxia is typically related to the process of eutrophication, the use of this model allowed students to directly observe mineral diagenesis, over time, visually in the sediments. Spatio-temporal visual changes were direct evidence of these processes, including the formation of Fe-Sulfide minerals such as framboidal pyrite or loss of Fe-Oxide minerals such as hematite due to both biogenic and non-biogenic reduction-oxidation reactions, or the development of visible colonies of variably colored microorganisms. Millivolt readings obtained by inserting wires at both ends of the sediment tubes and connecting them to a battery tester allowed students to directly measure potential energy changes in the columns with time as metabolic shifts occurred (aerobic to anaerobic), aiding the students' understanding of thermodynamics and the redox sequence in sediments.

Students explored a number of specific questions because they could manipulate several variables, including addition of organic substrate (molasses or oil), aeration (anoxic or oxic), nutrients (sulfate or nitrate), or sediment type (marine or freshwater sediments), to evaluate the impact of these variables on microbial respiration over a three-week time span. Students then used digital photographs and voltammetric measurements they took of the columns over time to make interpretations. The physical model represented the small-scale biogeochemical processes in sediments of both

estuarine and wetland environments. The situation statement provided to the students during the initial physical model laboratory is shown in Fig. 4.4a.

The IT exercises incorporated the use of geographical information systems (GIS) and Excel© to analyze large-scale spatial contamination in two Texas estuaries, Galveston Bay and Corpus Christi Bay, and the South Platte, Colorado, watershed through access to large datasets. In these modules, students chose the data they deemed important to analyze according to their working hypotheses about the studied systems; hypotheses were generated prior to student research activities within collaborative student teams. Examples of data students could manipulate included monthly nutrient concentrations over a series of decades, monthly rainfall data over decadal time periods, land-use data for multiple regions and counties, seasonal salinity and turbidity data, and selected anthropogenic organic and inorganic pollutants, among others. The broad range of data was needed to support a variety of student research questions (Etheredge and Rudnitsky, 2003). Examples of the IT-based, inquiry modules situation statements are provided in Fig. 4.4b-c.

The summative learning products associated with the implemented laboratories included three written reports (incorporating the technologies of digital photographs, Excel©, and ESRI ArcGIS© maps) and a PowerPoint© animation (Table 4.2). These products were used to assess students' conceptual models and to expose students' imperfect conceptions and/or inabilities to incorporate observed data and trends into their conceptual models.

4A. The Physical Model- Winogradsky Column Module

You have been called by the US government to determine the affects of eutrophication on sediments. Specifically, your task is to test the response of the sediment biogeochemistry to different variables by building a Winogradsky column. You are to assemble into four scientific teams of four scientists per team. Your team is to choose a variable and determine an experimental design and hypotheses. The variables and methods of measurement that you may want to consider are included below (remember establishing controls and replication are important components of experimental design). You will report your results to the class in presentation form after three weeks of observation.

Recommended Variables	Recommended Methods of Measurement
Dissolved oxygen meter	mV meter
Sediment type (Terrestrial vs. Marine)	Non – galvanized nails
Oxygen level (Aeration vs. no Aeration)	Digital camera
Organic matter content (Oil vs. Molasses)	

4B. The IT- Texas Coastal Margins Module

You are a researcher for the EPA and your advisors have decided that further investigation of the Texas coastal margin is needed. You have been assigned to investigate two Texas estuaries, Corpus Christi Bay and Galveston Bay, which are both National Estuaries under protection. They would like you to determine the *nutrient contamination state*, by determining *seasonality and trends of the nutrient inputs*, *possible contamination sources (i.e. landuse patterns),* the impacts of the nutrients on the previously studied *estuarine biogeochemical processes*, by making *recommendations for change* to ensure a future healthy watershed. They would also like you to rank the estuaries in terms of nutrient contaminant severity and which estuary should be prioritized for financial support for restoration.

4C. The IT- South Platte, CO Module

You are an environmental consultant bidding and completing a project report for the federal government. In this project, you are going to write a proposal to study water quality in the South Platte River and then a report on your findings. Knowledge of the quality of the Nation's streams and aquifers is important because of the implications to human and aquatic health and because of the significant costs associated with decisions involving land and water management, conservation, and regulation. In 1991, the U.S. Congress appropriated funds for the U.S. Geological Survey (USGS) to begin the National Water-Quality Assessment (NAWQA) Program to help meet the continuing need for sound, scientific information on the extent of the water-quality problems, how these problems are changing with time, and an understanding of the effects of human actions and natural factors on water quality conditions. The NAWQA Program assesses the water-quality conditions of more than 50 of the Nation's largest river basins and aquifers, known as Study Units. Collectively, these Study Units cover about one-half of the United States and include sources of drinking water used by about 70 percent of the U.S. population. The objectives of this project are to identify and evaluate potential water quality and water resource problems within the South Platte River watershed in Colorado, Wyoming, and Nebraska.

Fig. 4.4. Examples of student situation statements to immerse students in the studied systems and to prompt student research: (A) the physical module (B) the Texas coastal margins IT-module and Barnet (C) the South Platte, CO IT-module.

Instrumentation and Reliability

We developed a rubric which provided a quantitative analysis of all students' written reports and conceptual model expressions (included in animated group presentations constructed in PowerPoint©). The rubric provided a pre-constructed set of standards and learning goals, where a point system was used to assess student performance. Our design of the assessment instrument was guided by the skills scientists utilize during authentic inquiry as summarized by Chinn and Malhotra (2002). Ten categories were determined as criteria for the rubric, including (i) content knowledge, (ii) construction of a hypothesis, (iii) experimental design, (iv) ability to think critically, (v) inclusion of references, (vi) comparison to water quality standards, (vii) ability to collect and report data, (viii) organization, (ix) creativity, and (x) inclusion of essential components to communicate research findings, following Chinn and Malhotra's (2002) suggestions.

A team of graduate students served as external evaluators and determined the reliability of the instrument. These evaluators, although enrolled in the departments of Teaching, Learning & Culture (TLAC) and Geology & Geophysics at Texas A&M University, were not involved in the implementation of the current research. Each evaluator graded the same three student reports which were randomly selected from a stratified report database. We assessed reliability by calculating the internal consistency coefficient (α) using the statistical package SPSS©. Table 4.3 illustrates the resulting inter-rater reliability where all rubric categories gave an internal consistency value of 0.83. When considering that in exploratory research a modest reliability of 0.50 to 0.60

is acceptable (Ravid, 1994), our final instrument showed acceptable reliability in differentiating students' products.

Table 4.3
Evaluators' rubric mean scores, range 0-2, standard deviations for selected student products, and total rubric inter-rater reliability (α), range 0-1

Rubric Category	**Mean**	**Standard Deviation**
Content Knowledge	1.30	0.61
Critical Thinking	1.42	0.61
Scientific Components	1.28	0.57
Data Collection	1.52	0.65
Hypothesis	0.70	0.76
References	0.22	0.58
Standards	0.24	0.59
Experimental Design	1.26	0.56
Creativity	1.64	0.60
Organization	1.76	0.56
Reliability Coeff. (α)	**0.83**	

Results

What Is the Role of Prior Knowledge in Mediating Students' Abilities to Think Like Scientists and Develop Conceptual Models about a Complex Earth System in an Inquiry-Based Learning Environment?

Variables used in answering this question are summarized in Table 4.4. Initial statistics using a one-sample Kolmogorov-Smirnov test of the dataset indicated that the data were normally distributed ($\rho > 0.05$) and equally varied ($\rho < 0.05$) as determined by Levene's homogeneity of variance test; therefore, the data satisfied the assumptions of the statistical tests utilized in the following analyses. A Student's *t*-test revealed no significant ($\rho > 0.05$) differences in inquiry-based learning (IBL) and conceptual model expression (CME) between the students in the high and low prior knowledge groups (Table 4.5). Therefore, the average scores for the two prior knowledge groups were considered statistically the same. Regression analyses were then performed on each prior knowledge group to assess the relationships between inquiry-based learning and conceptual model expression (Table 4.6). A significant ($\rho < 0.05$) relationship and a good linear fit ($r^2 = 0.606$) between student conceptual model expressions and inquiry learning was found in the high prior knowledge group. However, the low prior knowledge group did not show a significant ($\rho > 0.05$; $r^2 = 0.087$) relationship between these two variables. Therefore, the statistical results indicated a strong predictive relationship between conceptual model expressions and inquiry performance in the high prior knowledge group only, which may mean that these students were able to connect

their conceptual models to scientific inquiry and utilize them during problem-solving, whereas the low-prior knowledge groups were not. Implications of this finding in terms of the design of instruction for students with varying levels of prior knowledge are discussed below.

Table 4.4
The measured variables, the instruments and reliability, scoring range, student mean scores and standard deviations (s.d.) used in this research study

Variable	**Instrument**	**Reliability (α)**	**Range**	**Mean (s.d.)**
Prior Knowledge (PK) Levels	Courses Taken	-----	Low (< 3) High (≥ 3)	2.8 (1.6)
Inquiry Module Performance (IMP)	Scores on written reports (rubric)	0.83	0-2	1.08 (0.57)
Conceptual Model Expression (CME)	Scores on student animation & presentation (rubric)	0.83	0-2	1.31 (0.75)

Table 4.5
Paired Student's *t*-test to determine differences between prior knowledge groups (IMP= inquiry module performance; CME = conceptual model expression)

Pair	Paired-Difference Mean	Paired-Difference Std. Deviation	T	N	Sig. (2-tailed) ($\rho < 0.05$)
IMP – High & Low	-4.55	12.52	-0.961	7	0.374
CME- High & Low	1.91	13.64	0.371	7	0.724

Table 4.6
Regression analysis of inquiry module performance (IMP) and the conceptual model expression (CME) of the two prior knowledge student groups (Asterisks represent statistical significance)

Group	Mean	Std. Deviation	N	R^2	Sig. ($\rho < 0.05$)
High					
IMP	75.97	9.72	7		
CME	64.50	9.46	7	0.606	0.0398*
Low					
IMP	73.29	11.18	7		
CME	67.32	8.84	7	0.087	0.479

Do Imperfect Conceptions about Complex Earth Systems Persist, Even Within a Learning Environment Specifically Designed to Incorporate Scientific Inquiry and Enrichment Through the Use of Physical Models and IT-Based Multiple Representations?

In answering this question, we characterized the various student difficulties with the implemented modules through an analysis of the rubric scores on each implemented module and through the identification of student imperfect conceptions. First, we evaluated student average scores in each rubric category and each module (Table 4.7). Results indicated that the IT exercises presented more difficulty for students as compared to the physical model and animation exercises. Further students generally scored lower on the rubric categories of *inclusion of a hypothesis* (average rubric scores of 0.86), and *referral to references/standards* (average rubric scores 0.17) than on the other rubric categories (average rubric scores > 1.21). Next, several imperfect conceptions were revealed during student conceptual model development, including issues of scale, application of factual knowledge, and reasoning about systems. We preferred the use of the term "imperfect conception" over the term "misconception" in this study because "misconception" can imply a negative, incorrect student response, whereas "imperfect conception" implies a naive conception that is only partially correct in the examined situation but could be entirely correct in a different scenario. For example, many students believe that all rivers flow down from north to south.

Table 4.7
Average student (n=14) scores (0=low, 2=high) of rubric categories for each IBL module and the conceptual model expression (CME)

Rubric Category	Physical Model Avg. Score	IT- Texas Coastal Margins Avg. Score	IT- South Platte Avg. Score	CME Avg. Score	Total Avg. Score
Content Knowledge	1.07	1.27	1.20	1.29	**1.21**
Critical Thinking Skills	1.64	1.20	1.20	1.29	**1.33**
Scientific Components	1.93	0.93	0.87	1.71	**1.34**
Data Collection	1.64	1.40	1.07	2.00	**1.52**
Hypothesis	1.36	0.33	0.53	1.20	**0.86**
References	0.29	0.27	0.13	0.00	**0.17**
Standards	0.00	0.27	0.40	0.00	**0.17**
Experimental Design	1.71	0.93	1.07	1.57	**1.31**
Creativity	2.00	1.20	1.60	2.00	**1.69**
Organization/ Coherence	1.86	1.53	1.47	2.00	**1.71**
Class Avg. Score on Modules (%)	**76.4**	**57.6**	**61.4**	**72.8**	**67.1**

Some rivers may indeed flow in this direction, however, topography is the main factor that determines flow path; therefore in some cases it would be true if the student said a river ran north to south as long as the topographic gradient also decreased in this direction. Our definition of an imperfect conception in this work included reasoning, thinking, and using terms that may be correct when applied to other cases, but in the phenomena under study it was either inappropriate or misapplied. Student responses in Table 4.8 were descriptions of processes or mechanisms that explained observations in the Winogradsky column experiments. Student A's response is an example of an imperfect conception because "molecules" are not bigger than "cells" therefore their conceptual model did not account for scale. Student B's and student C's responses inappropriately used the course terminology in their explanation of the phenomena. The definition of a chemically "reduced" environment does not contain enough oxygen to sustain "aerobic processes" so student B's statement contradicted itself. Student C's statement was also contradictory because the formation of "pyrite" must occur in oxygen free environments, therefore "oxygen" can not be "readily available" during its formation. Student D's statement illustrated simplistic reasoning and the lack of consideration of alternative hypotheses. The student reasoned that the newly formed "black material" may have been "bacteria" based upon a limited understanding of links between biotic (bacterial respiration) and abiotic (precipitation of black Fe-Sulfides) processes, an important characteristic of most Earth systems. The above imperfect conceptions highlight student difficulties with this complex system and perhaps emphasize areas of needed student support in order to develop more accurate conceptual

models. Even though such imperfect conceptions were at first frustrating for the course instructors, we should note that these imperfect conceptions may have never come to our or the student's attention if we had not involved our students in such inquiry-based tasks and allowed opportunities for them to express their conceptual models. In contrast, during direct instruction, students often may not be aware of such imperfect conceptions nor have the appropriate time to revise them because of infrequent exams and the lack of model-driven exercises.

Table 4.8
Examples of student imperfect conceptions revealed during conceptual model development

Student	Response	Area of Limitation
A	"The reason bacteria degrade the molasses faster than the oil is because the oil **molecule** is bigger than the bacteria **cell.**"	Linking of scales
B	"A **reduced** environment has **aerobic** processes."	Content knowledge
C	"**Pyrite** exists where **oxygen** is readily available."	Content knowledge
D	"The **black** material produced in the sediment is **bacteria.**"	Reasoning

Discussion and Conclusions

Conceptual Model Expressions as Predictors of Student Inquiry Performance

In this pilot study, we attempted to implement, design and assess instructional modules in an undergraduate Earth science classroom using multiple representations (IT and physical models) and IBL in order to address known student learning difficulties involving conceptual model development of complex systems. We measured the predictability of student inquiry performance based on conceptual model expressions. We assessed learners based on prior knowledge levels. Our results showed that conceptual model expressions could be used as a predictor of IBL module performance in the students with high prior knowledge, even though no significant differences between high and low prior knowledge groups' performances were found on either of the two outcome measures, conceptual model expression and inquiry-based learning.

Conceptual models are a central component of scientific research. They are used to guide the development of hypotheses, make predictions, and communicate understanding to the scientific community (Dunbar, 1995). Students with high prior knowledge likely represented a group with greater expertise and abilities to effectively use conceptual models to solve ill-constrained problems. Students with low prior knowledge and less expertise likely had a wider range of learning issues to overcome in order to solve the same problems. For instance, the low-prior knowledge students seemed unable to connect their conceptual models to the inquiry task, which may

indicate that they did not understand the significance of the conceptual model or its role in solving scientific problems.

Student Difficulties During Implemented Inquiry Modules

As previously mentioned, students have difficulties reasoning about multivariable causality (Kuhn et al., 2000). Instructors can support the learning process by assisting their students to reach their full potential through the placement of cognitive scaffolds (aids that support student thinking, reasoning and remembering) in an instructional sequence (Vygotsky, 1978). Scaffolds can be instructional, curricular, or technological (Krajcik et al., 2000). For example, Goldman et al. (1999) suggests scaffolds such as tutoring strategies, modeling practice, visualizations and representations, and inquiry to assist students in their development of the knowledge representations, ways of thinking, and social practices that depict the Earth sciences. Further, Etheredge and Rudnitsky (2003) suggest utilizing the "inquiry cycle" when teaching IBL in the classroom. Their version of an inquiry cycle suggests that instructors strongly consider their students' backgrounds (prior knowledge) while creating an inquiry learning environment that sequences instruction to include a prolonged immersion experience in which the system is explored in depth before proceeding to the development of scientific questions about the phenomenon under study. Once scientific questions are developed, students then do their own research to answer one or more questions. Finally, the inquiry sequence ends with a consequential task (a final product in which the students are evaluated), which has been posed by the

teacher and requires the students to apply their understanding in a new yet similar context.

Although we took some major steps in aiding student conceptual model development of complex systems using IBL and multiple representations in this pilot study, our findings suggest that there may have been other areas where additional scaffolding could have been applied during implementation in order to further decrease student difficulties, especially in regard to the low prior knowledge students. We found that students had particular difficulties with imperfect conceptions of eutrophication and that they had difficulties with the use of the IT for modeling. We can speculate that the students may have had cognitive over-load issues while using IT (see Kalyuga and Sweller, 2004), but much more information would be needed in order to investigate the nature of these difficulties. Even in spite of the difficulties, we wish to note that the IT experience allowed students to visualize the large-scale societal/geological impacts on the studied system (e.g. ESRI ArcGIS™ software), and that no other instructional tool in the Earth sciences has allowed students to experience these impacts first hand.

Pedagogical Recommendations

In response to the student difficulties identified above, we suggest an intervention that includes a number of accommodations. Our recommendations to better support the development of external conceptual models of complex Earth systems among university students include refining the scaffolding (or support), and pedagogical content knowledge (the instructor's knowledge of a subject matter, students and possible

misconceptions, curricula, and general pedagogy) the teacher brings to the learning situation (Barnet and Hodson, 2001; Driel et al., 2001). We suggest an accommodation to provide more opportunities for students to express, share, and discuss their external conceptual models, more immediate feedback to students, and more time for reflection and revision of internal mental models (Goldman et al., 1999). We also suggest invoking more class discussions on models and how they are useful to the scientific community in order to further student understanding of their own expressed conceptual models, the nature of science, and the evolution of knowledge in science. To address the IT difficulties and imperfect conceptions we previously noted, we suggest utilizing a distributed-expertise (a network of the human resources of the learning community), collaborative (grouping of students with the intent to form cooperative teams), approach by pairing the more IT advanced students with those who are less advanced and pairing high prior knowledge students with low prior knowledge students during inquiry modules. We also suggest identifying students' imperfect conceptions prior to the intervention, thus allowing the opportunity to address them during the inquiry modules, perhaps through an open-ended pretest, through the use of probing questions, or through the use of student interviews to identify common misconceptions. Finally, we suggest implementing IBL modules that more closely follow the inquiry cycle suggested by Etheredge and Rudnitsky (2003) to assist students' understanding of the nature of scientific inquiry.

As Krajcik et al. (2000) reports, inquiry can pose challenges for teachers where it may require uses of time and types of instruction that are different from traditional

teaching methods. As educators in the Earth sciences, we must be willing to adapt our instruction in order to address student learning difficulties. Our determination of this pilot studies' effectiveness on different prior knowledge groups and the identification of the student imperfect conceptions that persist with this type of instruction are useful preliminary assessments aiding in our recognition of possible pedagogical improvements. In summary, we specifically recommend pinpointing students' imperfect conceptions prior to inquiry-based instruction, providing a wide variety of instructional scaffolding to facilitate understanding, differential pairing of students by prior knowledge and IT familiarity, and explicitly paying attention to the ways in which scientists think and do their own research when investigating complex environmental systems.

CHAPTER V

DEVELOPING NON-SCIENCE MAJORS' CONCEPTUAL MODELS OF COMPLEX EARTH SYSTEMS IN A PHYSICAL GEOLOGY COURSE

Overview

The purpose of this study was to characterize the impact of inquiry-based learning (IBL) coupled with multiple representations in fostering student conceptual model development and content knowledge of authentic environmental issues that innately exhibited complex behavior. The research was conducted in nine introductory physical geology laboratory sections (n =144) at Texas A&M University. Participants were randomly placed into experimental and control groups where experimental groups were exposed to IBL and multiple representations including both web-based learning materials (e.g. technology-supported visualizations and analysis of multiple datasets) and physical models, whereas control groups were provided with the traditional "workbook style" laboratory assignments. Assessment of pre- and post-test student performance indicates that significant ($\rho < 0.05$) gains in achievement (e.g. content knowledge and conceptual model development) occurred primarily within the experimental group. Analysis of variance suggests that student conceptual model constructions were significantly different ($\rho < 0.01$) between test groups. These results indicate that the use of IBL and multiple representations had a positive impact on student learning about complex environmental issues. Difficulties in students' abilities to reason about

complex systems were influenced by imperfect conceptions, as made explicit in expressed conceptual models obtained from student products.

Introduction

A principle objective of environmental education is to achieve scientific literacy among students where they are prepared for future participation in society as informed citizens and are able to make educated decisions about a rapidly changing and complex world (American Association for the Advancement of Science [AAAS], 1993; Jimenez-Aleixandre and Periero-Munos, 2002; NRC, 2000). Miller (1993, 1998) defines scientific literacy as a multidimensional construct which includes understanding of basic scientific vocabulary, the process of the nature of scientific inquiry, and the impact of science and technology on society. However, most introductory science and geoscience courses are generally designed to cover facts, theories, and techniques that do not relate to other fields, ignore higher order thinking, and do not support accurate conceptual model development, therefore leading students to develop a naïve view that science is an unproblematic accumulation of facts that describe the world (Dori and Hersovitz, 1999; Mathewson, 1962; Sandoval and Reisner, 2003). These student views may ultimately lead to the development of inaccurate conceptual understandings of complex Earth and environmental systems, the nature of science, and affect the ability to make sound decisions and arguments about socioscientific issues (Bell and Lederman, 2003; Sadler et al., 2004; Zeidler et al., 2002).

System Complexity and Student Conceptual Models

Students' ability to understand complexity has become a leading research strand in recent studies (Ekborg, 2003; Forrester, 1994; Grotzer, 1993; Kuhn et al., 2000; Raia, 2005; Sell et al., 2006) because most natural systems exhibit characteristics that are complex. These include (i) interactions between system components, (ii) changes in system state over space and time, and (iii) unpredictable self-organization that leads to feedbacks producing the emergence of structure or patterns (Colucci-Gray, 2006; Herbert, 2006; Sell et al., 2006). Eutrophication, the process in which excess nutrients (nitrogen and phosphorous) stimulate the growth of phytoplankton and indirectly the bacteria thriving on the seafloor that feed upon the sinking phytoplankton and other sources of particulate organic material (POM; e.g. phytoplankton, detritus, fecal pellets, etc.) which ultimately leads to the on-set of bottom water hypoxic conditions in some estuarine regions, is a good example of a complex process that occurs in natural Earth systems. We choose to focus on the environmental topic of eutrophication in this research study because it is an important socioscientific issue and it consistently occurs in the Gulf of Mexico, which is located less than 100 miles from the university, causing it to be a relevant topic for many of our students.

Solving complex problems, such as eutrophication, requires the development of accurate conceptual models. The term *conceptual model* will be used in this research to describe students' expressed mental models and can be defined as an accurate, reasonable representation of natural phenomena and indicates a level of expertise (Greca and Moreira, 2000; Libarkin et al., 2003). Poor conceptual models of complex

environmental systems have led stakeholders to poor environmental decisions and risk assessments. Variations in stakeholder conceptual models of environmental systems have contributed to environmental conflict during ecosystem management (Hurley et al., 2003) and water resources management (Sneddon et al., 2003). People's conceptual models, when applied to risk perception, are also often ill-structured leading to incorrect perceptions of risk due to global warming (Kempton et al., 1991), radon (Bostrom et al., 1993), and electric fields (Morgan et al., 1990). Similarly, understanding and manipulating conceptual models of complex Earth systems can present significant learning difficulties for many students (Herbert, 2006; Sell et al., 2006) where common issues include limited conceptual understanding of associated knowledge domains and the use of naïve models to guide explanations (Coll and Treagust, 2003; Guisasola et al., 2004; Harrison and Treagust, 1998; Sanger and Greenbowe, 1997). However, conceptual change among students is supported when they are engaged in authentic practices with their conceptual models (Carey, 1985; She, 2004; Vosniadou and Brewer, 1987). These practices can include scientific inquiry and using multiple representations.

Supporting Student Conceptual Models through Inquiry and Multiple Representations

In this research, we have embedded IBL as an instructional methodology because it has been shown to increase students' depth of knowledge and understanding of the nature of science (Bransford et al., 1999; NRC, 1996) and ultimately may support the development of accurate and complex conceptual models among non-science undergraduate students. We use the acronym IBL to describe the inquiry-based learning

module that was implemented in the classroom where we have aligned our framework to the following description of inquiry. Inquiry requires the asking and refining questions, designing and conducting investigations, gathering and analyzing data, making interpretations and conclusions, and reporting findings; it promotes the development, transformation, and representation of ideas; and it emphasizes depth and not breadth (Krajcik et al., 2000). Central to inquiry is that it be in the context of authentic scientific investigations (AAAS, 1993; NRC, 1996, 2000) where it mirrors real scientific research, practices, and activities (Brown et al., 1989; Chinn and Malhotra, 2002; Dunbar, 2000; Latour and Woolgar, 1986). Authentic inquiry is important for learners because it enables them to act meaningfully and purposefully (Brown et al., 1989) and assists in the development of scientific knowledge (Edelson, 1997; Goldman et al., 1999), while promoting active-learning, the development of communication skills, and reflection/revision (Brown et al., 1989; Edelson, 1997; Hart and Nolan, 1999).

In conjunction with IBL, student manipulation of multiple representations can support the development of higher quality conceptual models. Multiple representations include symbols, objects, pictures, and mathematical equations. Specific examples appropriate to our study include physical models (e.g. Winogradsky columns) and information technology (IT)-based representations (e.g. GIS). Research results indicate that physical models can assist students in developing rich understandings of science (Penner et al., 1998). Learning technologies expand the range of questions that can be investigated in the classroom, the type of information that can be collected, the kinds of data representations that can be displayed, and the products that can be created (Krajcik

et al., 2000) by providing students the tools to communicate, contextualize, visualize, and manipulate (Barab and Luehmann, 2003; Edelson, 1997, 2001). Instructional sequences and learning environments that stress the use of multiple representations, the development of student conceptual models, and IBL can enhance students' understanding of the nature of science and the development of cognitive and metacognitive skills such as higher-order thinking, communication, knowledge transfer, and decision-making (Boulter and Gilbert, 2000; Buckley and Boulter, 2000; Herbert, 2006).

Research Objectives

This research emphasized instruction that utilized IBL, within an authentic science setting, and focused on the use of multiple representations when teaching and learning about complex environmental systems in order to support scientific literacy in terminal science courses. The aim of this work was to influence the development of students' conceptual models and knowledge through instruction that was interactive, IT-rich, and used lab-scale models to represent field-scale systems. The guiding hypothesize included that instruction which guides students' exploration of authentic scientific questions embedded in situated and meaningful contexts and allowed for student manipulation of multiple representations (IT and physical models) would enhance student conceptual model development and content knowledge of eutrophication, a complex environmental process.

Materials and Methods

Participants and Context

This study was conducted in an introductory geology course that included 144 undergraduate students from Texas A&M University. Membership of the class was predominantly non-science majors (81.0%; Fig. 5.1) with diverse course backgrounds/content knowledge (Table 5.1). Pre-assessment surveys revealed that only ~ 6% of the students were interested in a career in the geosciences and ~ 70% claimed they learned best by hands-on experience. Furthermore, surveys indicated misconceptions in the students' previous understanding of the nature of science (Table 5.2) and attitudes about scientific citizenship, where ~ 10% of the class felt they should not be held responsible as citizens to make any geoscience related societal decisions and they should just learn enough to pass the course.

The class was a terminal science course for most students enrolled where it served as one of the university's science core courses. It was originally designed based on traditional introductory workbook-style laboratories focusing on building skill-sets and didactic knowledge in learners, where students were encouraged to memorize information. Further, lecture and laboratory materials/topics often did not align well.

Table 5.1
Student course backgrounds

Science Course	**Number of Students (n=144)**
High School Science Only	49
Collegiate Science	103
Introductory Chemistry	30
Introductory Physics	18
Geography	18
Oceanography	16
Atmospheric Science	6
Introductory Biology	24
Other	17

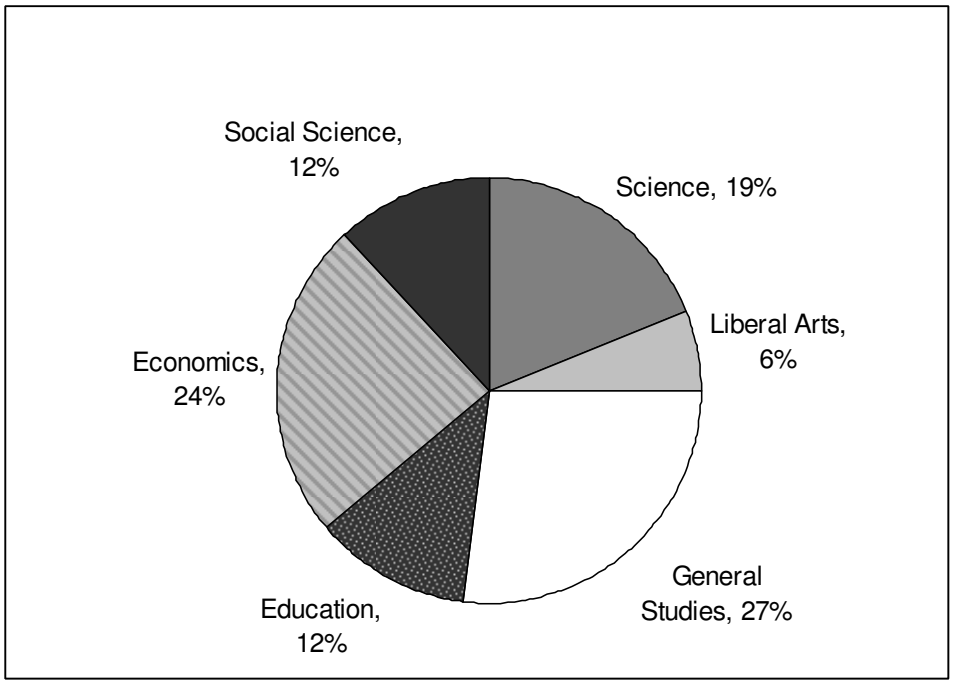


Fig. 5.1. The distribution of student majors from all nine laboratory sections.

Table 5.2
Student prior understanding of the nature of science according to initial survey (Asterisked statements are true)

Statement	Percent of Students that Agree (%)
A general and universal scientific method exists	53.0
Scientists employ an element of creativity to solve problems*	24.3
Evidence accumulated carefully will result in sure knowledge	6.6
Science and its methods can answer all questions	3.3
Scientific laws are absolute	7.2
Scientists do NOT adjust their results to fit their theory*	84.5

Instructional Sequence

There was a total of nine laboratory sections utilized in this research (n = 144), each having the same lecture session. Three laboratory sections were exposed to both the IT and the physical model, and will be referred to as *experimental* treatment groups. Six of the remaining laboratory sections were provided traditional workbook style assignments; three were taught by their regular TA, and will be referred to as *control – TA teaching*; and three were taught by the implementer, and will be referred to as *control – teaching*. All laboratory sections covered the same content domain of eutrophication where the instructional sequence included: (i) completing a pre-lab content knowledge test (ii) reading on-line materials and taking a quiz on the course web-page before class, (iii) constructing a pre-lab conceptual model expression, (iv) completing the lab

exercise, (v) submitting a post-lab report, (vi) constructing a post-lab conceptual model expression, and (vii) completing a post-lab content knowledge test.

The week-long, implemented, experimental laboratory module was designed to specifically facilitate student-guided inquiry as defined by Bonnstetter (1998), where it was geared around the NRC (1996) and Krajcik et al. (2000) definitions of inquiry (e.g. the diverse way scientists study the natural world and pose explanations based on evidence). In the IBL setting of the experimental laboratory module, students were encouraged to work in groups to complete assignments, to explore outside resources aside from their given text, and to use the scientific method during problem solving. The IT tools used by students included Excel©, QuickTime© videos, ESRI ArcView© GIS, and the World Wide Web. PowerPoint© lectures were used in association with laboratory exercise, as well as background readings and one-on-one small group help through Socratic inquiry methods. The physical model included a Winogradsky column, which is a clear tube of sediment that is allowed to go anaerobic through the utilization of O_2 during microbial respiration (Deacon, 2004). Since hypoxia is typically related to the process of eutrophication, the use of this model allowed students to directly observe mineral diagenesis in the sediments that usually occurs during hypoxia. Spatio-temporal visual changes were direct evidence of these processes, including the formation of Fe-Sulfide minerals such as framboidal pyrite or loss of Fe-Oxide minerals such as hematite due to both biogenic and non-biogenic reduction-oxidation reactions, or the development of visible colonies of variably colored microorganisms. Students choose among several variables, including addition of organic substrate (molasses or oil), aeration, nutrients, or

sediment type (marine or freshwater sediments) to evaluate the impact of these variables on microbial respiration over a three-week time span. Since time was limited, students observed QuickTime© movies of experienced learners (graduate students) building the columns. Students then made interpretations from digital photographs, taken by the experienced learners, of the columns. Once the introductory students made their hypothesis, observations, and conclusions, they could observe the experienced learners' scientific thought process during the same procedure as they explained their reasoning; the novice learners could then make modifications to their work. The physical model represented small-scale biogeochemical processes that occur in sediments of both estuarine and wetland environments and allowed for field-scale phenomena to be observed at the laboratory scale. The IT exercises incorporated the use of geographical information systems (GIS) and Excel© to study large-scale spatial contamination in two Texas estuaries through access to large data sets. In these modules, students chose from pre-constructed GIS maps and Excel© plots they deemed important to analyze according to their hypothesis about the system.

The control groups (both *teaching and non-teaching*) were provided traditional workbook-style laboratories where students were not provoked to participate in any activities atypical of their "normal" lab experience. The workbook exercises covered the topic of eutrophication where students were given the Mississippi River Bight case study to interpret. The specific activities included reading comprehension, short answer, graphing, and interpretation of 2-D graphics. A short power-point lecture was given at the beginning of the laboratory session, as was typical for the class.

Data Collection

The learning products that were developed from all laboratory treatment groups (control and experimental) included student background surveys, pre/post content knowledge tests, pre/post conceptual model drawings, and written reports. The product development allowed assessment of the impact of multiple representations and IBL on student conceptual model development and content knowledge. Examples of the pre/post content knowledge multiple choice exam questions are provided in Table 5.3 and examples of student post-conceptual model drawings are provided in Fig. 5.2.

Data Analysis

The quantitative data (e.g. pre/post tests and pre/post conceptual model drawings) were analyzed by SPSS 11.0 (the Statistical Package for the Social Sciences). A paired two-sample *t*-test for means was used to determine significance between students' performance on the pre/post-tests. Analysis of variance (ANOVA) was used to determine initial differences between test groups and to determine mean differences between groups in each of the rubric categories. The alpha levels used as criteria were selected *apriori* and were $\rho < 0.05$ for the *t*-test and $\rho < 0.01$ for the ANOVA.

Table 5.3

Example content knowledge pre/post multiple choice exam questions

Questions	Possible answers
What percent of the US population in 2010 will live within 50 miles of the coast?	A). 50% B). 25% C). 95% D). 80%
Eutrophication is most accurately described by the process of:	A). How algae grow in marine systems B). An enrichment of nutrients causing excess algal growth in marine systems C). Water color change D). Water salinity change
The manner in which humans choose to use the land (land-use) can lead to which of the following water quality issues:	A). Pesticide run-off B). Fertilizer run-off C). Drought D). A and B only
A non-point source pollutant is best described by:	A). A distinct source B). A source of multiple origins C). A source you can point to D). A source we do not care about
The common contaminants that cause eutrophication include:	A). Nitrate B). Carbonate C). Phosphate D). A and C only E). All of the above
Reduction describes the process in which:	A). An element losses electrons B). An element gains electrons C). An element losses protons D). An element gains protons
Microbes use which of the following to degrade organic matter:	A). O_2 B). SO_4^{2-} C). FeOOH D). NO_3^{2-} E). All of the above
What is the chemical formula for pyrite?	A). FeS B). FeS_2 C). $FeSO_4$ D). FeOOH
Organic matter is:	A). Composed of a carbon backbone B). Broken down by microbes and used as an energy source C). Needed by microbes to survive D). A and C only E). All of the above
If eutrophication in a coastal margin occurred, then the most likely affect on the microbial communities living in sediment would include:	A). More organic carbon would reach the seafloor (and the associated microbial communities) and the microbial metabolism would increase B). Less organic carbon would reach the seafloor (and the associated microbial communities) and the microbial metabolism would decrease C). More organic carbon would reach the seafloor (and the associated microbial communities) and the microbial metabolism would decrease D). All of the microbes would die from too much food availability

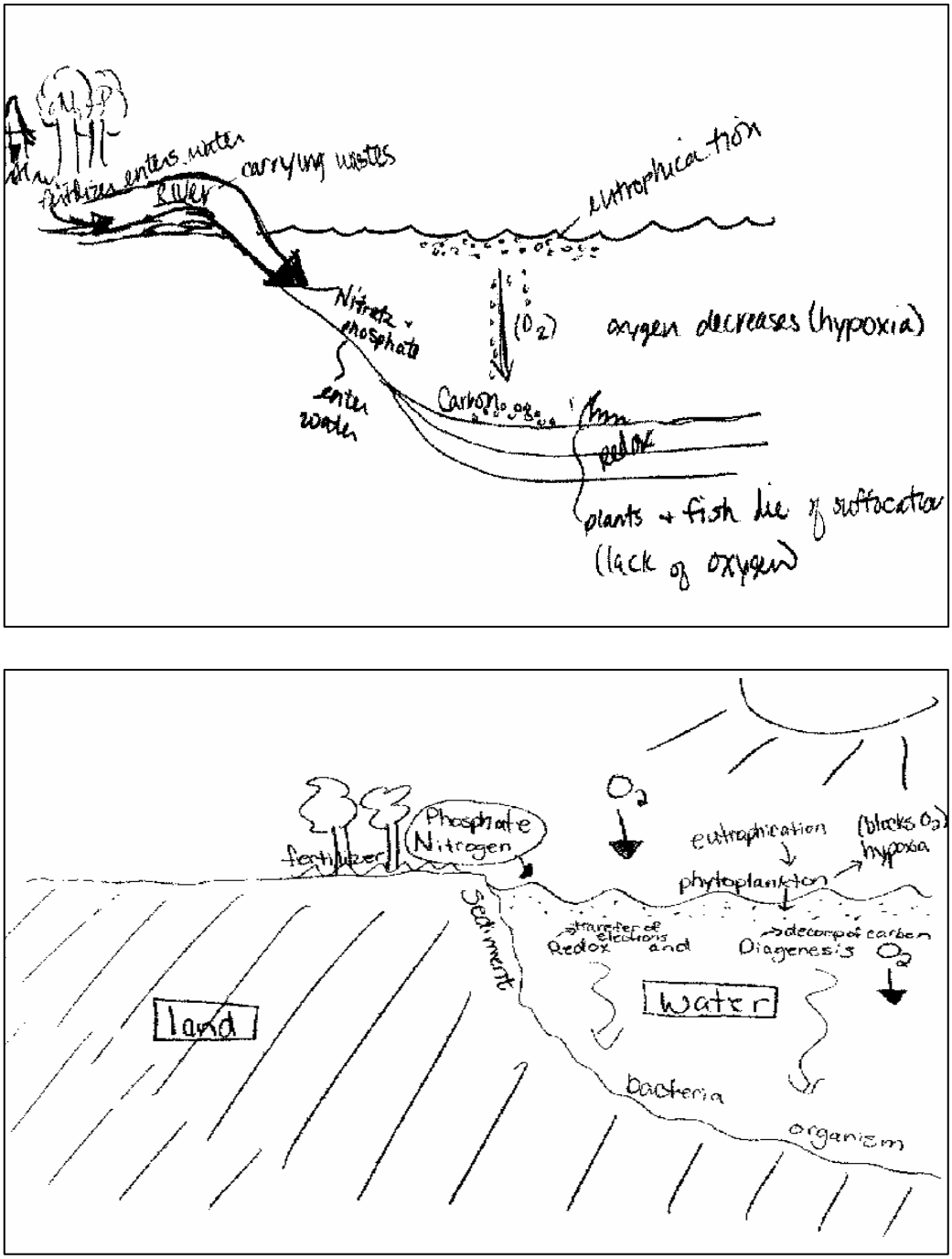


Fig. 5.2. Examples of student post-conceptual model drawings of eutrophication.

Instrumentation and Reliability

Development of two rubrics, one for the conceptual model expressions and one for the reports, enabled quantitative analysis of student's work through a pre-constructed set of standards and learning goals, where a point system was designed to assess student performance (Table 5.4). The rubrics acted as the instrument to assess learner knowledge in relation to the cognitive skills needed to perform the inquiry task. Our design of the assessment instruments was guided by the skills scientists utilize during authentic inquiry as summarized by Chinn and Malhotra (2002), where ten total categories of cognition were determined as criteria for the rubrics including: (i) content knowledge, (ii) construction of a hypothesis, (iii) experimental design, (iv) understanding of system behavior, (v) understanding of scale, (vi) ability to think critically, (vii) inclusion of scientific literature, (viii) ability to collect and report data, (iv) writing communication skills, and (x) ability to accurately represent/explain system complexity.

Reliability of the instrument was performed by two teams of graduate students serving as external evaluators. The evaluators were not involved in the implementation of the current research but were participants and graduate students in the Information Technology in Science Centre for Learning and Teaching at Texas A&M University. Three sample student products were randomly selected from a stratified report and conceptual model construction database where each evaluator graded all three. Reliability was assessed by calculating internal consistency values using the reliability coefficient (α) within the statistical package SPSS.

Table 5.4
Inter-rater reliability for each rubric category for both the report and conceptual model drawing rubrics (Asterisks represent below acceptable limits)

Rubric Category	**Acronym**	**Reliability (α) Reports**	**Reliability (α) Drawings**
Understanding of Scale	Scale	N/A	0.91
Understanding of System Processes	Systems	N/A	0.88
Accuracy	Accuracy	N/A	0.88
Content Knowledge	Content	0.25*	0.97
Critical Thinking	Think	0.73	0.78
Communication	Comm	0.67	N/A
Hypothesis	Hypoth	0.91	N/A
Experimental Design	Design	0.93	N/A
Inclusion of Data	Data	0.83	N/A
Scientific Literature	Sci. Lit.	0.74	N/A
	Average (α)	**0.84**	**0.88**

Table 5.5
Analysis of variance results for initial performance differences between treatment groups in both content knowledge and conceptual model expressions

	N	**Mean**	**Std. Dev.**	**Sig. ρ < 0.05**
Content Knowledge	9	43.65	3.88	0.755
Conceptual Model	9	2.71	1.17	0.135

Table 5.5 illustrates the resulting inter-rater reliability, where all but one rubric category gave acceptable reliability scores. When considering that in exploratory research a modest reliability of 0.50 to 0.60 is acceptable (Ravid, 1994), the final instruments showed good reliability in differentiating students' products ($\alpha = 0.84 – 0.88$).

Results

An ANOVA was conducted on the student populations to establish no initial differences ($\rho > 0.05$) between treatment groups (Table 5.5). Summative data satisfied all assumptions including homogeneity of variance ($\rho > 0.05$) and normal distributions ($\rho < 0.05$). The paired *t*-test determined differences between the means of the students' pre/post- content knowledge scores and conceptual model development for all treatment groups. Table 5.6 indicates that the only group that achieved significant ($\rho < 0.05$) differences for both of these scores was the experimental group (exposed to inquiry and multiple representations). The control-TA teaching treatment group indicated significance in only content knowledge and the control – teaching treatment group indicated no significant differences.

Table 5.6
Statistical results of student's pre-test and post-test for all treatment groups (Asterisks indicate statistical significance)

Group	**Pair (Pre-post)**	**Pre-test Mean (S.D.)**	**Post-test Mean (S.D.)**	**t-value**	**Significance ρ < 0.05**	**Effect Size**
Experimental	Conceptual model	37.77 (8.57)	50.00 (10.83)	5.36	0.033*	0.53
Experimental	Content knowledge	42.76 (1.65)	65.10 (7.78)	4.62	0.044*	0.89
Control – Teaching	Conceptual model	24.51 (13.65)	36.20 (15.27)	2.86	0.103	0.37
Control – Teaching	Content knowledge	42.99 (4.57)	60.60 (18.23)	1.94	0.192	0.55
Control – TA Teaching	Conceptual model	19.47 (4.72)	27.90 (20.83)	0.72	0.549	0.27
Control – TA Teaching	Content knowledge	45.15 (5.59)	78.15 (2.35)	16.39	0.004*	0.97

Table 5.7
Average rubric scores and analysis of variance results for the three treatment groups for the report and conceptual model rubric categories (Asterisks indicate statistical significance, R = reports, M = conceptual model drawings)

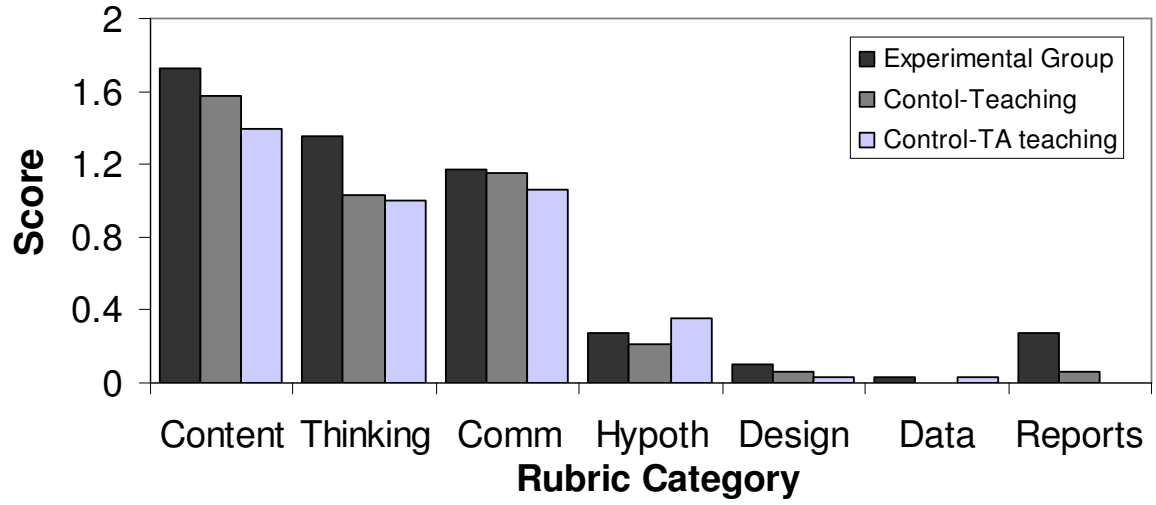
Report Rubric Categories							
Average Scores	Content^{R}	Think^{R}	Comm	Hypoth	Design	Data	Sci. Lit.
Experimental	1.73	1.35	1.17	0.27	0.10	0.03	0.27
Control Teaching	1.58	1.03	0.15	0.21	0.06	0.00	0.06
Control Non-Teaching	1.39	1.00	1.06	0.35	0.03	0.03	0.00
Sig. ρ < 0.05	*0.136*	*0.005**	*0.675*	*0.568*	*0.558*	*0.579*	*0.006**

Conceptual Model Rubric Categories					
Average Scores	Content^{M}	Accuracy	Think^{M}	Scale	Systems
Experimental	1.17	0.87	1.03	0.9	0.87
Control Teaching	1.00	0.67	1.03	0.73	0.51
Control Non-Teaching	0.77	0.48	0.65	0.58	0.29
Sig. ρ < 0.05	*0.054*	*0.930*	*0.055*	*0.247*	*0.003**

Moreover, the effect size results for the experimental group indicated medium to high effects of the inquiry activity where the average score in the post-test for the conceptual model drawing was 0.53 standard deviations greater than the average score on the pre-test (effect size = 0.53) and post-test results for the content knowledge test was 0.89 standard deviations greater than the average score on the pre-test (effect size = 0.89). The control treatment groups also showed pre-post gains in both the conceptual model drawing and content knowledge test, however, most of the gains were low to medium (effect size = 0.23 to 0.55), with the exception of the control-TA teaching group which had high gains in the pre-post content knowledge test (effect size = 0.97). Student performance on written reports and post-conceptual model constructions were then examined and results indicated that experimental groups preformed better than control groups in five of the seven written report rubric categories and four of five conceptual model rubric categories (Fig. 5.3). The non-parametric (Krustal – Wallis) ANOVA was utilized because individual rubric categories within treatments were not normally distributed. Table 5.7 shows that rubric categories with significant ($\rho < 0.01$) differences between groups included critical thinking, inclusion of scientific literature, and understanding of system processes.

Several imperfect conceptions were revealed during student conceptual model development, including application of factual knowledge, issues of scale, understanding of system processes, and reasoning.

(A)



(B)

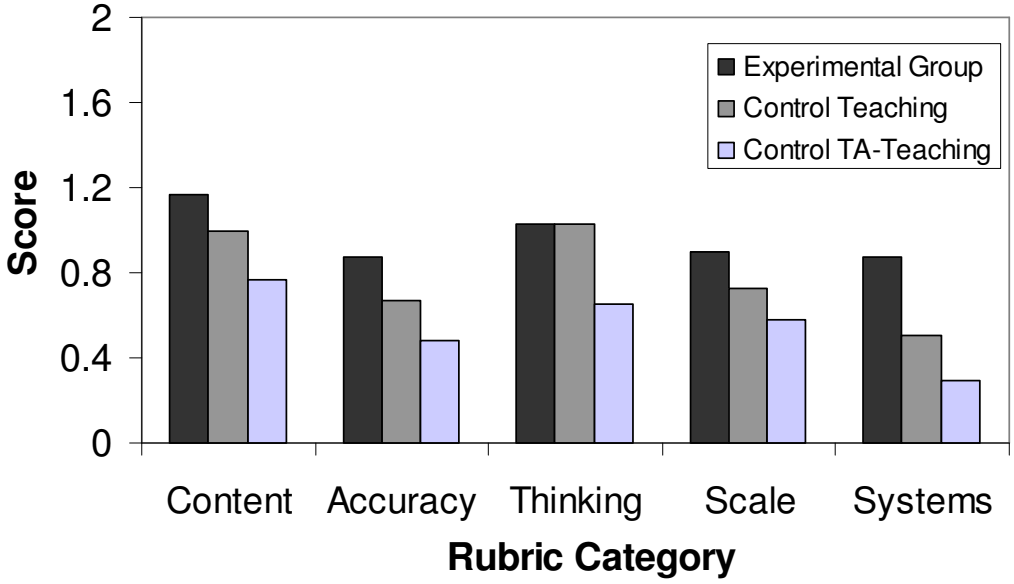


Fig. 5.3. Treatment groups written report performance (A) and post-conceptual model drawing performance (B) as according to rubric categories.

Table 5.8
Examples of student imperfect conceptions revealed during the conceptual model development

Student	Response	Area of limitation
A	"The **phytoplankton** may then **die** because of this **lack of oxygen**…"	Content knowledge
B	"For example, more **phytoplankton** means **less oxygen** and **more carbon dioxide**".	Content knowledge
C	"After the organic carbons degrade redox occurs… ".	System understanding
D	"If there is no oxygen in the water, any biological life that can not use the **redox ladder** dies such as **fish and crabs** ".	Understanding of scale and content knowledge
E	"…the (redox) ladder shows different reactions with the electrons and converts **aerobic water** to **anaerobic water**".	Content knowledge
F	"I think that the **phytoplankton** will eventually **eat away at the banks** causing them to erode".	Reasoning and understanding of scale

Examples of typical imperfect conceptions in this work included student response scenarios as found in Table 5.8. Students A, B, and E responses were imperfect conceptions which were lacking appropriate use of the content material and associated terminology. Student A was incorrect because "phytoplankton" do not utilize "oxygen" therefore they can not die from a lack of it. Student B was incorrect because "phytoplankton" uptake carbon dioxide and not oxygen. Student E was incorrect because "aerobic" and "anaerobic" are descriptions of processes and not "water". Student C's response was an imperfect conception that indicated a misunderstanding about system processes because the degradation of "organic carbon" and "redox chemistry" describe metabolic processes that proceed in concert and not in a linear sequence. Student D's response inappropriately used the "redox ladder" in relation to

larger biological life, where misunderstanding of scale was evident (the redox ladder is a term describing the microbial degradation of organic carbon though a series of energetic processes). Finally, Student F did not reason appropriately about scales when he/she stated that the "phytoplankton" will "erode the banks" by eating it. The above imperfect conceptions indicate that students need to be challenged to think critically and synthesis scientific information in order to have a strong understanding of the nature of science and to accurately explain complex Earth and environmental systems. Even though such imperfect conceptions may be frustrating for the instructor, it should be noted that without allowing student to express their conceptual models, these imperfect conceptions may never come to the attention of the instructor or the student and therefore can not be revised.

Discussion and Conclusions

Change in introductory geology courses is needed in order to support learners' understanding of scientific inquiry and to ease their learning difficulties about complex earth systems. Calls for such reform have focused on five general areas including content and curriculum; pedagogy and assessment; problem solving and critical analysis; scientific literacy; and computer – aided instruction (Ireton et al., 1997; Resnick, 1987; Stout et al., 1994; The Boyer Commission, 1998). Following these reforms are particularly important for introductory courses, which are typically terminal science courses for many undergraduates and therefore the best chance to increase scientific literacy of college students (Miller, 1998; Stout et al., 1994). Finding ways to enhance

terminal science courses is a primary objective of this work, in order to support collegiate geoscience education reform. We have included the environmental issue of eutrophication as the educational context because it may support students' intrinsic motivation to learn, as these issues are challenging and are considered to be socioscientific (Bransford et al., 1999). We have aspired to produce an instructional framework relating to environmental issues that encompasses IBL and multiple representations. Our hypothesis which stated that exposing students to this instructional context enhances student's conceptual model development and content knowledge of complex Earth systems has been accepted though data collected in this research. We have found that introductory geology students in experimental groups (those exposed to inquiry and multiple representations) significantly ($\rho < 0.05$) improved in their content knowledge and conceptual model development of eutrophication. The data has also shown that significant differences ($\rho < 0.01$) in rubric category performance between experimental and control (those exposed to traditional workbook style labs) groups are evidence for the experimental group's ability to think more critically, refer to the scientific literature more frequently, and to understand system behaviors better than control groups.

Moreover, student imperfect conceptions (Sell et al., 2006) were revealed from their conceptual model development. As Cheng et al. (2005) notes, a teacher gains insight to the students understanding of new material taught by identifying student misconceptions where the teacher will be able to adjust the teaching and supply more useful materials when needed. Hills (1989) suggests that misunderstandings of major

environmental issues arise from the general lack of knowledge or in-depth exposure to such issues. Student imperfect conceptions, revealed in the current research, illustrated that sufficient exposure was essential to forming logical scientific arguments in research reports, suggesting that core knowledge and adequate time for reflection and revision needed to be provided in order for students to proceed with a complex thinking process (Goldman et al., 1999; Jonassen, 1999). Therefore, future modifications to this implementation would include longer exposure to eutrophication and coastal margin processes where more than one laboratory session would be donated to student learning about this complex Earth system. This result has significant implications in the commonly followed instructional practices of collegiate introductory geology courses, where courses frequently do not spend more than one week on any given topic. We believe that had we been able to overcome existing departmental course scheduling limitations, our results would have been even more significant. Therefore, in order to meet calls for undergraduate education reform, we argue that introductory courses should provide adequate opportunities for students to explore relevant socioscientific issues where instruction using inquiry and multiple representations (coupling IT and physical models) are utilized in order to support student development of accurate conceptual models of complex Earth and environmental systems.

CHAPTER VI

SUMMARY AND CONCLUSIONS

Perspectives on Achieving Synergy

This research model has incorporated both geoscience education research and biogeochemical research. As a result, synergy has been achieved between my teaching and research efforts by integrating the two. I have been able to bring my own scientific research of complex Earth systems to the classroom though the use of IT and physical models, hence providing my students an opportunity to experience authentic scientific research. In the classroom, I have been able to design, implement, and evaluate education research studies and ultimately disseminate them to the geoscience community which has added to my scholarship and productivity. My teaching experience has also informed my own pedagogy. For example, I have become acutely aware of my own "expert-blind spots" and have made efforts to fill in those gaps in order to successfully communicate with all students regardless of their prior knowledge or experience. It is my hope that this research model illustrates the value of creating leadership in science that embraces educating and communicating effectively with the public where the importance of scholarship in both the scientific and education enterprises is appreciated.

Research Summary

Volatile Organic Compound Research

This research was the first to measure the production of VOC metabolites in soil microcosm studies, while simultaneously measuring CO_2 emissions and microbial community shifts via FAME and CSUP methods during perturbations of environmental factors and during temporal and spatial dynamics in microcosm studies. It has shown that soil VOC metabolites are valid proxies for measuring shifts in microbial community composition by utilizing a plethora of multivariate statistical procedures. Specifically, factor and cluster analysis showed that the VOC method indicated shifts in microbial community composition similarly to the traditional FAME and Biolog methods. Statistically significant ($\rho < 0.05$) differences in VOCs over time and space and GIS analysis indicated that VOC production was dependent on landscape position of soils at the LANWR in southern Texas and the associated microbial communities and soil characteristics. Data collected from this research also illustrated that VOC production increased over time much like CO_2 emissions. In a multitude of ways, this work evidenced that the VOC method is advantageous over the traditionally utilized CO_2 method to monitor temporal ecosystem dynamics because it is an information rich indicator that not only records the soil microbial metabolism but also the associated community composition shifts and utilized carbon sources. Overall, this research has laid the foundation for future work utilizing VOCs as spatial and temporal indicators of microbial community composition.

Geoscience Education Research

This research has illustrated how scientists and undergraduate students reason about complex Earth and environmental issues. It has shown that simulated research activities (e.g. laboratory methods and/or physical models) provide an opportunity for students to participate in authentic inquiry activities. Furthermore, this work has provided example inquiry activities and research results that validate the use of inquiry and multiple representations in upper and lower division undergraduate geology classrooms. The results of this research have shown that expressed conceptual models are significant ($\rho < 0.05$) predictors of inquiry performance of high prior knowledge students. Through comparing experimental and control groups this work has also evidenced that the use of these pedagogical strategies provides significant ($\rho < 0.05$) learning gains in students exposed to the intervention, specifically in regard to the development of critical thinking skills, the use of scientific literature and references, and the understanding of system behavior. Moreover, students in experimental groups had significant ($\rho < 0.05$) pre-post gains in both their conceptual model development and content knowledge. Overall, this work has provided data to support the future use of pedagogical strategies that incorporate authentic inquiry and multiple representations in the undergraduate classroom. Limitations of the designed study and recommendations to increase the effectiveness of the classroom modules have been recommended including the implementation of collaborative student teams with distributed expertise, the extension of laboratory sessions to more than one week intervals per Earth science topic, and the use of the inquiry cycle as outlined by Etheredge and Rudnisky (2003).

Future Study Recommendations

Volatile Organic Compound Research

During this research biogenic VOCs from soil have been shown as a possible significant source of atmospheric carbon. However, further investigation is required to quantify the contribution of VOCs from soils to the atmosphere in various soil types over spatial landscapes. More research is also needed to determine the large-scale spatial variations in VOC production during seasonal change and the abiotic controls on their release to the atmosphere. In order to facilitate the above research plans we propose using *in-situ* techniques that would enable the direct collection of soil VOCs while simultaneously measuring the full suite of abiotic and biotic characteristics of the soil environment. Additionally, we recommend that future field and microcosm experiments be conducted in order to track the different types of VOCs that are emitted under anoxic and oxic conditions. This experiment would require the tracking of oxygen using a redox probe or oxygen sensor. We also suggest that the use of stable carbon isotope and polymerase chain reaction (PCR) techniques be applied to direct culture and soil microcosm studies in order to detail the carbon degradation pathways and subsequent production of VOCs by specific soil microorganisms.

Geoscience Education Research

This geoscience education work can easily extend to the K-12 setting where the inquiry modules designed in this research may be modified to fit the state and national

learning standards in science across these grade levels. Future aspirations include the design of on-line inquiry activities that would provide teachers the appropriate teaching materials and allow students to utilize information technology in their classroom within an authentic inquiry setting. Additionally, the design of field activities for K-12 students that would provide students the opportunity to participate in authentic research and collect field data for on-going scientific research is a future goal and a way to further integrate science and education research. Furthermore, this work likely extends beyond the classroom where some of our understanding of how students learn about complex environmental systems in formal educational settings can be applied to develop an understanding of stakeholder and citizen learning of the same systems under more informal conditions, with an objective of improving environmental management and decision processes. Citizens and stakeholders must understand how scientists (experts) think in order to make informed decisions. To facilitate this knowledge transfer, governmental agencies (e.g. EPA) have made considerable efforts to involve citizens and stakeholders in policy reform and management decisions (Allen et al., 2001; McDaniels and Gregory, 2004; NRC, 1999; Renn, 1999). This work can assist in the understanding of common misconceptions concerning environmental issues and aid in the development of educational materials that support informal learning about complex Earth systems.

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