

Electronic Thesis and Dissertation Repository

---

8-13-2012 12:00 AM

## Impacts of agricultural disturbance on communities of selected soil fungi (Agaricomycetes)

Jessie R. Wong  
*The University of Western Ontario*

Supervisor  
Dr. R. G. Thorn  
*The University of Western Ontario*

Graduate Program in Biology  
A thesis submitted in partial fulfillment of the requirements for the degree in Master of Science  
© Jessie R. Wong 2012

Follow this and additional works at: <https://ir.lib.uwo.ca/etd>



Part of the [Agriculture Commons](#)

---

### Recommended Citation

Wong, Jessie R., "Impacts of agricultural disturbance on communities of selected soil fungi (Agaricomycetes)" (2012). *Electronic Thesis and Dissertation Repository*. 708.  
<https://ir.lib.uwo.ca/etd/708>

This Dissertation/Thesis is brought to you for free and open access by Scholarship@Western. It has been accepted for inclusion in Electronic Thesis and Dissertation Repository by an authorized administrator of Scholarship@Western. For more information, please contact [wlsadmin@uwo.ca](mailto:wlsadmin@uwo.ca).

IMPACTS OF AGRICULTURAL DISTURBANCE ON  
COMMUNITIES OF SELECTED SOIL FUNGI (AGARICOMYCETES)

Spine title: Agricultural Disturbance on Communities of Agaricomycetes

(Thesis format: Monograph)

By

Jessie R. Wong

Graduate Program in Biology  
and Environment and Sustainability

A thesis submitted in partial fulfillment  
of the requirements for the degree of  
Master of Science

The School of Graduate and Postdoctoral Studies  
The University of Western Ontario  
London, Ontario, Canada

© Jessie R. Wong 2012

THE UNIVERSITY OF WESTERN ONTARIO  
The School of Graduate and Postdoctoral Studies

**CERTIFICATE OF EXAMINATION**

Supervisor

Examiners

\_\_\_\_\_  
Dr. R. G. Thorn

\_\_\_\_\_  
Dr. Hugh Henry

Supervisory Committee

\_\_\_\_\_  
Dr. Hugh Henry

\_\_\_\_\_  
Dr. Marc-André Lachance

\_\_\_\_\_  
Dr. Sheila Macfie

\_\_\_\_\_  
Dr. Richard Gardiner

The thesis by

**Jessie Rachel Wong**

entitled:

**Impacts of agricultural disturbance on  
communities of selected soil fungi (Agaricomycetes)**

is accepted in partial fulfillment of the  
requirements for the degree of  
Master of Science

Date: \_\_\_\_\_

\_\_\_\_\_  
Chair of the Thesis Examination Board

## **Abstract and Keywords**

The objective of this study was to use phylogeny-based and community-based analyses to compare the community composition of Agaricomycetes among four different agricultural treatments at the Kellogg Biological Station Long Term Ecological Research (KBS LTER) site. A phylogenetic tree that included 591 ribosomal DNA sequences previously obtained from KBS LTER documented the composition of Agaricomycete communities in each treatment. Sequences from KBS LTER were placed into 472 OTUs (putatively species-level operational taxonomic units defined by 99% or greater sequence similarity) and these were dominated by the Agaricales (with 330 OTUs), Cantharellales (39 OTUs), Hymenochaetales (29 OTUs), and Polyporales (23 OTUs). Multivariate statistical analyses incorporating phylogenetic information showed never tilled successional grasslands to be the most phylogenetically distinct treatment. The trend that phylotype and clade diversity decreased with increasing disturbance by tillage was consistent with results from previous individual studies emphasizing the importance of protecting remnant untilled grassland habitats.

### **Keywords**

Agaricomycetes, agriculture, KBS, multivariate statistics, phylogenetics, soil, sustainability, tillage, UniFrac

## **Dedication**

I dedicate this work to my supervisor, mentor and friend, Dr. Greg Thorn, without whom I would have never been able to complete my MSc. degree in a timely fashion and in such a nurturing academic environment.

## **Acknowledgements**

I thank and express my gratitude to my supervisor, Dr. Greg Thorn. His support, guidance, patience, and advice have been invaluable to my project and MSc. experience. Dr. Thorn always encouraged me and made my graduate experience enjoyable and fulfilling. I am grateful for all of the lessons he has taught me and for the positive influence he has had on my life.

My advisory committee members, past and present, Dr. Sheila Macfie, Dr. Hugh Henry, and Dr. Mark Bernards have provided me with insights and suggestions that have helped facilitate my project and strengthen my thesis. Each member has taken the time to help me through the inevitable road blocks that come up whilst conducting scientific research. For that I thank them. Furthermore, to all the professors that taught my graduate courses, I am grateful for learning from such a distinguished suite of academics.

I would like to express my thanks to previous Thorn lab students, Michael Lynch and Barbara Bahnmann, for providing me with the basis and data on which my MSc. was conducted.

I would like to recognize Jennifer McDonald, currently from the Thorn lab, and Ani Hoque, previously from the Thorn lab, for always being available to answer my questions; I would also like to express thanks to my good friends, Nikita Eskin and Vanessa Yoon, who both helped me with my initial research. I want to acknowledge all volunteers, visiting students, and work-study students of the Thorn lab for the laughs and conversations that made coming into work fun.

I would like to express my sincerest thanks to Jerad Furze, whose love and support has lighted the pathway of my MSc. journey. But most especially, I would like to thank my mother and father, Teresa and Albert Wong, and my friends for their support, love and constant encouragement through this process.

## Table of Contents

<b>Certificate of Examination</b> .....	ii
<b>Abstract and Keywords</b> .....	iii
<b>Dedication</b> .....	iv
<b>Acknowledgements</b> .....	v
<b>List of Tables</b> .....	x
<b>List of Figures</b> .....	xi
<b>List of Appendices</b> .....	xii
<b>List of Abbreviations</b> .....	xiii
<b>CHAPTER 1: INTRODUCTION</b> .....	1
1.1 Soil-its role in terrestrial ecosystems.....	2
1.2 Soil organisms.....	3
1.3 Agaricomycetes.....	5
1.4 Mechanisms by which agricultural practices affect fungal community structure.....	8
1.5 Sustainable agriculture and its link to Agaricomycetes.....	11
1.6 Phylogenetics and multivariate statistics.....	14
1.7 Thesis rationale and objectives.....	15
<b>CHAPTER 2: MATERIALS AND METHODS</b> .....	18
2.1 Study site.....	18
2.1.1 Soil.....	18
2.1.2 Agricultural treatments.....	19
2.1.3 Plant communities.....	19
2.2. Soil sample collection, washing, and isolation of fungal cultures or DNA...20	



2.2.1 PCR amplification and TA cloning.....	21
2.3 Phylogenetic analysis.....	22
2.4 Statistical methods and multivariate analysis.....	24
<b>CHAPTER 3: RESULTS.....</b>	<b>28</b>
3.1 Phylogenetic tree.....	28
3.2 UniFrac significance and P test.....	28
3.3 Jackknife environment cluster.....	59
3.4 Principal coordinates analysis (PCoA).....	61
3.5 Taxonomic representation in treatments studied.....	64
<b>CHAPTER 4: DISCUSSION.....</b>	<b>67</b>
4.1 Broad phylogenetic placement.....	67
4.2. Tulasnellales.....	67
4.3 Cantharellales.....	68
4.4 Polyporales.....	69
4.5 Agaricales.....	69
4.5.1 Clavarioid.....	70
4.5.2 Stephanosporaceae.....	70
4.5.3 Agaricoid clade.....	71
4.5.4 Tricholomoid clade .....	71
4.5.5 <i>Schizophyllum-Lachnella</i> clade.....	72
4.5.6 Marasmioid clade.....	72
4.5.7 Pluteoid clade.....	73
4.5.8 Hygrophoroid clade.....	73

4.5.9 Pterulaceae clade.....	74
4.6 Thelephorales.....	74
4.7 Hymenochaetales.....	75
4.8 Corticiales.....	76
4.9 Geastrales.....	76
4.10 Gomphoid/Phalloid.....	77
4.11 Sebacinales.....	77
4.12 Russulales.....	78
4.13 Auriculariales.....	79
4.14 Trechisporales.....	79
4.15 Non-represented clades.....	80
4.16 Representation in CT plots.....	81
4.17 Representation in NT plots.....	82
4.18 Representation in HTS plots.....	83
4.19 Representation in NTS plots.....	84
4.20 Conservation of fungi through sustainable practices.....	85
<b>CHAPTER 5: CONCLUSION.....</b>	<b>87</b>
5.1 Direction of future research.....	87
<b>References.....</b>	<b>90</b>
<b>Appendix 1.....</b>	<b>105</b>
<b>Appendix 2.....</b>	<b>106</b>
<b>Curriculum Vitae.....</b>	<b>112</b>

## List of Tables

Table 2.1.	Cover crop planted on conventional till (CT) and no till (NT) plots during sampling years.....	20
Table 3.1.	Environmental distance P-Values for each environment tested individually.....	58
Table 3.2.	Major clade distribution in CT, NT, HTS, NTS treatments at KBS LTER.....	65

## List of Figures

Figure 3.1.	Neighbour-joining tree of reference sequences and sequences recovered from soils in KBS LTER sites.....	30
Figure 3.2.	Jackknife environment cluster for all environments.....	60
Figure 3.3.	Principal coordinates analysis of KBS LTER sites based on UniFrac values from KBS and reference sequences.....	64

## **List of Appendices**

Appendix 1. Main Cropping System Experiment (MCSE) at Kellogg Biological Station Long Term Ecological Research (KBS LTER) site.....	105
Appendix 2. GenBank accession numbers for reference sequences used in phylogenetic analysis.....	106

### **List of Abbreviations**

BLAST	Basic Local Alignment Search Tool
CT	Conventional Till
DNA	Deoxyribonucleic acid
ECM	Ectomycorrhizal
GenBank	Nucleotide database of the National Center for Biotechnology Information
HTS	Historically Tilled Successional
ITS	Internal Transcribed Spacer
KBS	Kellogg Biological Station
LTER	Long Term Ecological Research
LSU	Large Subunit
MCSE	Main Cropping System Experiment
MEGA	Molecular Evolutionary Genetics Analysis
MUSCLE	Multiple Sequence Comparison by Log-Expectation
NGS	Next Generation Sequencing
NJ	Neighbour-joining
NT	No Till
NTS	Never Tilled Successional
OTU	Operational Taxonomic Unit
P-test	Parsimony test (in UniFrac)
PAUP	Phylogenetic Analysis Using Parsimony
PCoA	Principal Coordinates Analysis

PCR	Polymerase Chain Reaction
P1	Principal component 1
P2	Principal component 2
P3	Principal component 3
RFLP	Restriction Fragment Length Polymorphism
RNA	Ribonucleic Acid
rRNA	Ribosomal Ribonucleic Acid
SINA	SILVA INcremental Aligner
UniFrac	Unique Fraction

## CHAPTER 1: INTRODUCTION

*“The role of the infinitely small in nature is infinitely great.”  
- Louis Pasteur*

Ecosystems are complex and are made up of a number of interacting biotic and abiotic components; an ecosystem is the entire biological community, from viruses to vertebrates, and the community's non-living (abiotic) environment. Each component in an ecosystem is not separate from the others but they are intertwined. In order to maintain ecosystem health, there must be an understanding of how components function individually and more importantly, how they interact with one another. Each component has an influence on the ecosystem. However, the ways in which it affects the ecosystem are intricate, as different ecosystems are made up of different sets of components acting at various degrees and multiple spatial and temporal scales. The definition of ecosystem health remains complex and we must take into account our limited knowledge and ability to provide definitive sets of measures and criteria on which to assess ecosystem health (Schaeffer et al. 1988). So, although there are many definitions of ecosystem health, a contextual and more appropriate definition, for the purpose of this study, describes ecosystem health as how stable and sustainable an ecosystem is, with regards to its ability to maintain organization and autonomy over time and be resilient to stress (Costanza 1992). More generally this means how well an ecosystem is able to respond to disturbance and how quickly it can recover from such disturbance.



Ecosystem health requires an evaluation of the components that dictate a healthy ecosystem. Terrestrial abiotic components are water, the atmosphere, sunlight, and soil. Terrestrial biotic components include plants, animals, and microorganisms and each component is sensitive to environmental and anthropogenic changes.

### **1.1 Soil – its role in terrestrial ecosystems**

The solid matter of soil consists of mineral components derived from parent rock material and chemically complex organic components derived from living organisms (Carlile et al. 2001). A healthy soil also includes water, air, and living soil organisms (Doran et al. 1994). Soil provides an ecosystem with essential functions such as physical stability and support of aboveground biomass, filtering and buffering, water relations, and provides a habitat in which nutrient cycling may take place. Soil is of paramount importance as it supports our biosphere by supporting the production of food and fiber, and maintenance of overall ecosystem quality (Doran and Parkin 1994, Karlen et al. 1997, Doran and Zeiss 2000). It is important to consider the quality of a soil as an indicator of soil health, and its relationship to and as a basis for primary productivity. “Soil health is defined as capacity of soil to function as a vital living system, within natural or managed ecosystem boundaries, to sustain plant and animal productivity, maintain or enhance water and air quality, and promote plant and animal health” (Doran and Zeiss 2000). The health of the soil largely dictates the health, resiliency, and biodiversity of a terrestrial ecosystem. Soil quality is not only important to maintain aboveground primary productivity but also overall environmental quality, as primary producers support higher trophic levels. Soil quality is controlled by chemical, physical,

and biological components. For the purpose of this study, only the physical and biological components will be considered (Kennedy and Papendick 1995, Doran 2002).

## **1.2 Soil organisms**

The most influential soil organisms include bacteria, nematodes, and fungi. Their roles in soil structure and function have been correlated to beneficial parameters that define soil quality, including water storage, soil humus formation, decomposition and nutrient cycling, detoxification of toxicants, suppression of noxious and pathogenic organisms, and maintenance of ecosystem resiliency and soil tilth, which is defined as soil that has the proper structure and sufficient amounts of nutrients (Lynch and Bragg 1985, Tisdall 1991, Doran and Zeiss 2000, Kibblewhite et al. 2008). Soil structure is controlled in part by soil organisms, including mycorrhizal fungi whose hyphae physically entangle soil and also bind fine mineral and organic particles into aggregates with exudates such as glomalin (Miller and Jastrow 1990, Rillig 2004). Soil organisms, as determinants of soil health and ecosystem functioning, have been considered more seriously in recent years as the quality of our agricultural soils has been severely degraded through unsustainable agricultural practices such as chemical inputs in the form of pesticides, herbicides and fertilizers, as well as mechanical processes that have contributed to the loss of soil organic matter. Mechanized processes that turn or dig up soil, such as ploughing and tillage, can also lead to loss of top soil through erosion (Oldeman 1994, Kibblewhite et al. 2008). Soil organisms can be sensitive to variations in land management, are well correlated to beneficial soil functions, and can be useful for elucidating ecosystem processes (Doran and Zeiss 2000). However, soil organisms can be

difficult to study; many are difficult to observe, isolate, and identify and it is challenging or impractical to study their activities under realistic conditions (Fenchel 1992, Wardle and Giller 1996, Brussaard et al. 1997, Freckman et al. 1997). The opaque, chemically complex, and highly heterogeneous nature of soil creates difficulty when studying soil organisms (Parkin 1993, Ranjard et al. 2003). For this reason, they are often not included in ecological surveys, nor are they included as indicators of soil health. Traditionally, soil organisms have been studied at the process level, in terms of soil respiration rate and enzyme activities or very gross community measures such as total numbers of organisms (Parkinson and Coleman 1991). Detailed responses at the community level have not been studied as thoroughly (Kennedy and Smith 1995). However, the numbers of studies on soil organisms' responses, at the community level, have increased through the years (Wardle et al. 2004). Process level measurements are critical in our understanding of how soil organisms respond to environmental variations, but they may be too insensitive to detect changes on a finer scale due to the complexity of relationships within a community (Kennedy and Smith 1995). Process level measurements further lack information that could provide explanations for the patterns or changes observed.

A comprehensive understanding of soil health and ecosystem health necessitates an understanding of soil organisms and especially their responses to land management practices, as their presence has large implications for the quality and quantity of agricultural production. The composition, abundance, and activity levels of soil organisms have been shown to be markedly different in agricultural systems when compared to the natural ecosystems from which they were derived (Lavelle et al. 1994, Matson et al. 1997). In order to accurately assess land management practices and their

resulting effects on soil organisms, a variety of soil organisms must be sampled, if possible, and observed. These include soil bacteria, nematodes, and fungi, as these organisms function in nutrient cycling, decomposition, soil structure maintenance, and contribute to soil organic matter (Kibblewhite et al. 2008).

### **1.3 Agaricomycetes**

Agaricomycetes are a class of fungi in the phylum Basidiomycota; approximately 21,000 of 30,000 accepted species of Basidiomycota are Agaricomycetes, and many of these live in soil (Hibbett et al. 2007; Kirk et al. 2008). The Agaricomycetes is a diverse class in both nutritional mode and fruiting body form (fruiting body being the macroscopic spore-bearing structure of a fungus) (Carlile et al. 2001). Among the Agaricomycetes are species that are saprotrophs (organisms that feed by decomposing organic matter) and form mutualist symbioses with the roots of various plants [ectomycorrhizae (ECM)]. Filamentous fungi, including ECM start with the basic unit of a hypha (plural hyphae), which usually consists of a chain of elongated cells, to build a highly branched three-dimensional network called a mycelium (Bartnicki-Garcia et al. 1969, Gooday 1971, Steele and Trinci 1975). Some Agaricomycetes are important pathogens of timber, vegetable crops, and even humans (Hibbett 2006). Many species of Agaricomycetes act as primary decomposers of wood and other plant litter (Hibbett 2006, Lynch and Thorn 2006). Primary decomposers are characterized as organisms that possess the enzymes needed to degrade complex polymers, including lignin and cellulose, found in plant litter (Blanchette 1991).

Lignin degradation is the rate-limiting step in nutrient turnover in soils (Ohkuma et al. 2001). Lignin is an amorphous, heterogeneous, and highly refractive polymer of various proportions of ester-linked aromatic alcohols (*p*-coumaryl, sinapyl, and coniferyl) that is present in all plant tissues to some degree, but more abundantly in woody plant tissues (Hatakka 1994). Lignin solidifies plant cell walls, provides strength and rigidity, and protects wood from microbial attack (Hatakka 1994). It is estimated that lignin is the second most abundant aromatic compound on Earth, second to cellulose (Ohkuma et al. 2001). Cellulose is an unbranched homopolysaccharide of  $\beta(1\rightarrow4)$  linked D-glucose that is fibrous and water-insoluble (O'Sullivan 1997). It is found in the cell walls of plants, in roots, stems, and leaves (O'Sullivan 1997). A large proportion of Agaricomycetes are the primary agents of lignocellulose degradation because these organisms possess the necessary enzymes, such as laccase, lignin peroxidases, and manganese peroxidases, needed to degrade lignin (Hatakka 1994).

There are two major modes of wood decay performed by Agaricomycetes. Brown-rot occurs when the lignin component of woody materials is not appreciably degraded and only cellulose and hemicelluloses are degraded (Morgenstern et al. 2008). In white rot, the lignin component is efficiently degraded either simultaneously or in advance of the degradation of other woody components (Morgenstern et al. 2008, Ohkuma et al. 2001). Extracellular class II peroxidases secreted by Agaricomycetes include manganese peroxidases, lignin peroxidases, and versatile peroxidases, which, along with laccases, aid in lignin depolymerization (Morgenstern et al. 2008, Ohkuma et al. 2001). Lignocellulose-degrading Agaricomycetes make plant litter more available to other fungi, or more palatable, and (together with the fungal biomass) nutritious to

detrivorous soil animals. As decayers of organic matter, Agaricomycetes help to recycle nutrients, and make them available to plants and other organisms.

Another important role that Agaricomycetes fulfill is that of a symbiont and more specifically, forming mycorrhizal associations with plants. These groups of Agaricomycetes are known as ectomycorrhizal (ECM) fungi. ECM fungi form associations with living plant root tips; a collection of fungal hyphae surrounds the root tip and penetrates the intercellular space between cortical root cells, forming what is known as a 'Hartig net' (Smith and Read 2008, Brundrett 2002). ECM fungi form associations with roots of angiosperms such as *Eucalyptus*, *Betula*, *Populus*, *Fagus*, and *Shorea* and gymnosperms such as *Pinaecea* (Brundrett 2004). ECM fungi help to transport water and nutrients; they mobilize nitrogen (N), phosphorus (P), calcium (Ca), and magnesium (Mg) from solid mineral substrates through organic acid excretion (Landeweert et al. 2001) and from organic substrates by enzymatic digestion (Bending and Read 1995, Tibbett and Sanders 2002). Fungal hyphae provide a low-cost method, relative to much more massive plant roots, that increases the volume of soil explored for soil nutrients. The diversity of roles that Agaricomycetes play are mirrored by the range in size of their fruiting bodies, from 0.1 mm in diameter to the largest fruiting body of all fungi found to date, *Fomitiporia ellipsoidea*, a polypore found in China with an estimated volume of 40.5 m<sup>3</sup> and weight of 400-500 kg (Dai and Cui 2011). The mycelium of another Agaricomycete, *Armillaria bulbosa*, was estimated in Michigan to occupy 15 hectares and to weigh approximately 9700 kg (Smith et al. 1992). Mycelia (individuals) of Agaricomycetes range from centimeters to tens or hundreds of meters in diameter, very large when compared to moulds and yeasts, which range from a few  $\mu\text{m}$  to

tens of  $\mu\text{m}$  in diameter (Walker 1998). Given the various important roles of Agaricomycetes, it is necessary to study the factors that determine their presence and community composition in soil ecosystems.

#### **1.4 Mechanisms by which agricultural practices affect fungal community structure**

Agricultural expansion has been called the single most destructive form of land-use change and one of the most significant anthropogenic alterations to the global environment (Matson et al. 1997). From 1700-1980, the total area of cultivated land increased 466% (Matson et al. 1997). From 1980 onwards, agricultural expansion has slowed, as less natural habitat is available for conversion to agricultural lands, but yields have increased dramatically. The increase was exponential during the “Green Revolution” in the 1960s and onwards, due to intensification of agriculturally managed lands, accomplished through high-yielding and pest-resistant crop varieties, chemical fertilizers and pesticides, large-scale irrigation, and mechanization of agriculture (Matson et al. 1997). Agriculture has local and global environmental consequences; these include loss of biodiversity, emissions of pollutants contributing to global climate change, habitat fragmentation and degradation, pollution of ground water, eutrophication of surrounding freshwater and/or marine habitats, increased erosion, soil acidification, and loss of soil fertility.

Agricultural soils have been better studied by mycologists than soils of undisturbed ecosystems (Carlile et al. 2001). Changes in soil fungal community structure due to agriculture can be attributed to the initial conversion of natural habitats to managed agricultural lands and subsequent land management practices, influencing soil

quality (Matson et al. 1997). Agricultural lands differ from undisturbed soils in containing artificially higher levels of N, P, and K (Carlile et al. 2001). Agricultural soils are subject to monoculture cropping systems and disturbance by cultivation of land and



chemical properties of soil that greatly alter the matrix supporting soil organisms (Kennedy and Smith 1995). A disruption and loss of diversity of beneficial soil fungi may profoundly alter biological regulation of decomposition and nutrient availability in soil.

Even in the absence of tillage, chemical changes due to inputs of fertilizers and lime (to increase soil pH) can alter species composition and plant-fungi symbioses. Both nitrogenous and phosphatic fertilizers have been reported to reduce mycorrhizal colonization of plant roots (Hayman 1980, Harinikumar and Bagyaraj 1989). Higher amounts of fertilizer may inhibit mycorrhizae due to a decreased dependence by plants when the soil is nutrient saturated (Coleman et al. 1983). Plant preference for fertilizer is due to the decreased energy expenditure to acquire N and P; comparatively, mycorrhizal associations with plant roots are more energy expensive (Coleman et al. 1983). Although plant species differ in the degree to which they support ectomycorrhizal relationships (Brundrett 2009), fertilizer application may inhibit ectomycorrhizae formation by Agaricomycetes.

Some biological functions provided by soil organisms have been substituted by land management practices that speed up or intensify the some of the same services, such as the use of fertilizers and tillage (Matson et al. 1997). These practices marginalize the free ecosystem services provided by fungi that can act to improve soil fertility and plant health. Management practices, however, can be designed to maximize the presence and function of soil biota (Matson et al. 1997).

### **1.5 Sustainable agriculture and its link to Agaricomycetes**

Sustainable agriculture cannot be easily defined and many interpretations of the concept exist - the term itself can encompass environmental, social, economic, and public health factors and emphasis cannot be placed on any one single factor. Nonetheless, the basis of sustainable agriculture must depend on the resources that support the production of crops for food and fiber. A broad definition of sustainable agriculture states that it “...is one that, over the long term, enhances environmental quality and the resource base on which agriculture depends; provides for basic human food and fiber needs; is economically viable; and enhances the quality of life for farmers and society as a whole” (Weil 1990). For the purposes of this report, sustainable agriculture will be considered in the biological context with emphasis on environmental quality and the resources that affect environmental quality.

The first steps to sustainable agriculture must be the identification of the end goals (Doran 2002). The strategies or course by which we will attain these goals must be developed and indicators or benchmarks to mark progress will help determine if strategies are indeed working (Doran 2002). One concern surrounding sustainable agriculture is that policy-makers, conservationists, land-managers, and producers cannot agree on any one set of goals. Conflicting views hamper the process of defining specific goals and there is a need from the mycologist’s and ecologist’s point of view to include the enhancement or preservation of all relevant organisms that provide necessary ecosystem services, when developing strategies for sustainable agriculture.

Sustainable agriculture is ever more necessary now as our population has surpassed 7 billion. In order to preserve the resources that feed the global population,

certain practices will need to be adopted in order to maintain biological productivity for future generations. As mentioned previously, soil, the keystone of our agricultural systems, must be preserved. Also, in alignment with sustainable agricultural goals, soil must remain productive in ways that do not degrade it. Sustainable agricultural practices that can maintain soil quality include crop rotation, planting of cover crops, and reduction of tillage (Tilman et al. 2002).

Sustainable agriculture goals, with emphasis on preserving soil fungi, can include reducing the degree to which soil is conventionally tilled, known as conservation tillage. This practice reduces the severity of the physical effects at the soil surface and the top layer of soil as well as increases organic inputs through crop residue retention (Matson et al. 1997). Low-till or no till is often cited as one of the best promoters of a more complex and dynamic fungal community structure for this reason (Matson et al. 1997). Fungal hyphae remain intact and as a result, colonization of plant roots and litter is not retarded by physical alterations. As well, low-till or no till can be adopted to promote other abiotic factors that can support a complex fungal community structure. For example, reduced tillage can help to conserve water, restore soil fertility, and reduce soil erosion caused by conventional intensive management practices, which are fundamentally due to the removal of crop residue at the soil surface (Johansson et al. 1994, Alguacil et al. 2008). Reduction of tillage can also increase early-season P uptake in crops, especially in low-input farming systems (McGonigle and Miller 1996a). This is because associations between mycorrhizal fungi and crops may form earlier in the season, which contributes to improved crop growth and development (McGonigle and Miller 1996a).

Historically, fungal surveys or identification of fungal species in soil have been restricted to culture-based sampling methods and identification through morphology. These methods were largely restrictive because similar morphologies may lead to misidentification of fungal species and culture-based methods fail to recover major groups of fungi (including saprotrophic and ectomycorrhizal Agaricomycetes) and often sample the same mould or yeast individual repeatedly (Straatsma et al. 2001). Furthermore, most groups of Agaricomycetes are difficult to study because they are hard to detect due to their transient fruiting bodies and are difficult to identify when not fruiting due to lack of distinguishing morphological characters (Hawksworth 2001, Hawksworth and Lagreca 2007, Matheny et al. 2006, Moncalvo et al. 2002). The inability to culture the majority of Agaricomycetes from soil contributes to the difficulty of assessing their presence and diversity in soils (Hawksworth 2001). However, DNA-based methods of sampling have greatly resolved these issues simply because they are reliant on soil-extracted DNA rather than visual identification. The phylogenies that result from DNA-based methods are very different from traditional morphological classifications and the use of DNA-based methods is resolving current controversies in fungal taxonomy (Hibbett 2006, Moncalvo et al. 2000).

Previous studies by Lynch (2004) and Bahnmann (2009) used DNA-based methods to assess the community composition of Agaricomycetes in Michigan agricultural soils. Bahnmann (2009) repeated DNA-based methods used by Lynch (2004) and concluded that DNA-based surveys of Agaricomycetes are repeatable across years.

## 1.6 Phylogenetics and multivariate statistics

One way to study changes at the community level is through the use of phylogenetics. Phylogenetics is the study of evolutionary relationships of a group of organisms (Nei and Kumar 2000). Phylogeny can be inferred from morphological characteristics, which are often not reliable as many different species have developed similar but non-homologous characteristics through convergent evolution. Phylogeny is more confidently inferred using molecular evidence, such as DNA, RNA, or protein, because these can be represented as short standardized sequences that can distinguish species and higher taxa based on genetic variation (Hajibabaei et al. 2007). Phylogenetic relationships of genes or organisms are usually presented in a phylogenetic tree, most commonly represented as a cladogram, which is a tree that depicts branching order (Gregory 2008). Any phylogenetic tree is merely a hypothesis, to be further tested with additional data; the true phylogeny is almost always unknown. The topology of the tree may be altered by a larger or different taxon sample or by changes to the parameters of the phylogenetic analysis

A phylogenetic tree can be analyzed using multivariate statistical analyses which are useful for analyzing large, complex data sets (Ramette 2007). The basic aim of multivariate, or exploratory analyses, is to represent the (dis)similarity between objects (eg. samples, sites) based on values of multiple variables associated with them (Ramette 2007). Multivariate analyses are useful to reveal patterns in large data sets with many interacting variables, but do not directly explain why those patterns exist (Ramette 2007). Traditionally, multivariate analyses are not able to incorporate phylogenetic data.

However, UniFrac, a program used in this study, is a suite of tools used that can run multivariate analyses on phylogenetic data.

### **1.7 Thesis rationale and objectives**

Agaricomycetes provide free ecosystem services that maintain and alter soil quality by decomposing plant litter, forming mutualistic associations with plants, and by maintaining soil structure. Current farming practices dictate that much of agricultural land is subject to soil homogenization through tillage and ploughing and further alteration through chemical inputs such as pesticides and fertilizers. In order to utilize the ecosystem services that Agaricomycetes provide, a more comprehensive understanding on how tillage specifically affects agaricomycete community structure is the goal of this study. Previous studies by Lynch (2004), Bahnmann (2009), Thorn et al. (1996), Vranic and Thorn (unpublished (2005-2007)), and H. Deacon and Thorn (unpublished (2006-2009)) provide a larger rDNA data set from samples taken from KBS LTER in different years, and provided the basis for this study. Lynch's (2004) phylogenetic analysis revealed increased species and clade diversity with decreasing disturbance and certain species and clades were found only in certain treatments. Bahnmann (2009) had similar findings but repeated molecular sampling indicated that only some clades are stable and persistent throughout sampling years.

The objectives of this study were:

- i) to detect patterns in the phylogeny that could not be detected in individual previous studies

- ii) to use phylogeny-based and community-based analyses to compare composition of Agaricomycetes among four different agricultural treatments

With the use of a larger data set and new phylogenetic tools, and under the assumption that tillage, aboveground plant community composition, and chemical inputs affect community composition of soil Agaricomycetes, the goal of this thesis is to determine what differences in community composition can be seen among different land management practices, from conventional tillage to no tillage to successional plant communities. The Kellogg Biological Station Long Term Ecological Research (KBS LTER) site provided an ideal location to test the relationship between soil tillage and soil Agaricomycetes because experimental plots at KBS represent different degrees of agricultural disturbance by tillage as well as successional plant communities. This thesis will be more comprehensive than past research because it is based on a larger set of pooled data, whereas previous studies by Lynch (2004) and Bahnmann (2009) were conducted on individual sample sets. Furthermore, since 2004-2009, there has been an increase in the number of reference sequences available in GenBank, increasing the chances of related reference sequences for more of the unknown soil sequences. New trends may be resolved in this study due to advancements in phylogenetics and placement of previously unknown taxa of Agaricomycetes based on published (Hibbett et al. 2007, Matheny et al. 2007) and unpublished data (Thorn and Hibbett). Finally, phylogenetic analysis in combination with multivariate statistics that have not been previously applied to them may provide novel insights not recognized in past studies by Lynch (2004) and Bahnmann (2009). These tools will give more detailed insight into how tillage may affect the community composition of soil Agaricomycetes. Understanding this relationship must

underlie the analysis of consequences of anthropogenic land use change in agroecosystems.



## CHAPTER 2: MATERIALS AND METHODS

### 2.1 Study Site

Data were obtained from soil samples collected at the Kellogg Biological Station Long Term Ecological Research site (KBS LTER). KBS is one of 26 sites that belong to the national LTER network. It was established in 1989 as a long-term research site for ecological and environmental studies on agroecosystems. KBS LTER is located in southwest Michigan, 50 km east of Lake Michigan (42° 24' N, 85° 24' W, elevation 288 m). The site covers 1600 ha of cropping systems, successional communities, and small lakes. The area surrounding the KBS site is mainly a rural landscape, where vegetation ranges from cultivated fields to early successional fields to old growth forests. A more detailed site description can be found on the KBS website ([http://lter.kbs.msu.edu/about/site\\_description/index.php](http://lter.kbs.msu.edu/about/site_description/index.php)). Sampled sites at KBS include conventional till (CT) and no till (NT) plots of either corn, soybean, or wheat in alternating years, plots tilled until 1989 then allowed to enter succession (HTS), and a successional community that has never been tilled (NTS) (Appendix 1).

#### 2.1.1 Soil

Soil in southwestern Michigan is characteristic of a mature glacial outwash plain and moraine complex as initially formed by the Wisconsin glaciation. Nowadays, soils in the region are mostly sandy-loam and silty clay loams of moderate fertility. Soils found at KBS fall into 15 soil series representing 4 general soil orders: alfisols, entisols, histosols, and mollisols. The dominant soil type at KBS is sandy-loam alfisol in the Main Cropping System Experiment (MCSE) (Broughton and Gross 2000).

### **2.1.2 Agricultural treatments**

Experimental plots at KBS include different cropping systems, with a range of chemical-input intensities, and successional communities. Soil samples were collected by Thorn in June and July 1993, Lynch in June and October 2002, Bahnmann in November 2004 and November 2005, and Deacon in June 2006 from replicate plots of four treatments: conventional till (CT), no till (NT), historically tilled successional (HTS), and never tilled successional (NTS), located within the MCSE.

CT plots are tilled annually by chisel plow before seed planting in May. NT plots have not been tilled since 1989, and row crops were drill seeded. HTS plots have not been tilled since 1989 and have been allowed to turn into successional meadow, maintained by periodic burning. Finally, NTS plots are represented by a meadow that has not been tilled in recorded history and has been maintained by annual mowing since 1989. Each replicate plot is approximately 1 ha, except for NTS plots, which are approximately 0.1 ha in area and approximately 200 m off-site. Both CT and NT plots receive chemical inputs of fertilizer (N, P, K, lime) at agronomically relevant levels depending on the crop of the year. HTS and NTS plots have not received fertilizer since 1989; however, there is an N-subplot within NTS that was fertilized since 2001; no samples for this study came from that subplot. No pesticides have been applied to CT, NT, HTS or NTS plots.

### **2.1.3 Plant communities**

Corn, soybean or wheat are planted each year in CT and NT plots and rotated among years (Table 2.1).

Table 2.1 Cover crop planted on conventional till (CT) and no till (NT) plots during sampling years

	1993	1994	2002	2004	2005	2006
Corn	X		X		X	
Soybean		X				X
Wheat				X		

HTS is an old field dominated by goldenrods (*Solidago canadensis*), clover (*Trifolium*), and non-native grasses (*Elymus*, *Poa* and *Phleum*). NTS is a meadow dominated by non-native grasses (*Bromus*, *Poa*, and *Arrhenatherum*), goldenrods (*Solidago canadensis*), brambles (*Rubus*), and clover (*Trifolium*). More detailed plant community information during years of sampling can be found by downloading the complete data table on the KBS website (<http://lter.kbs.msu.edu/datatables/237>).

## 2.2 Soil sample collection, washing and isolation of fungal cultures or DNA

All procedures described in this section were carried out by previous investigators (Thorn et al. 1996, Lynch 2004, Bahnmann 2009, Deacon, Vranic and Thorn, unpublished) and are reported as background information to my study. Soil cores (2.5 cm diameter by 15-30 cm depth) were collected from 5 or more locations within 4-6 replicate plots of each treatment, and were kept at 4°C until processed. Subsamples of 10 g (fresh weight) from pooled soil samples from each plot (Thorn et al. 1996, Bahnmann 2009, Deacon unpublished) or from individual sampling sites within plots (Lynch 2004) were suspended in 125-150 ml of 0.1 M sodium pyrophosphate, shaken for 5 minutes and washed through sieves of decreasing pore size: #16 (1.18 mm), #60 (0.25 mm), and #270 (0.053 mm), which were washed and sterilized with 70% ethanol between samples.

Washed organic material collected on the 0.053 mm mesh sieve (250-1000  $\mu$ L) was used to isolate cultures of Basidiomycetes on selective agar media (Thorn et al. 1996, Vranic unpublished). Soil DNA was extracted using a bead beating protocol with either the Power Soil DNA extraction Kit  $\text{\textcircled{R}}$  (Mo Bio Laboratories, Inc.) (Bahnmann 2009) or UltraClean<sup>TM</sup> Soil DNA Kits (Mo Bio Laboratories, Inc.) (Lynch 2004) and the FastPrep<sup>TM</sup> FP120 instrument (Bio101) with 4 cycles of 30 seconds at setting number 4 with breaks of 5 minutes on ice in between cycles (Lynch 2004, Bahnmann 2009, Deacon unpublished).

### **2.2.1 PCR amplification and TA cloning**

Amplification of soil-extracted fungal DNA was carried out using primers with specificity for Basidiomycota (Lynch and Thorn 2006). The primers used were B001 (5' – GCTTTACCACATAAATCTGA – 3') and B2R+ (5' – TACCGTTGTAGTCTTAACAG – 3') (Lynch and Thorn 2006, Gardes and Bruns 1993). These primers yielded an amplification product approximately 2.4 kb in length spanning the nuclear ribosomal ITS region and approximately 1000 bp into the small (SSU or 18S) and large (LSU or 25S) rRNA genes. Amplification used a standard PCR protocol with 20-30 seconds at an annealing temperature of 55°C and 120 seconds extension at 72°C. Successful amplifications were gel-purified and used as a template for TOPO TA cloning (Invitrogen Corp., Mississauga, Canada). Twelve to 20 randomly selected clones from each cloning reaction were subjected to restriction fragment length polymorphism (RFLP) screening using either *RsaI* or *MspI* restriction enzymes (Fisher Scientific Ltd., Nepean, Canada). Clones with unique RFLP banding patterns within a cloning reaction

were sequenced using LR3 (5' – GGTCCGTGTTTCAAGAC – 3') sequencing primer described by Vilgalys and Hester (1990), yielding approximately 5' 600bp of the LSU gene.

### **2.3 Phylogenetic analysis**

This section represents my own study methods. A set of 102 LSU sequences of Agaricomycetes representing the major phylogenetic groups of Agaricomycetes (Hibbett et al. 2007, Thorn and Hibbett, unpublished) were downloaded from GenBank to provide references and clustering points for KBS sequences. A list of reference sequences with GenBank accession numbers is included (Appendix 2). Five hundred ninety-one sequences of Agaricomycetes collected from KBS LTER and 123 of their closest nucleotide-nucleotide BLAST (Basic Local Alignment Search Tool) matches (<http://www.ncbi.nlm.nih.gov>) were compiled. Reference sequences, unknown sequences and their closest BLAST matches were the basis on which phylogenetic analysis was conducted.

All sequences were compiled into one FASTA file and were initially aligned using the ClustalW algorithm in MEGA5 (Tamura et al. 2011). However, the alignment was unsuccessful, probably due to the phylogenetically broad taxon sample and ragged sequence matrix (with some taxa having sequences that started or ended beyond others). To solve the latter issue, sequences were trimmed at the 5' end of the LSU gene using the identifier sequence CAAATCAG, and (where present) after the LR5 primer TTTCCCTCAGGA (Vilgalys and Hester 1990). The sequence region retained includes only LSU data, removing the highly variable ITS region; the ITS region was difficult to

align across the phylogenetically broad set of taxa included. The data matrix included up to approximately 900 bases of each sequence from most reference taxa, and 600 bases from unknown sequences from KBS LTER.

The file was split into two separate files to facilitate alignment, with the same three out-groups defined in each file: *Tremella aurantia*, *Trichosporon dulcitum*, and *Dacrymyces chrysospermus*. Species in the out-groups are from the classes Tremellomycetes and Dacrymycetes, of the sub-phylum Agaricomycotina. Both files were converted from FASTA to multi-FASTA format using DNA Baser (DNA Baser Sequence Assembler 2011) for alignment in SINA (v.1.2.9), which uses ribosomal structure to guide alignment of small and large subunit ribosomal RNA sequence data (Pruesse et al. 2007). The two alignment files were then merged using MUSCLE (Edgar 2004) and the alignment subsequently confirmed by visual inspection in SeaView (Gouy et al. 2010). The aligned file contained 816 sequences by 1274 bases, of which 259 were invariant and 794 were phylogenetically informative.

A constraint tree was defined for reference sequences such that they were constrained to a topology derived from multiple gene phylogenies of the Basidiomycota (Hibbett et al. 2007), while the unknown sequences were allowed to join the tree wherever their placement was optimal under Neighbor Joining (NJ) in PAUP 4.0b10 (Swofford 2002, Tamura et al. 2004). An NJ analysis with enforced backbone constraint was run in PAUP, using the BioNJ algorithm (Gascuel 1997). Bootstrap support was calculated for the NJ tree based on a heuristic search of 100 replicates, each with 10 random order sequence addition replicates (Swofford 2002). The resultant tree was edited in ClarisDraw to improve fonts and divide the tree into page-size portions. Operational

taxonomic units (OTUs) were defined as a single sequence or groups of sequences that had less than ~1% change in sequence identity.

## **2.4 Statistical methods and multivariate analysis**

All community analyses were completed using the Unique Fraction (UniFrac) suite of software (Lozupone et al. 2006). UniFrac is a diversity measure that uses phylogenetic information to compare environmental samples by measuring the distance between communities based on the lineages they contain (Lozupone and Knight 2005; Lozupone et al. 2011). The web application required two input files; the phylogenetic tree generated in PAUP in NEXUS format and an environmental file describing the source of each sequence in the phylogenetic tree as a text file.

The UniFrac metric was used to determine whether communities in the sampled plots ('environments') were significantly different (Lozupone and Knight 2005). The UniFrac significance metric was calculated once using the "each environment individually" option and "all environments together" option. The "each environment individually" option measures each sample against the rest of the tree, treated as a single "other" environment and determines if that particular environment has a more unique branch length than expected by chance (Lozupone and Knight 2005). Its output is a table of P-Values for each separate environment indicating whether it is significantly different from the rest of the tree. This allows one to determine which particular environment is contributing to the unique branch length. The "all environments together" option determines whether or not sequences from all different environments, when measured against each other, are significantly different from each other (Lozupone and Knight

2005). In each case, the output is a corrected P-Value and a raw P-Value. A corrected P-Value is the raw P-Value that has been corrected for multiple comparisons when there are two or more. In each case, the number of tree permutations used to generate the random distribution of values to which the value of the true tree is compared when calculating a P-Value was set at 100. The number of permutations is a parameter that can be adjusted within this analysis that can re-analyze the data up to 100 times (Lozupone and Knight 2005).

The Parsimony test (P-test) in UniFrac was also performed (Martin 2002). The P-test determines whether the differences between environments are significant using phylogenetic information (Martin 2002). This was done in support of the UniFrac significance test. The P-test is more likely to give a significant result than the UniFrac significance metric when many sequences are very closely related to one another but are unique to one particular environment (Lozupone and Knight 2005). UniFrac determines two P-Values, raw and corrected. The Bonferroni correction is used to correct P-Values for multiple comparisons. Parsimony is employed by characterizing the tree by computing the minimum number of changes to explain the observed distribution of sequences between the different environments in the tree (Martin 2002). The output is a P-test value that is a fraction of random permutations of the labels that require the most parsimonious explanation. Therefore the P-test, using tree topology, tests the hypothesis that fewer changes from one environment to another are required to explain the observed distribution of sequences than would be required if the environments were randomly assigned to each sequence (Martin 2002). Again, the number of permutations was set at 100.



A jackknife environment cluster analysis was performed on the tree. Jackknifing is a statistical re-sampling technique that determines whether or not the same results could be found using only a random sub-sample of the data (Lozupone and Knight 2005). The number of permutations can be set to re-cluster the data 10 or 100 times (Lozupone and Knight 2005). The number of permutations was set at 100. Jackknifing is used to determine how robust the analysis is to both sample size and evenness by assessing how often the cluster nodes are recovered when smaller, even sets of sequences are sampled from each environment (Lozupone and Knight 2005). Jackknifing minimizes within-group variation and maximizes between-group variation in order to reveal well-defined groups or clusters of environments and therefore reduce the dimensionality of the data set to a manageable group of rows (Ramette 2007). Clustering environments are useful to determine patterns that could not have been determined from the pattern of significant differences alone (Lozupone and Knight 2005). The result is a tree-like diagram that shows how samples relate to one another by how strongly they are clustered, and this allows one to be more confident of clusters that are well-supported (Lozupone and Knight 2005).

While cluster diagrams are useful for showing which environments are most closely related to one another, principal coordinates analysis (PCoA), also known as metric multidimensional scaling, is useful to show how environments are distributed along multiple axes of variation (Lozupone and Knight 2005, Schmit and Lodge 2005). PCoA uses a linear mapping of distance or dissimilarities between objects, 'sites' or 'replicate environments' or 'treatments' for the purposes of this research, onto the ordination space (projection in a Cartesian space) so that similarities and differences

between replicate environments, which are represented as points in the ordination space, can be more easily visualized (Ramette 2007). Eigenvalues are used to measure how much variance is accounted for by the largest synthetic variables, or principal components (Ramette 2007). PCoA uses presence-absence data, which is useful as a variety of distance measures can be used (Schmit and Lodge 2005). PCoA was completed in UniFrac by calculating the distance matrix for each pair of environments using the UniFrac metric (Lozupone and Knight 2005). Distances are represented by points in a 2D space with a number of dimensions one less than the number of samples (Lozupone and Knight 2005). UniFrac provides an output of raw data with eigenvalues and eigenvectors (Lozupone and Knight 2005). PCoA summarizes the data with many variables to a smaller set of synthetic, derived variables, or principal components. The principal components, in descending order, describe how much of the variation each of the axes in this new space explains (Lozupone and Knight 2005). The first principal component separates out the data as much as possible, the second principal component provides the next most separation, and so on (Lozupone and Knight 2005). The reduction often leaves residual variation, information in the original data that is not retained by the new principal components in order, which allows for easier visualization.

## CHAPTER 3: RESULTS

### 3.1 Phylogenetic Tree

Generation of a phylogenetic tree in PAUP 4.0b10 (Figure 3.1) using KBS sequences and GenBank reference sequences, provided the basis on which statistical tests and multivariate analysis were conducted. Bootstrap support for nodes was represented by numbers on branches to the left or right of sequence names. Bootstrap numbers ranged from 0 to 100, where 51 was the lowest bootstrap value reported, indicating weak support, and where 100 was the highest bootstrap value possible, indicating very strong support. Operational taxonomic units (OTUs) were indicated to the right of sequence names. Conventional till plots are coloured in red, no till plots are coloured in orange, historically tilled successional plots are coloured in yellow, and never tilled successional plots are coloured in green. A total of 472 OTUs were defined based on the 591 sequences recovered from the four focal treatments at KBS LTER and the major clade Agaricales accounted for 330 (70%) of all OTUs. The remaining OTUs were assigned to 12 of the 19 major clades of Agaricomycetes: Tulasnellales – 19 OTUs; Cantharellales – 39 OTUs; Polyporales – 23 OTUs; Thelephorales – 3 OTUs; Hymenochaetales – 29 OTUs; Corticiales – 2 OTUs; Geastrales – 4 OTUs; Gomphoid/Phalloid clade – 3 OTUs; Sebaciniales – 9 OTUs; Russulales – 2 OTUs; Trechisporales – 4 OTUs; Auriculariales – 5 OTUs.

### 3.2 UniFrac significance test and P test

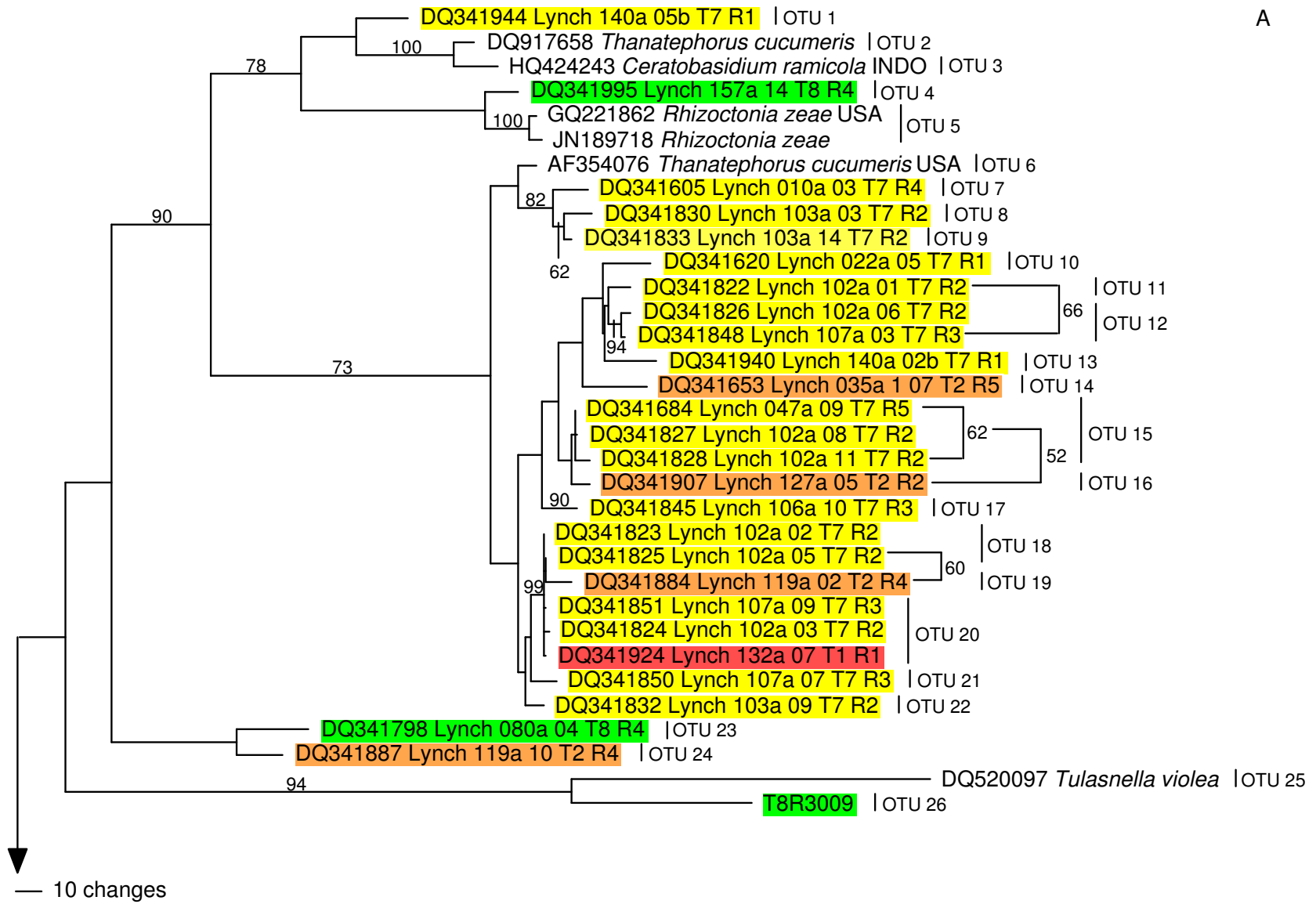
When sequences from all environments were tested individually using the UniFrac significance metric, the UniFrac P-Value given for each environment ranged

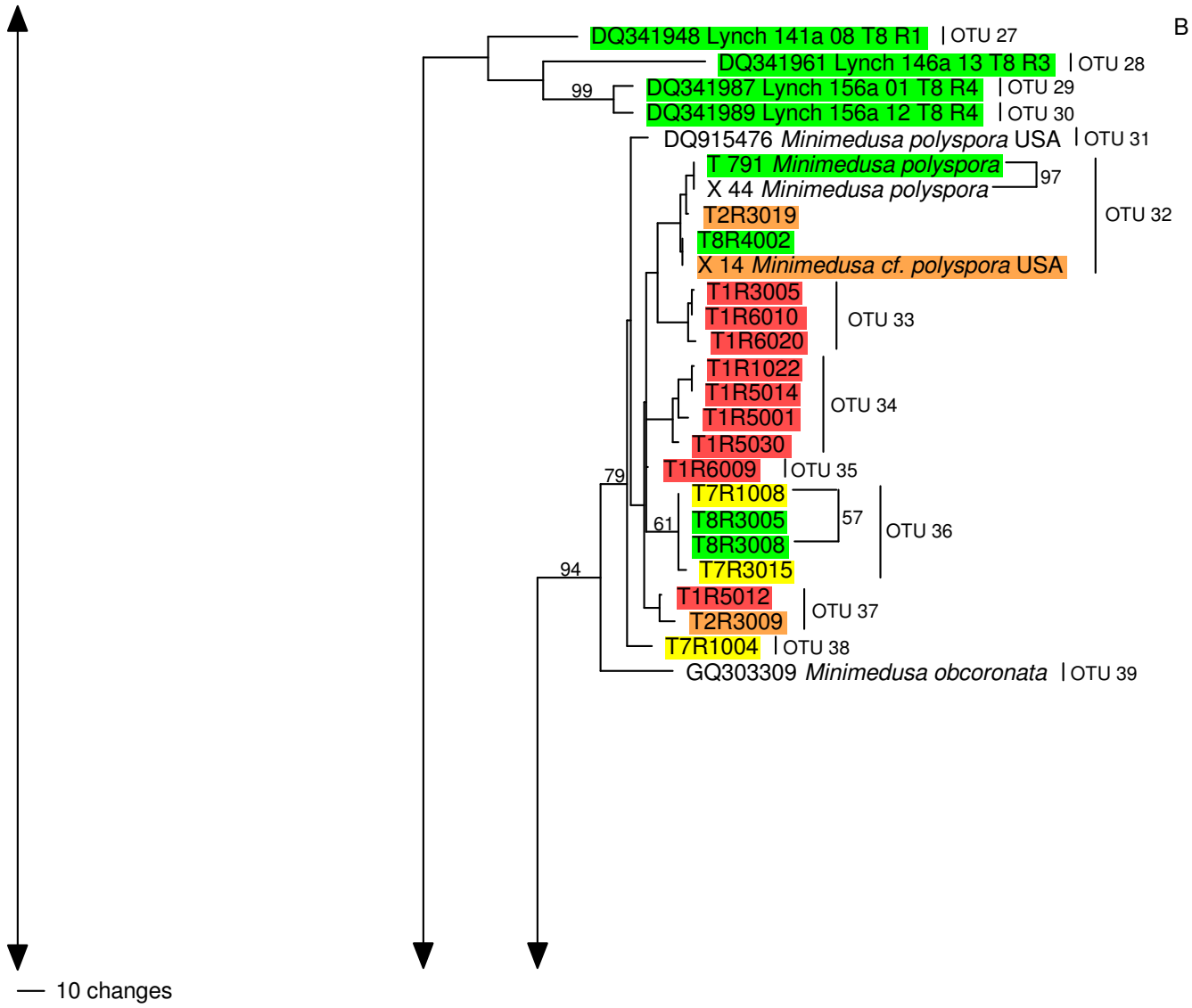
from  $\leq 0.01$  to 1.0 (Table 3.1). There was no significant difference for most environments when they were measured against the rest of the tree (treated as a single “other” environment). However, plots NT T2R2, HTS T7R4, HTS T7R5, and NTS T8R1-R4 had P-Values of  $\leq 0.01$ , indicating that these seven plots were uniquely different in the phylogenetic makeup of their communities.

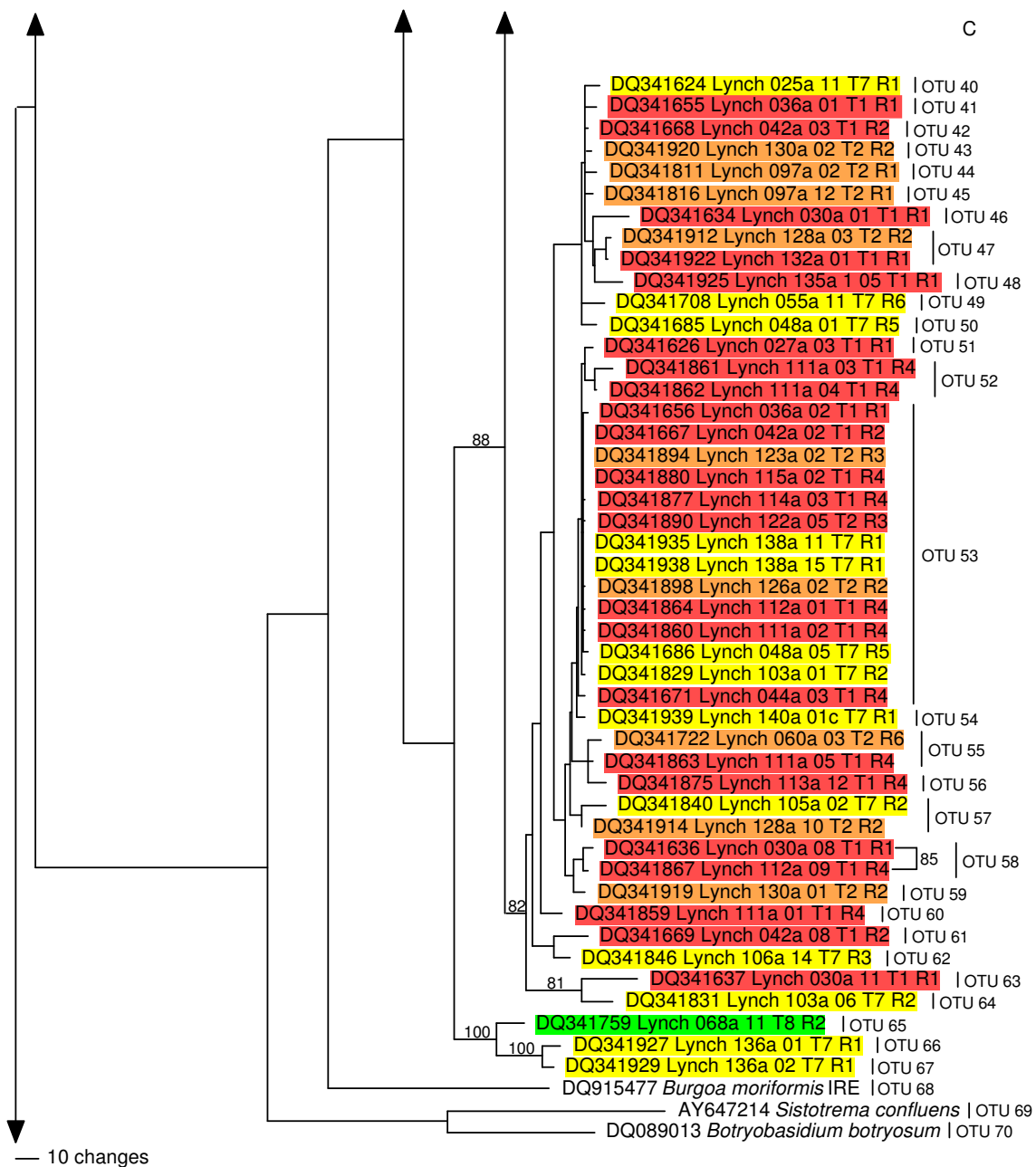
Figure 3.1. Neighbour-joining tree of reference sequences and sequences recovered from soils in KBS LTER sites: conventional till (CT); no till (NT); historically tilled successional (HTS); no till successional (NTS). Names coloured in red indicate CT; names coloured in orange indicate NT; names coloured in yellow indicate HTS; names coloured in green indicate NTS. Uncoloured names are reference sequences from GenBank, or other sequences not from the four study treatments at KBS LTER. The tree is scaled. The scale bar represents branch length corresponding to 10 changes for every 1000 bases. Major clades are (A) Tulasnellales; (B,C) Cantharellales; (D-F) Polyporales; (G-J) Agaricales: Clavarioid and Stephanosporaceae; (K-N) Agaricales: Agaricoid; (O,P) Agaricales: Tricholomoid; (Q) Agaricales: Schizophyllum-Lachnella, Maramioid; (R,S) Agaricales: Pluteoids; (T) Agaricales: Hygrophoroid, Tricholomoid; (U) Agaricales: Pterulaceae, Amylocorticales, Boletales, Atheliales, Jaapiales; (V) Thelephorales, Hymenochaetales, Corticiales, Gloeophyllales; (W) Geastrales, Gomphoid-Phalloid, Hymenochaetales; (X) Sebacinales, Russulales; (Y) Hymenochaetales; (Z) Dacrymycetes, Trechisporales, Auriculariales; (AA) Tremellomycetes

\*“*Coprinus*” *cordisporus* name currently unresolved (Redhead et al. 2001)

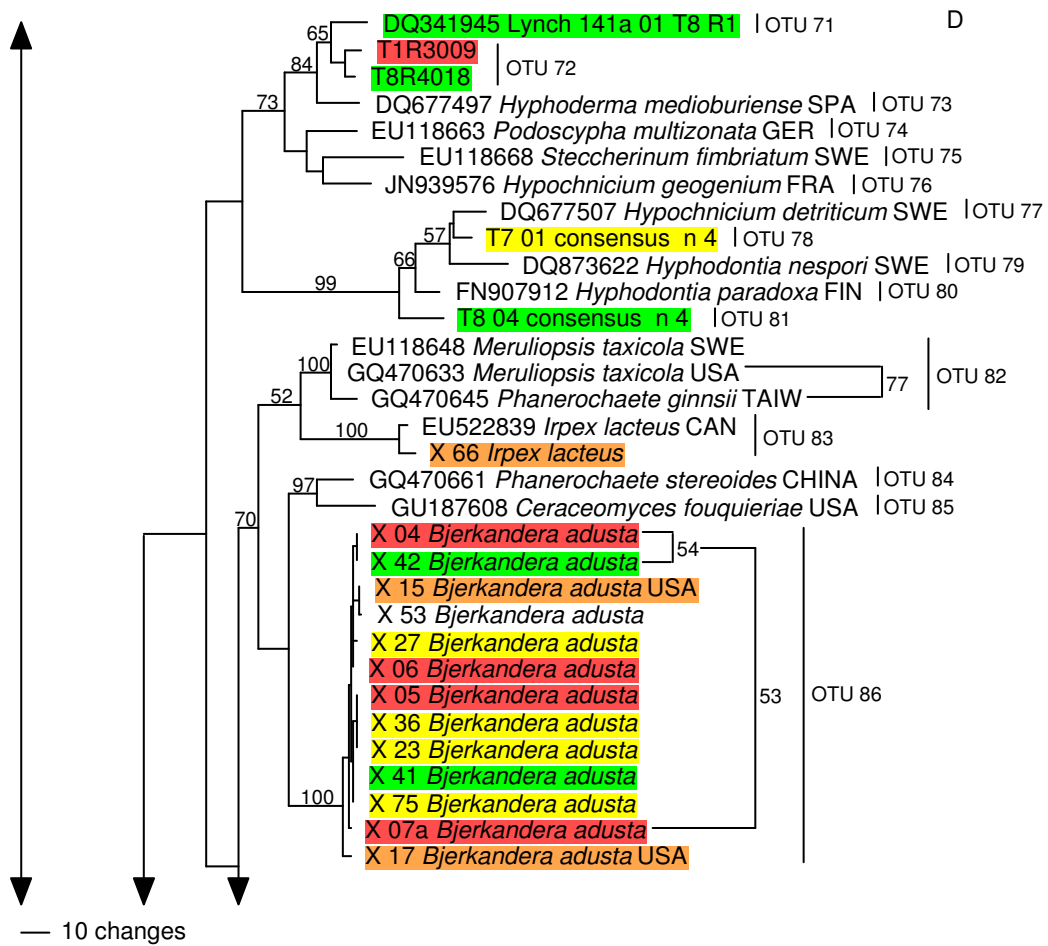
A



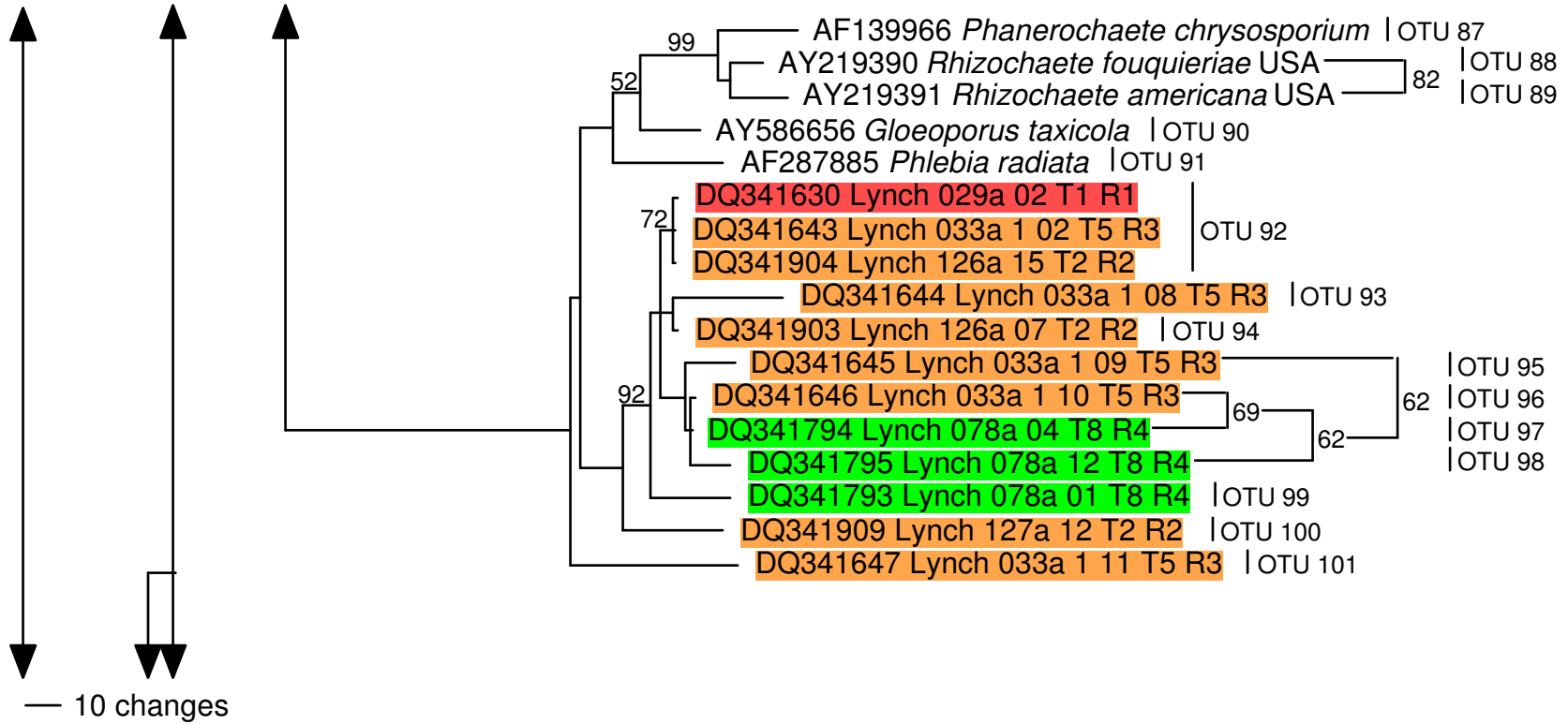


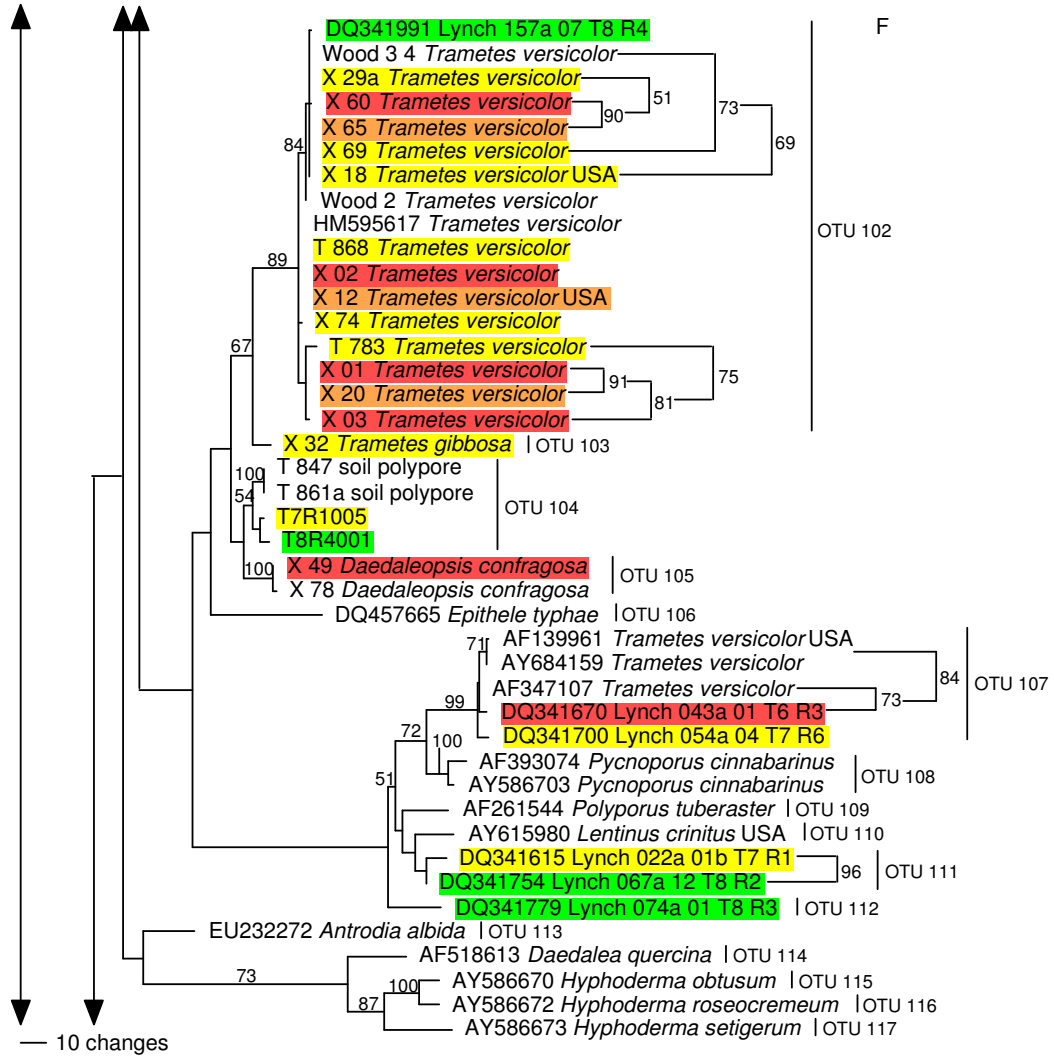


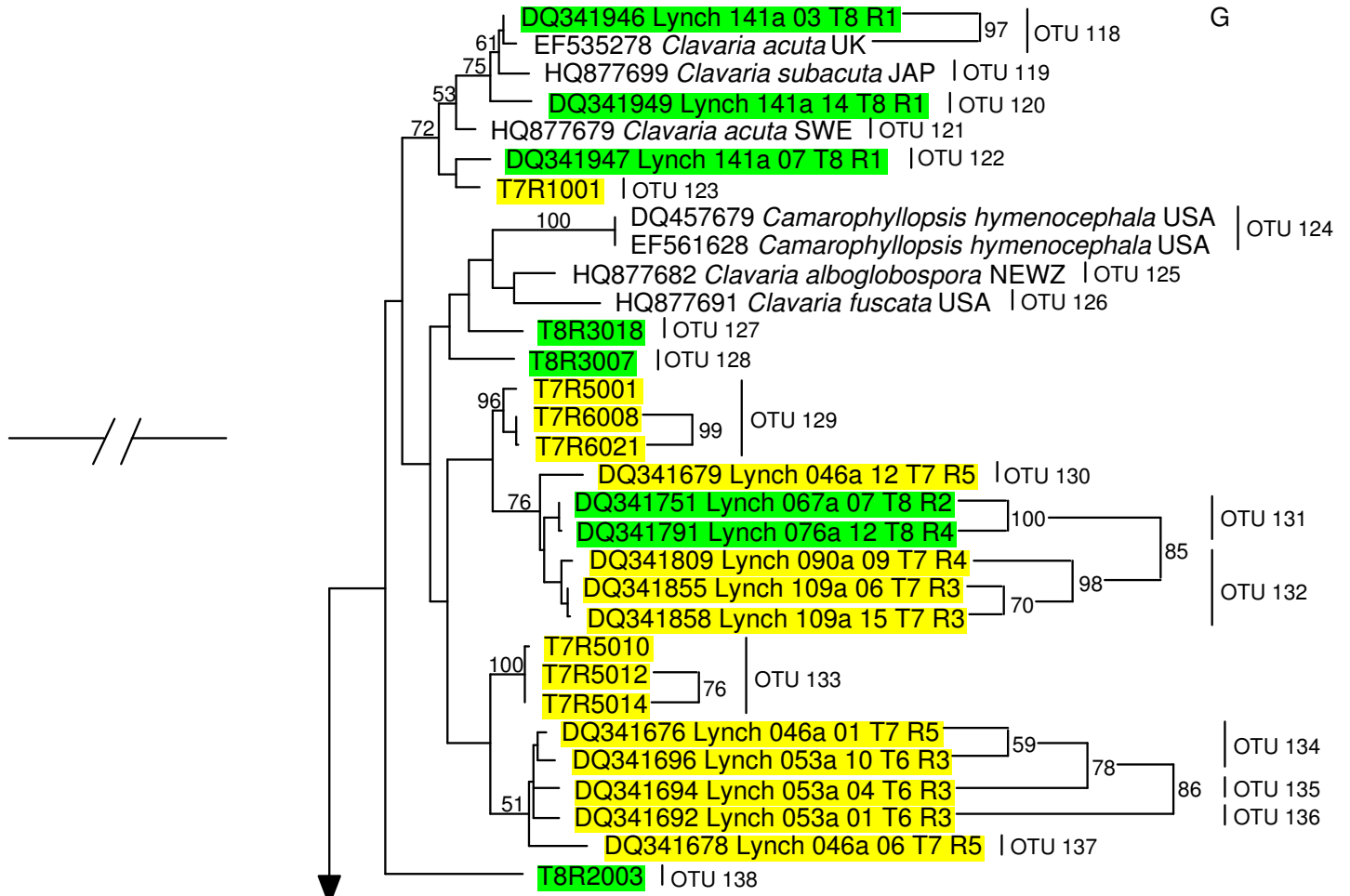
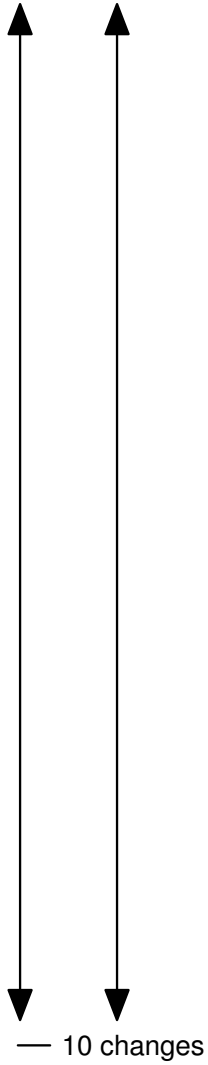


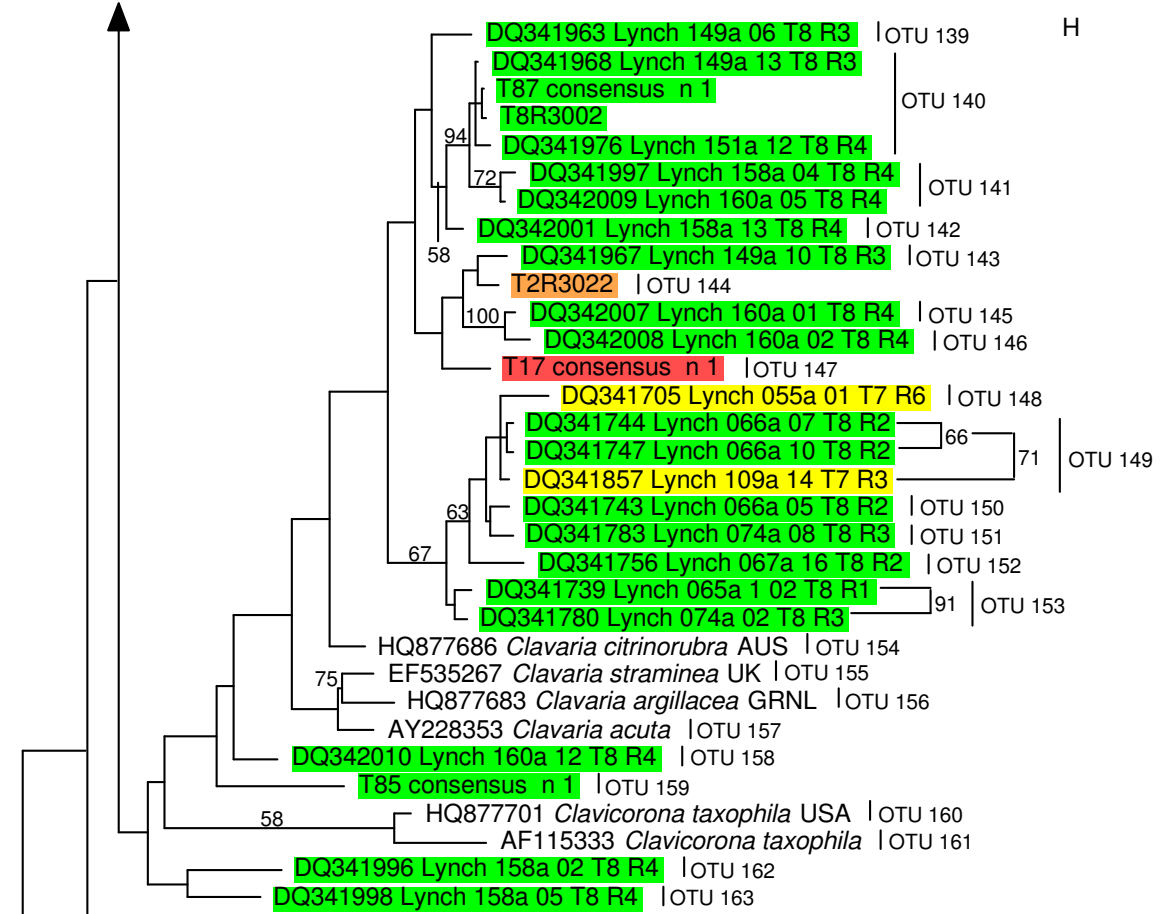
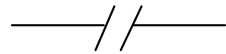
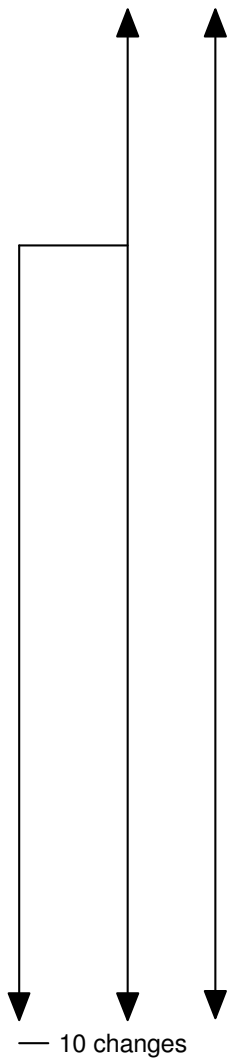


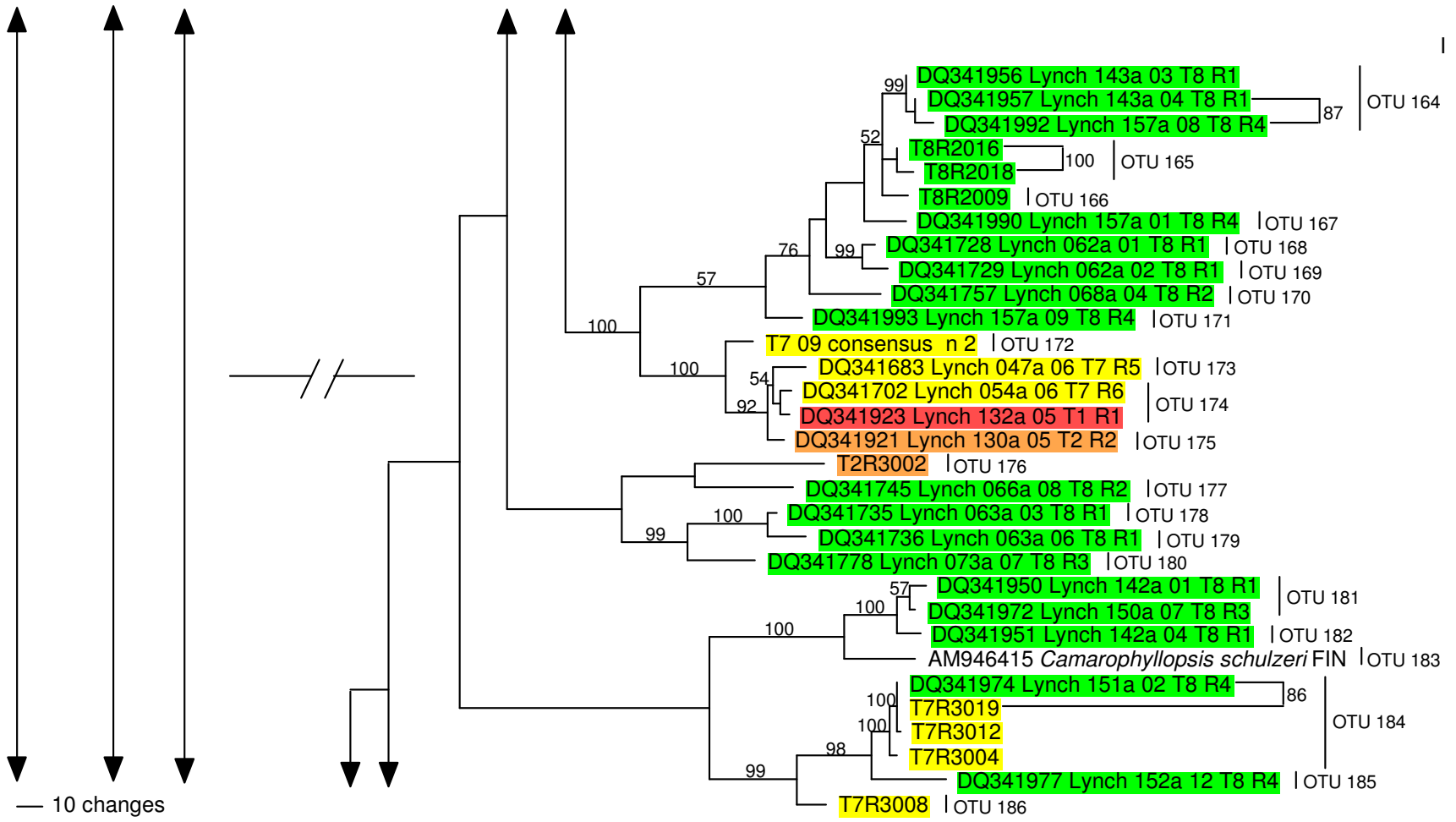
E

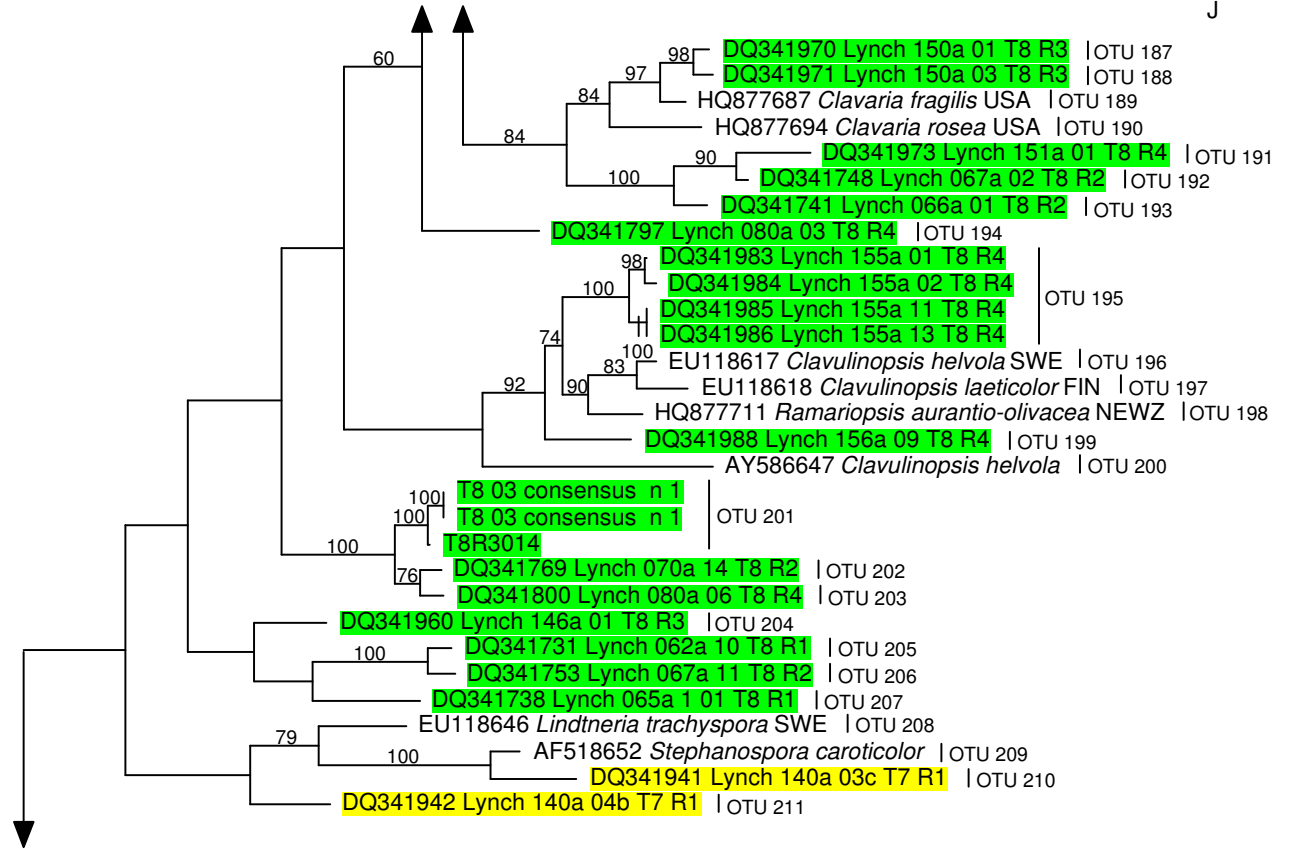
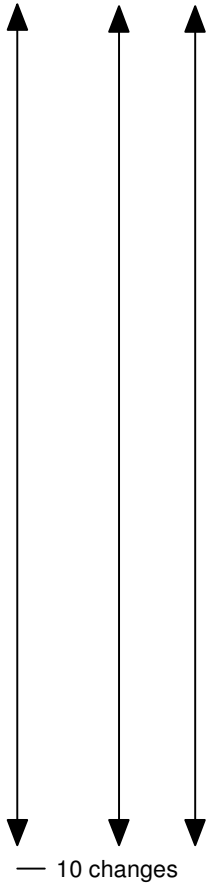


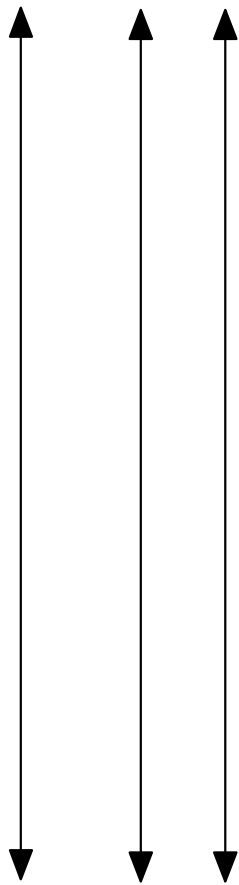




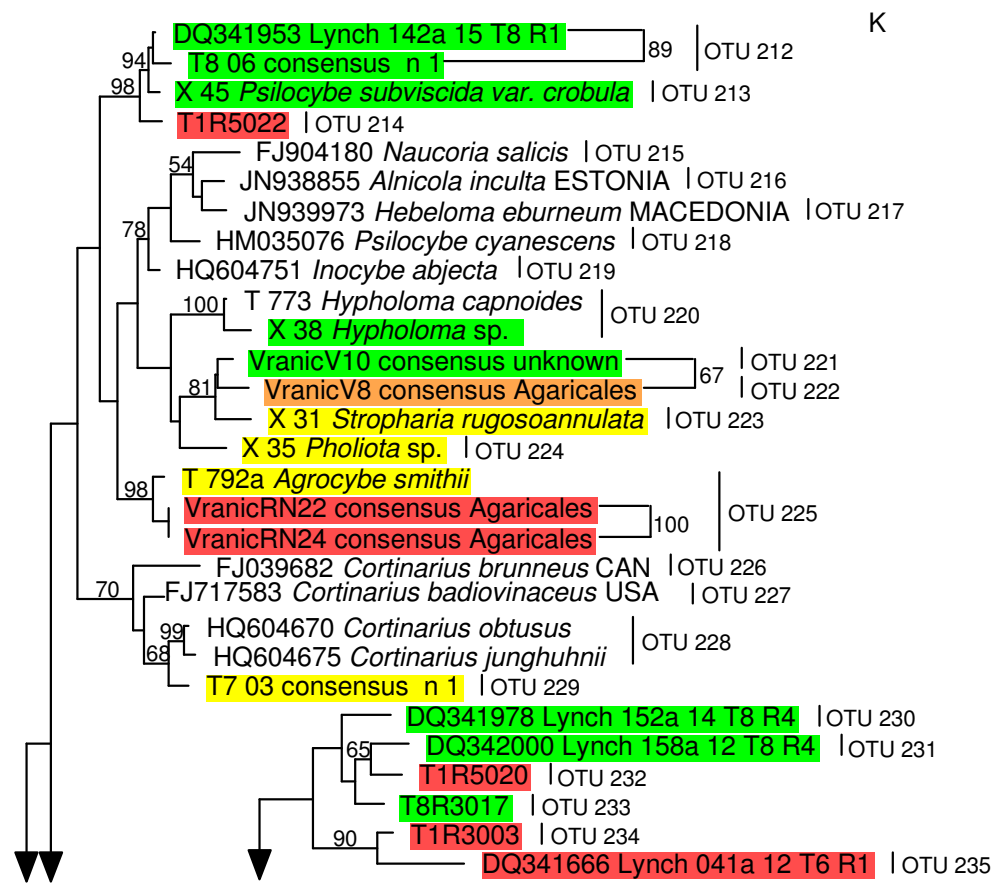




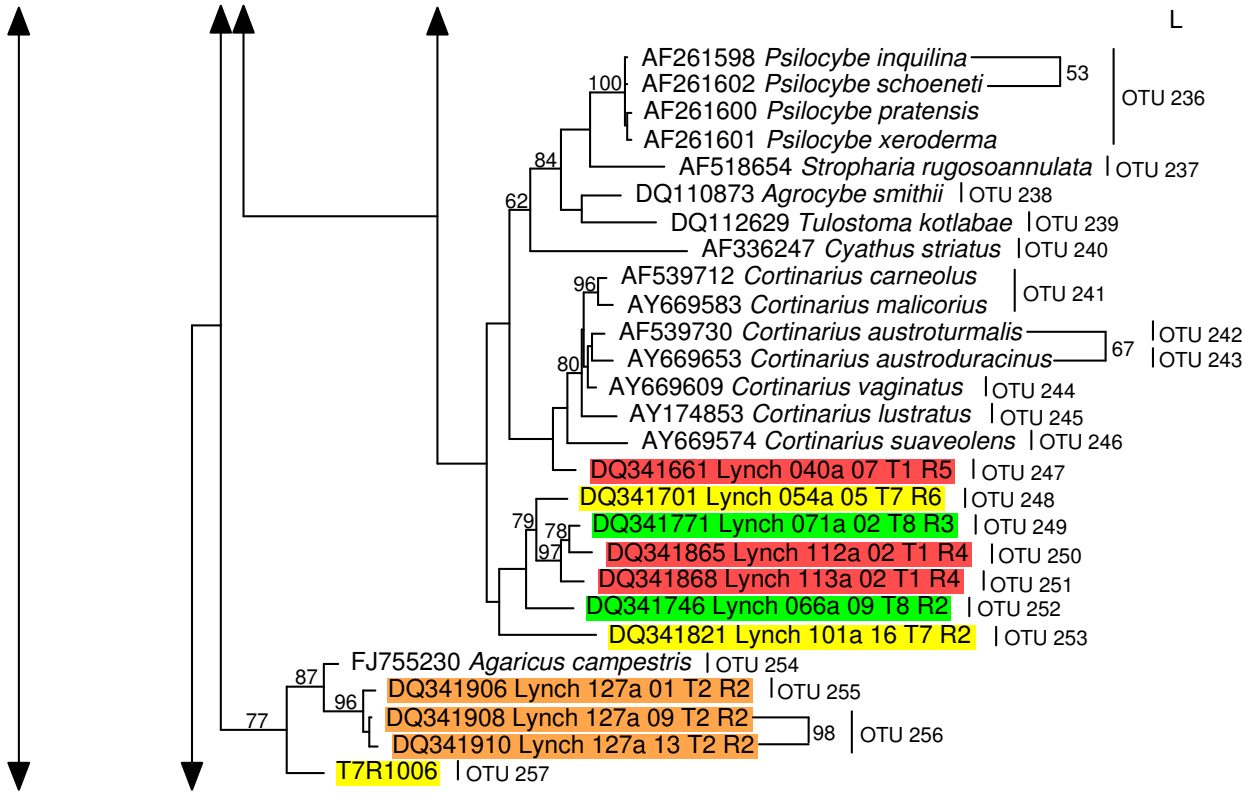
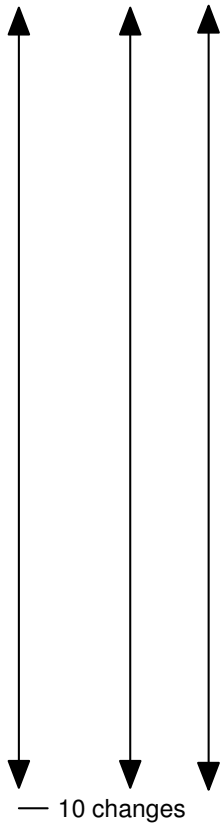


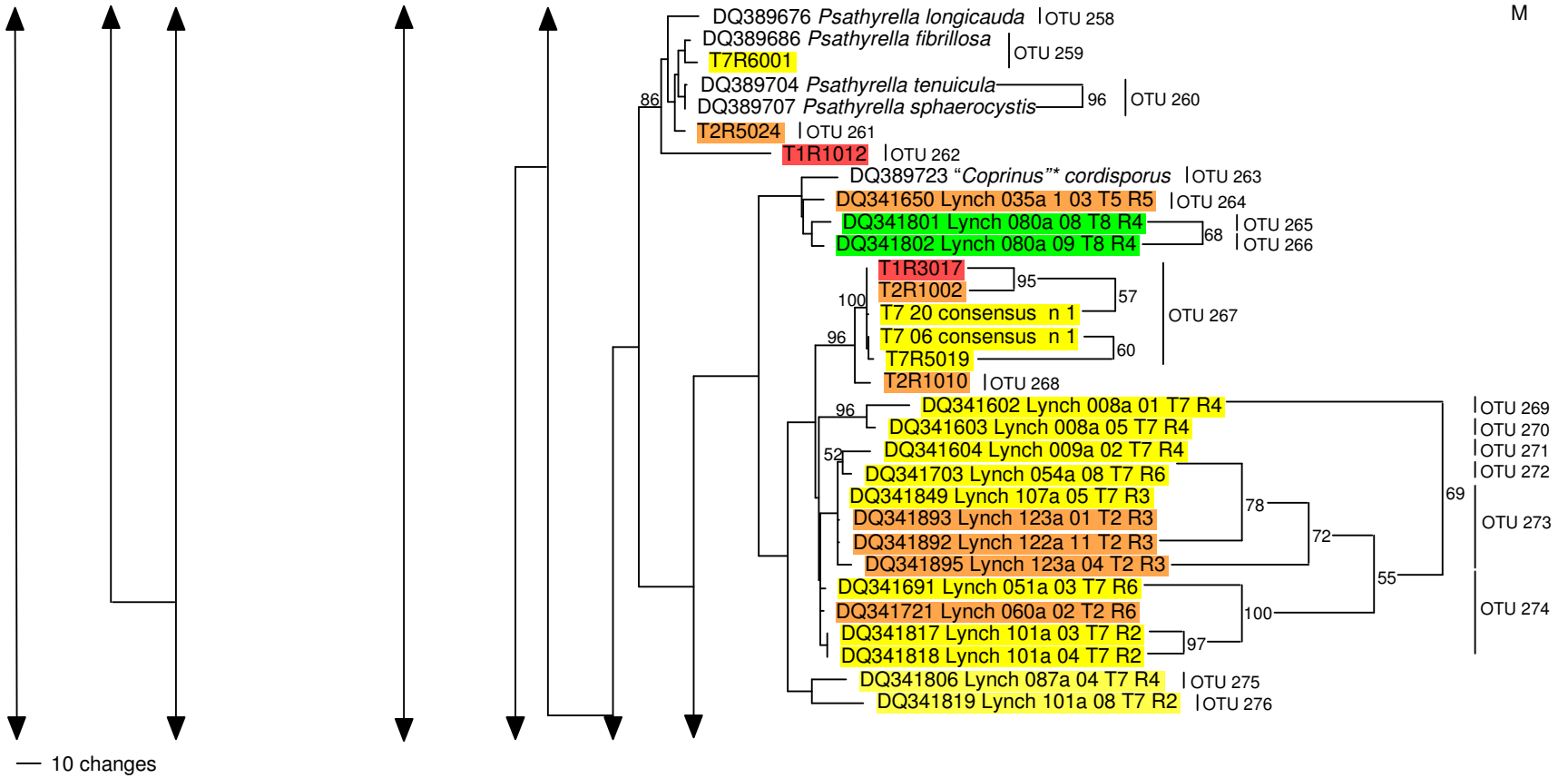


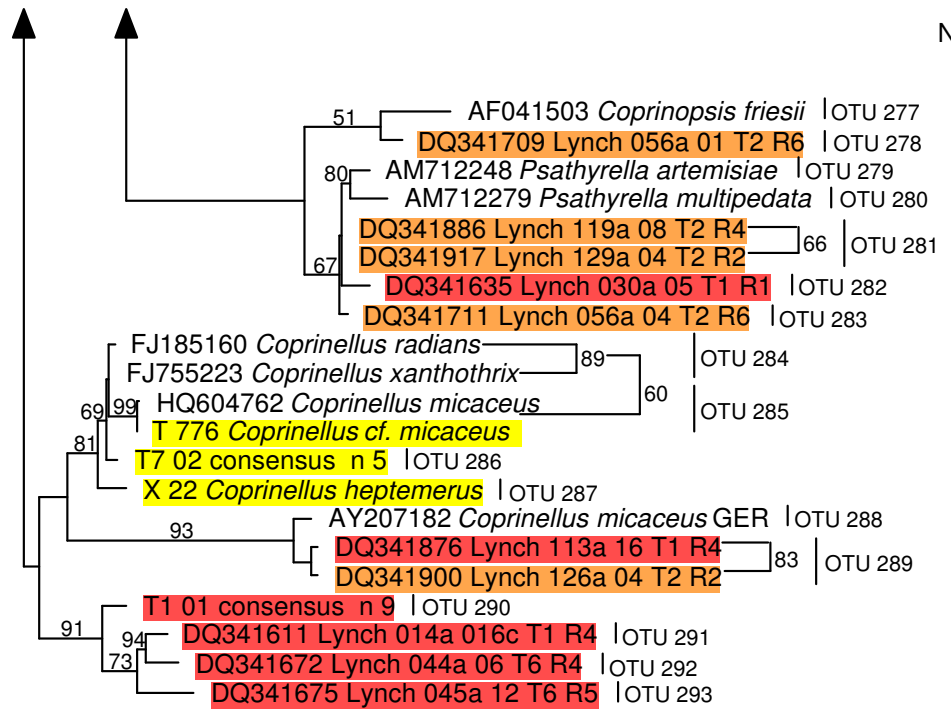
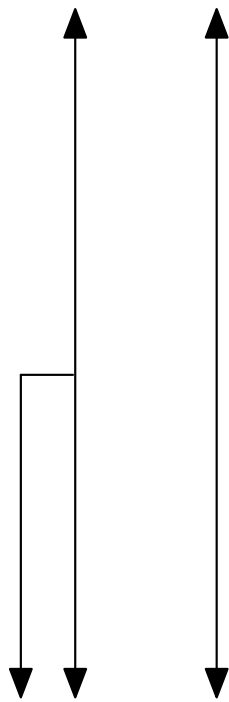
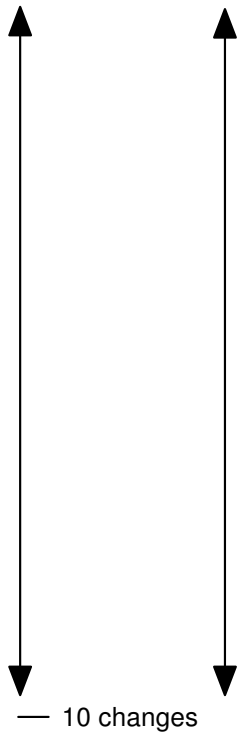
— 10 changes



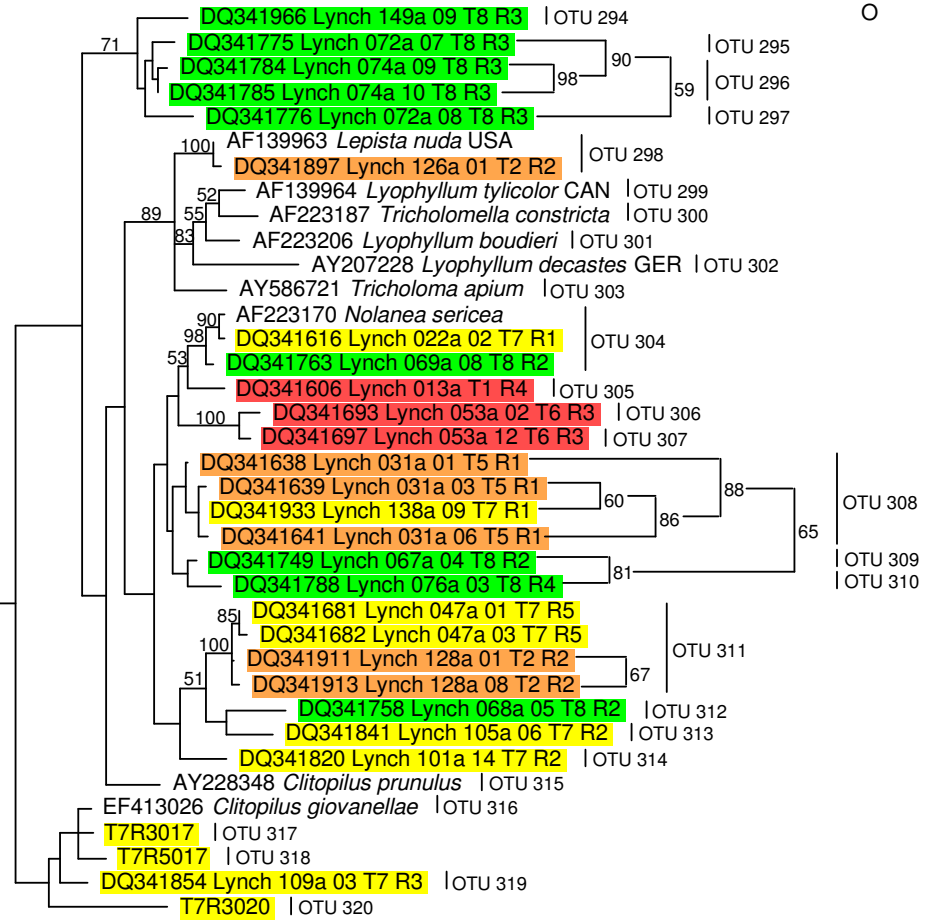
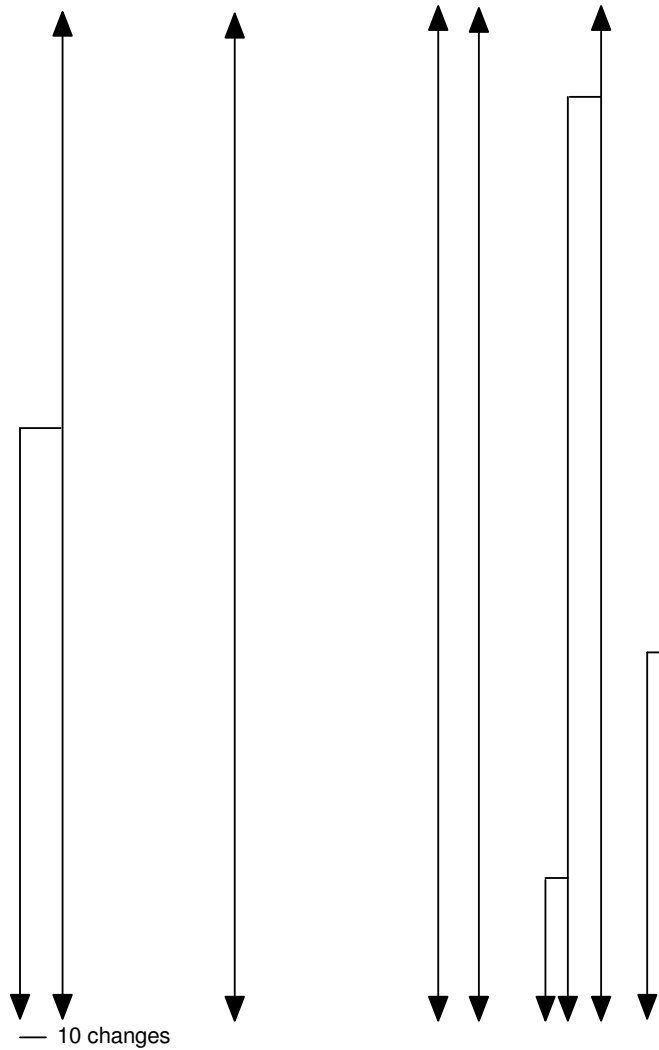


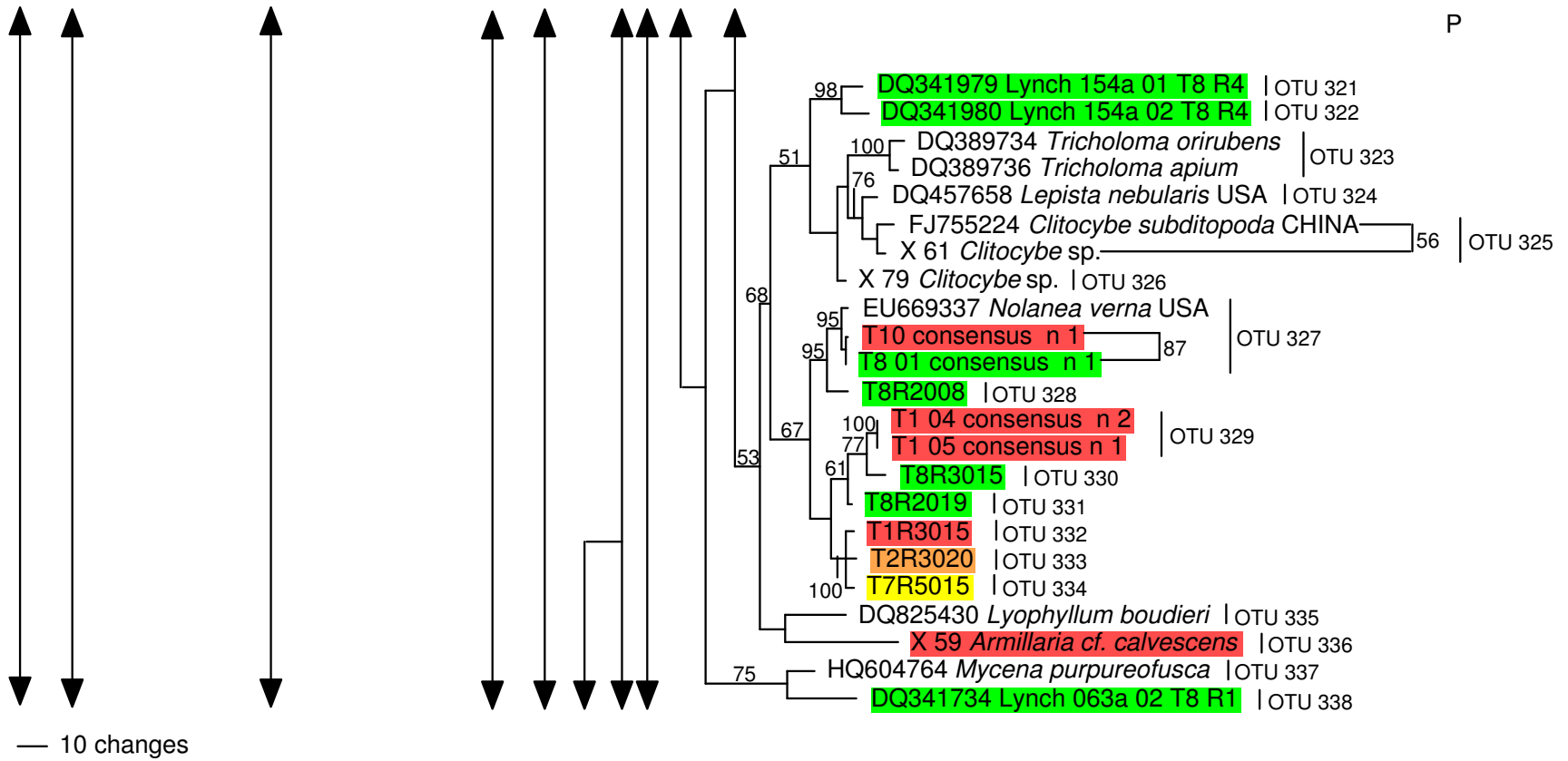




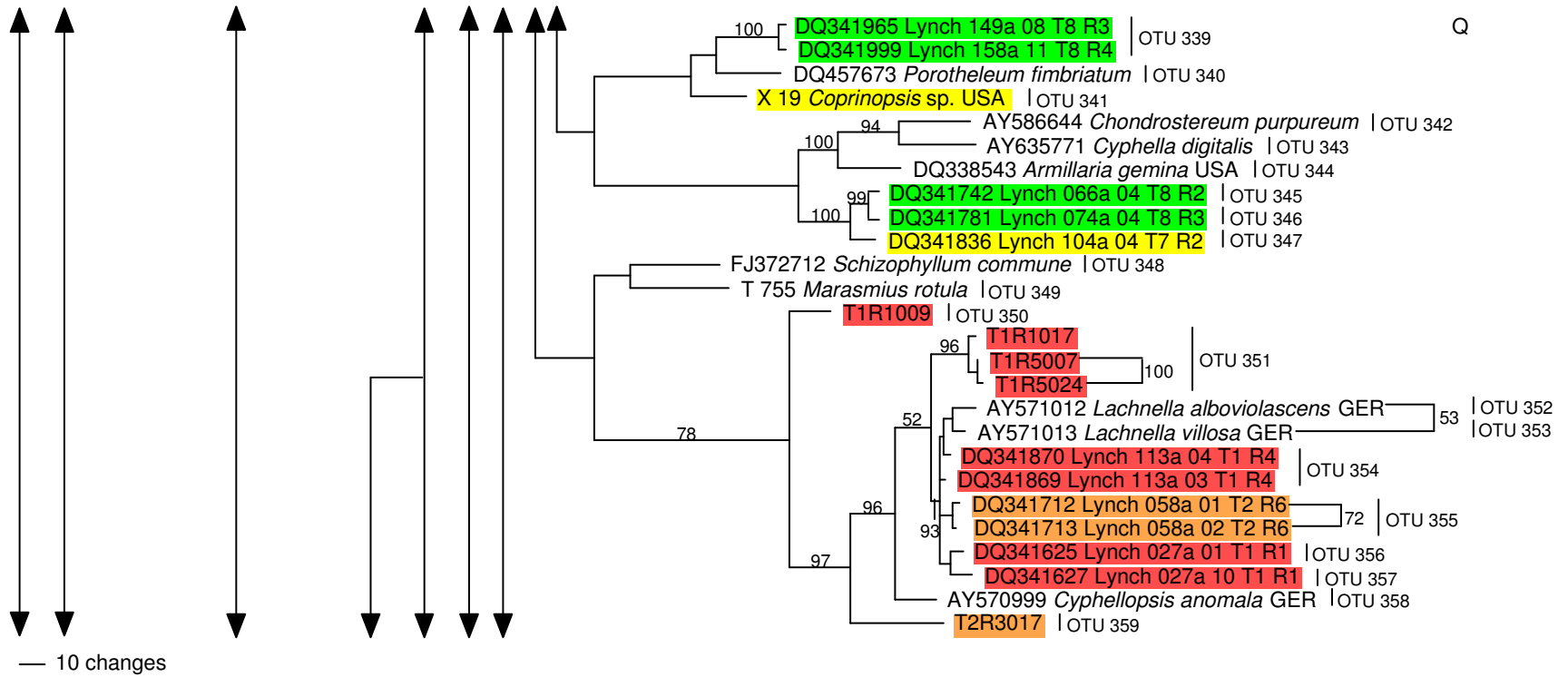


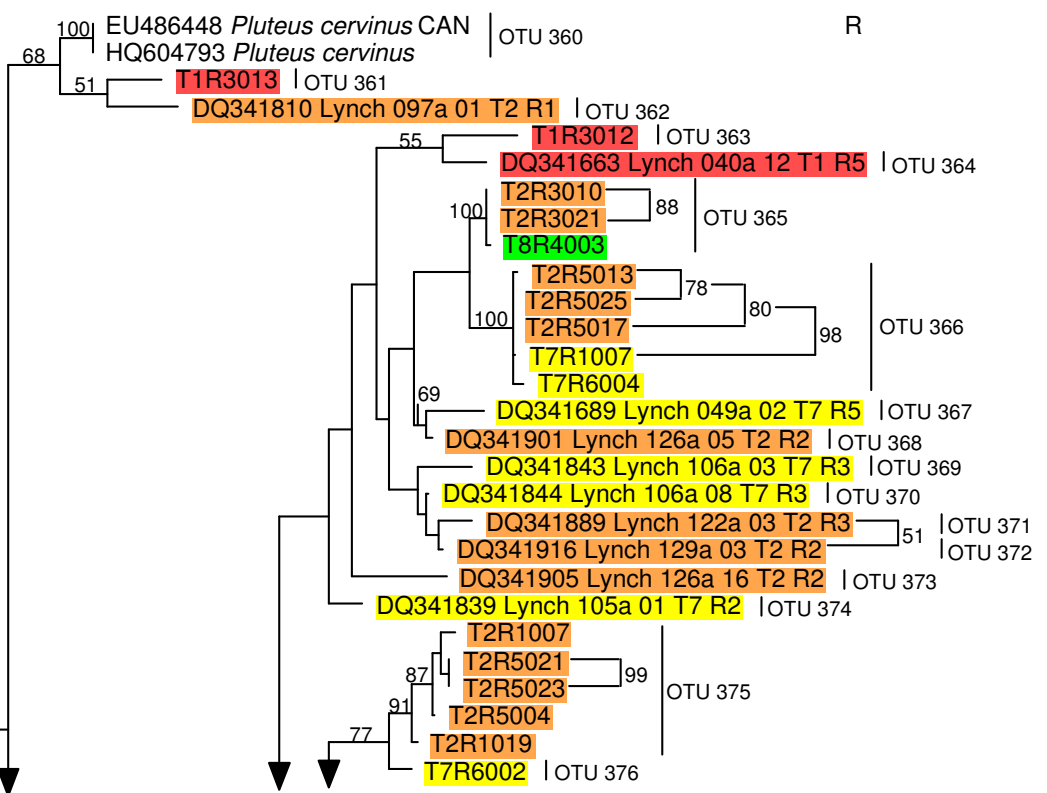
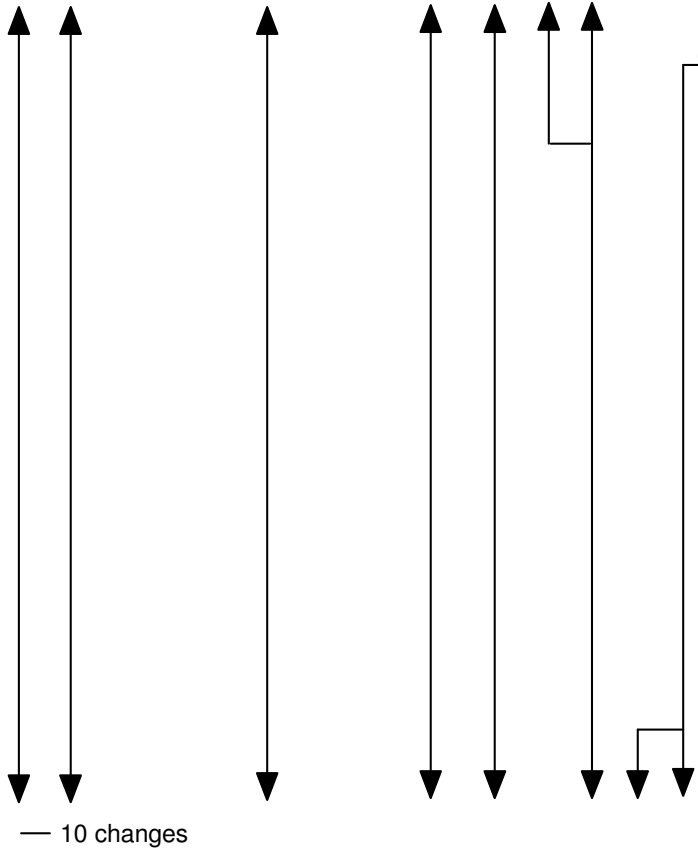
N

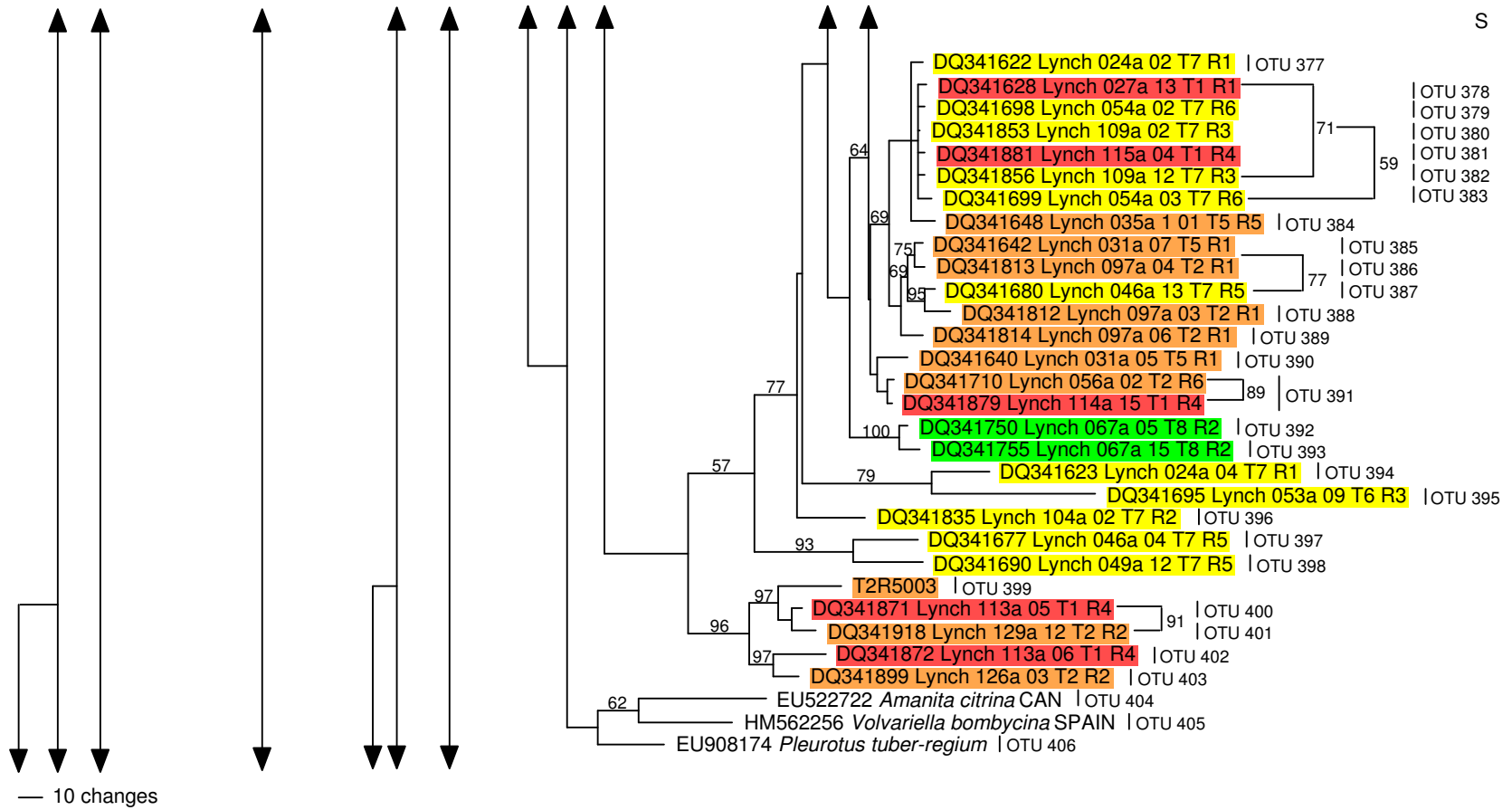




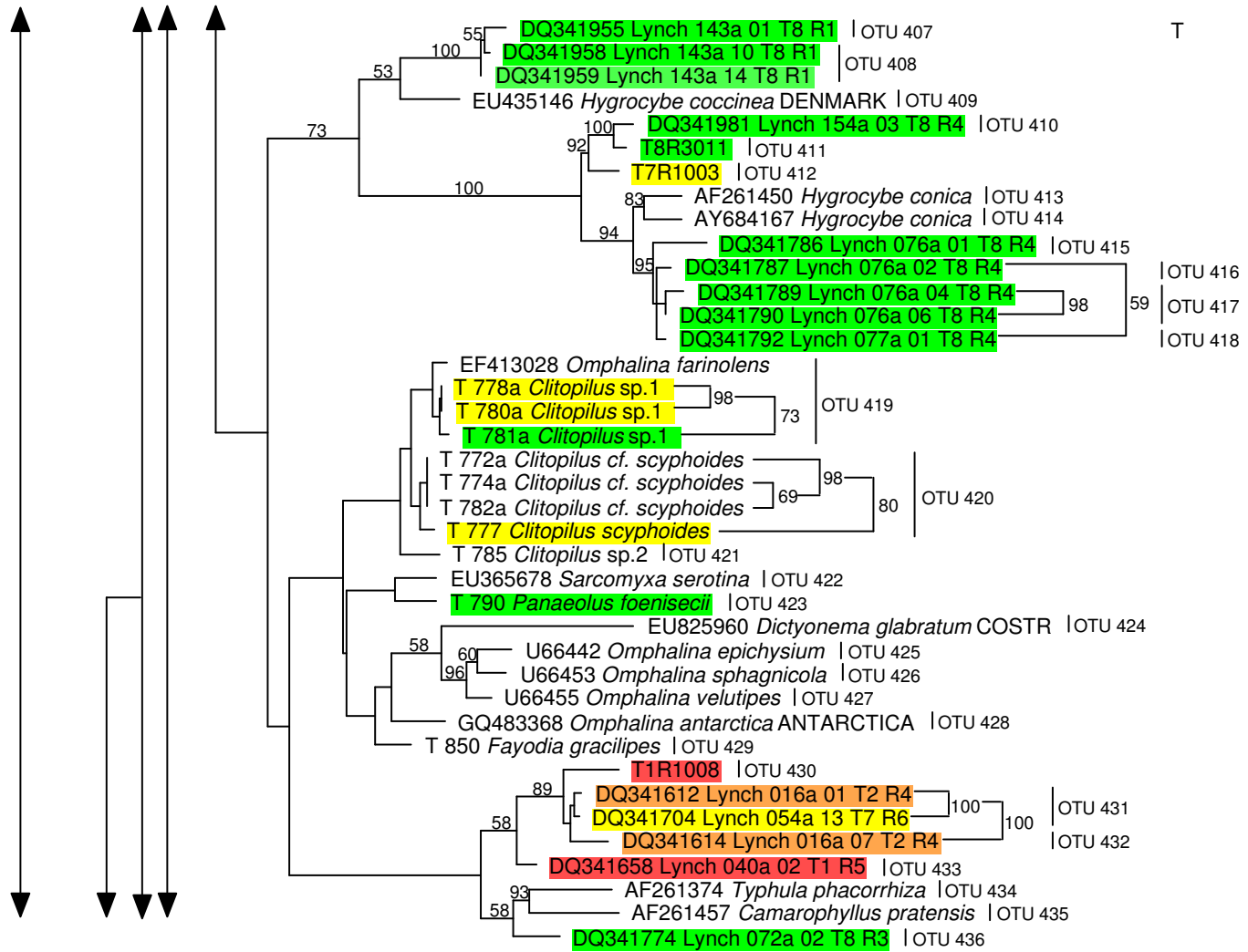
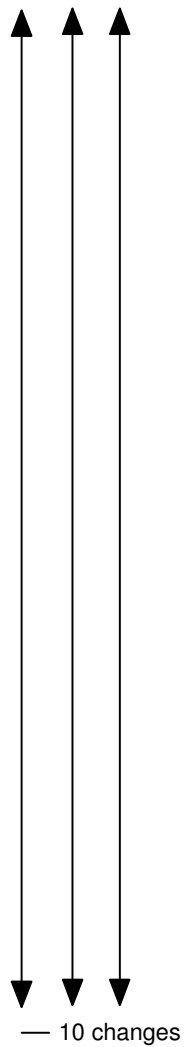
P

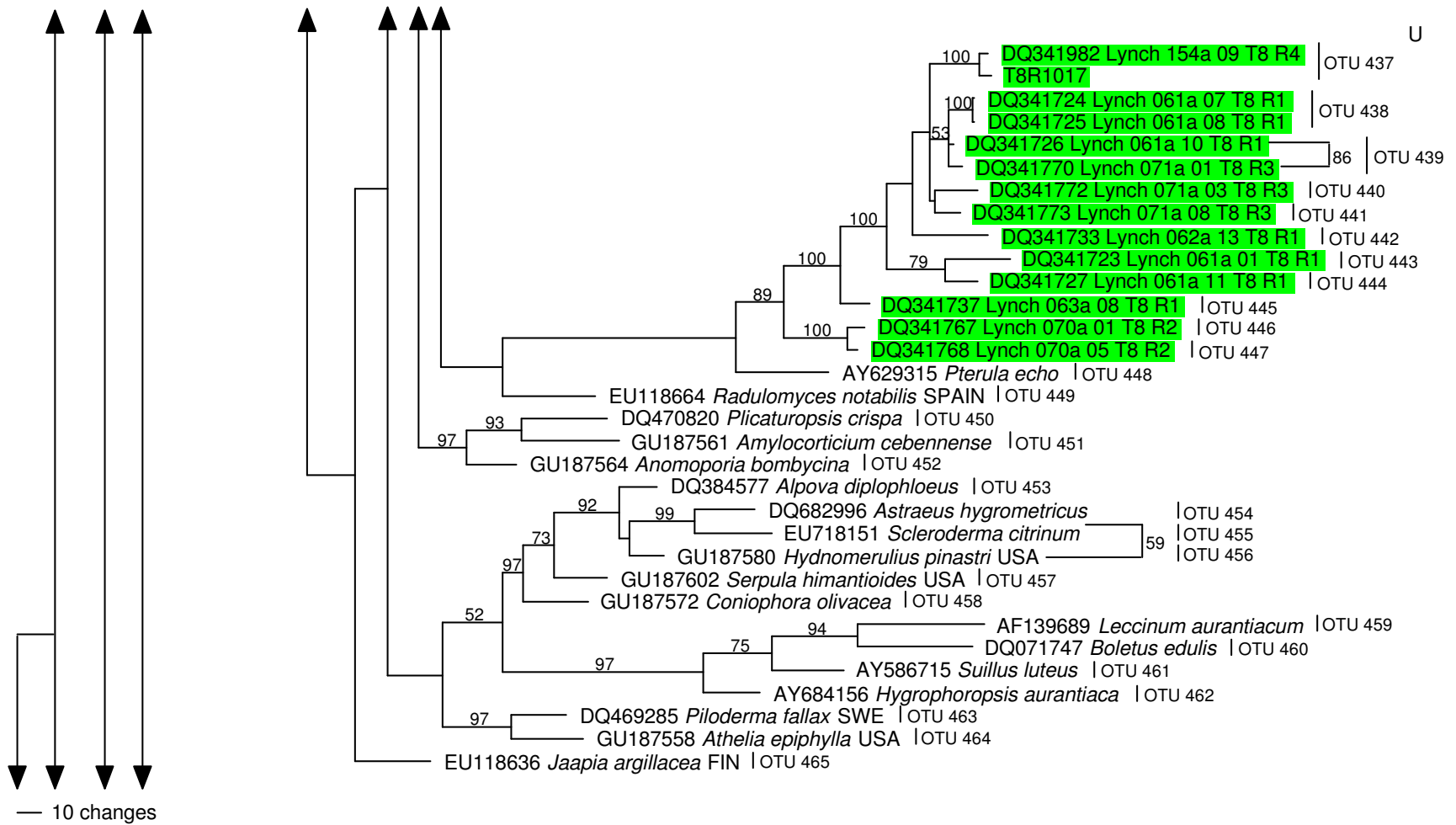


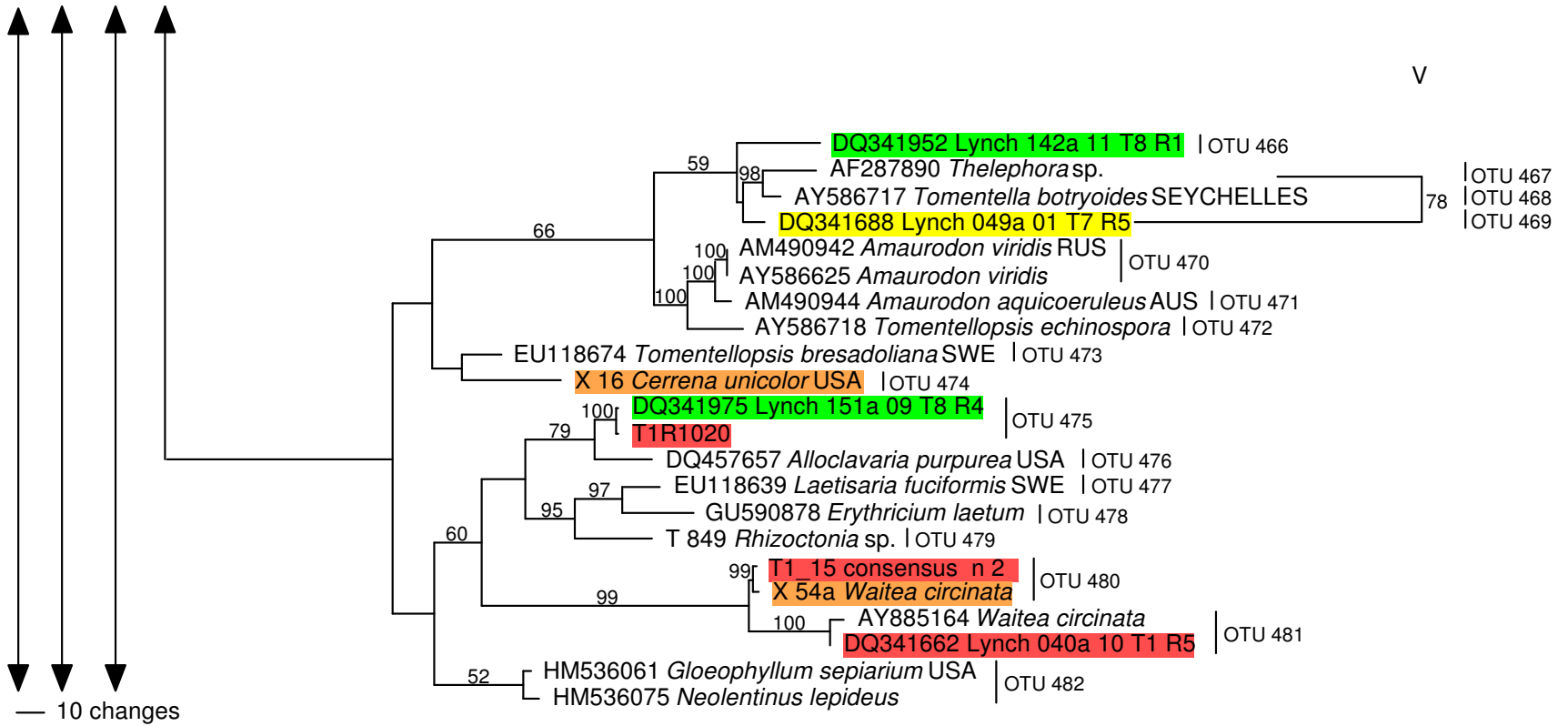


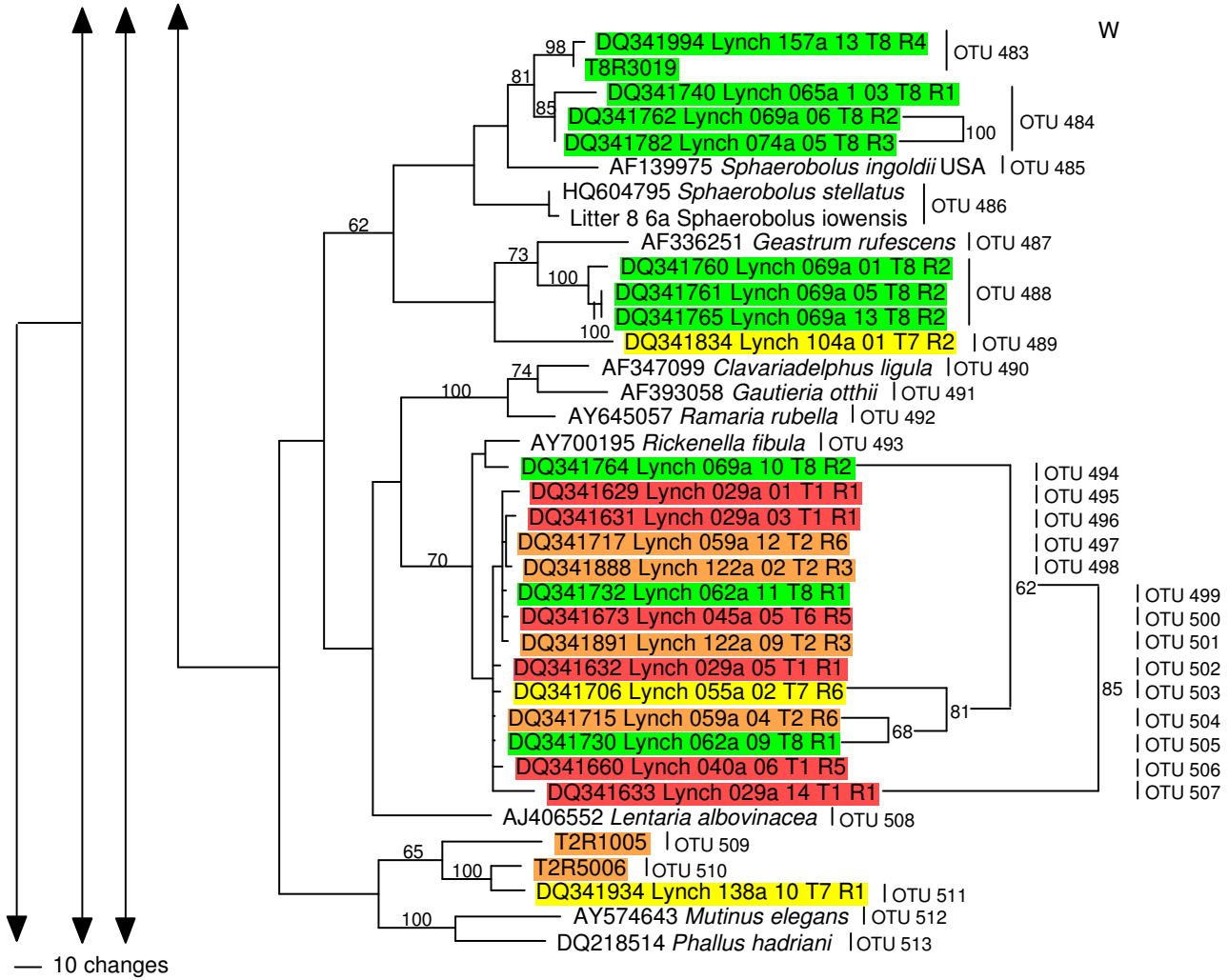


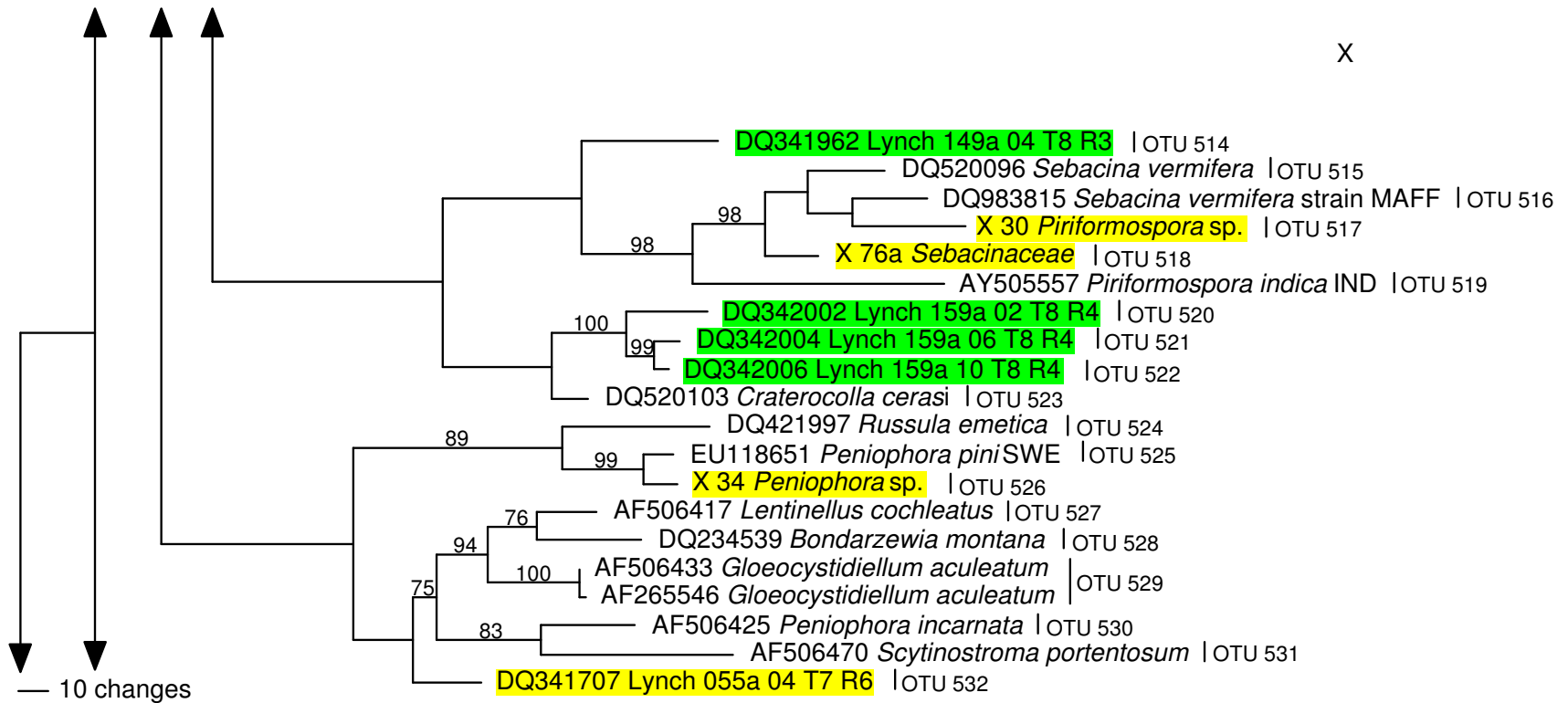


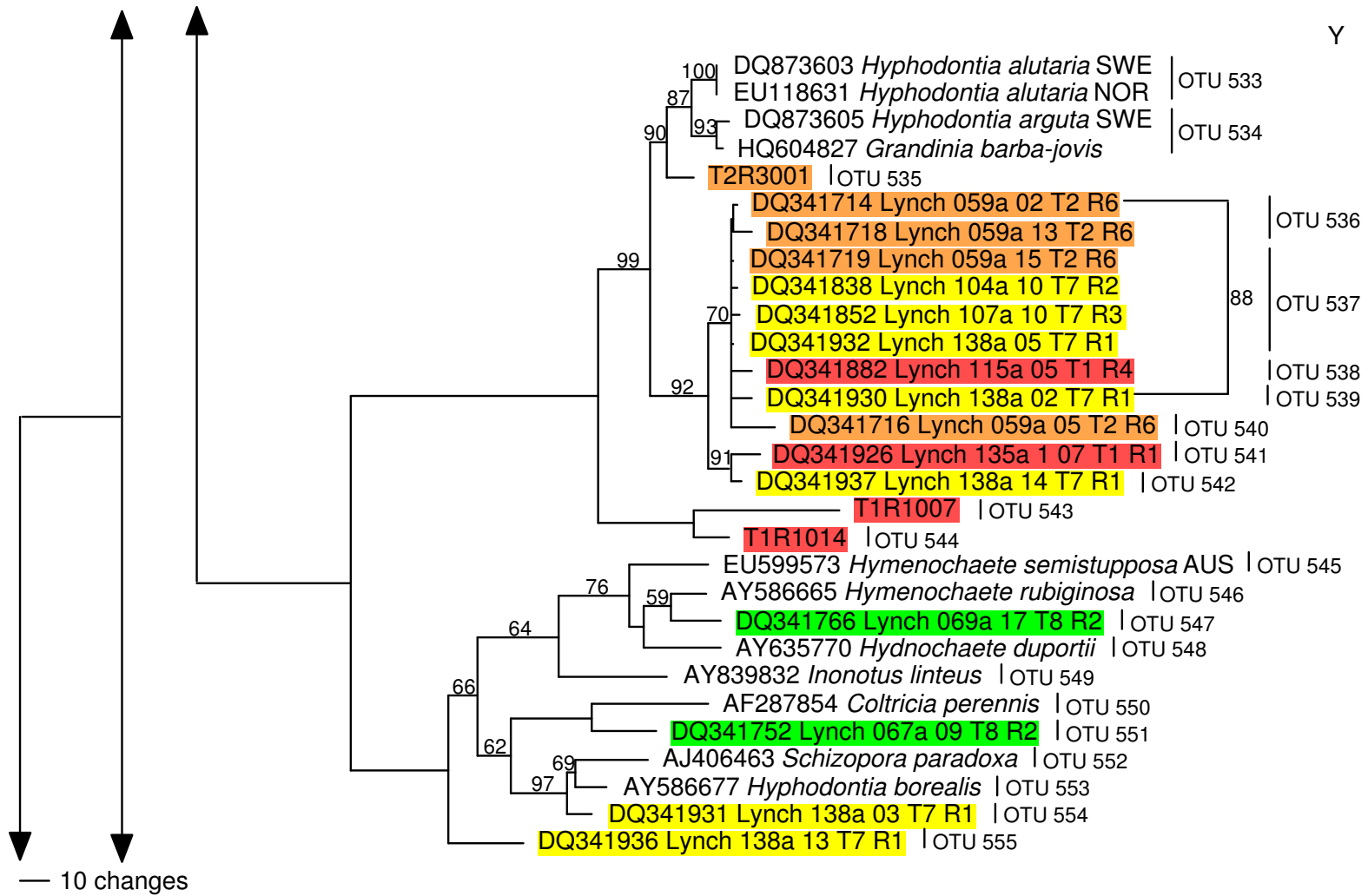


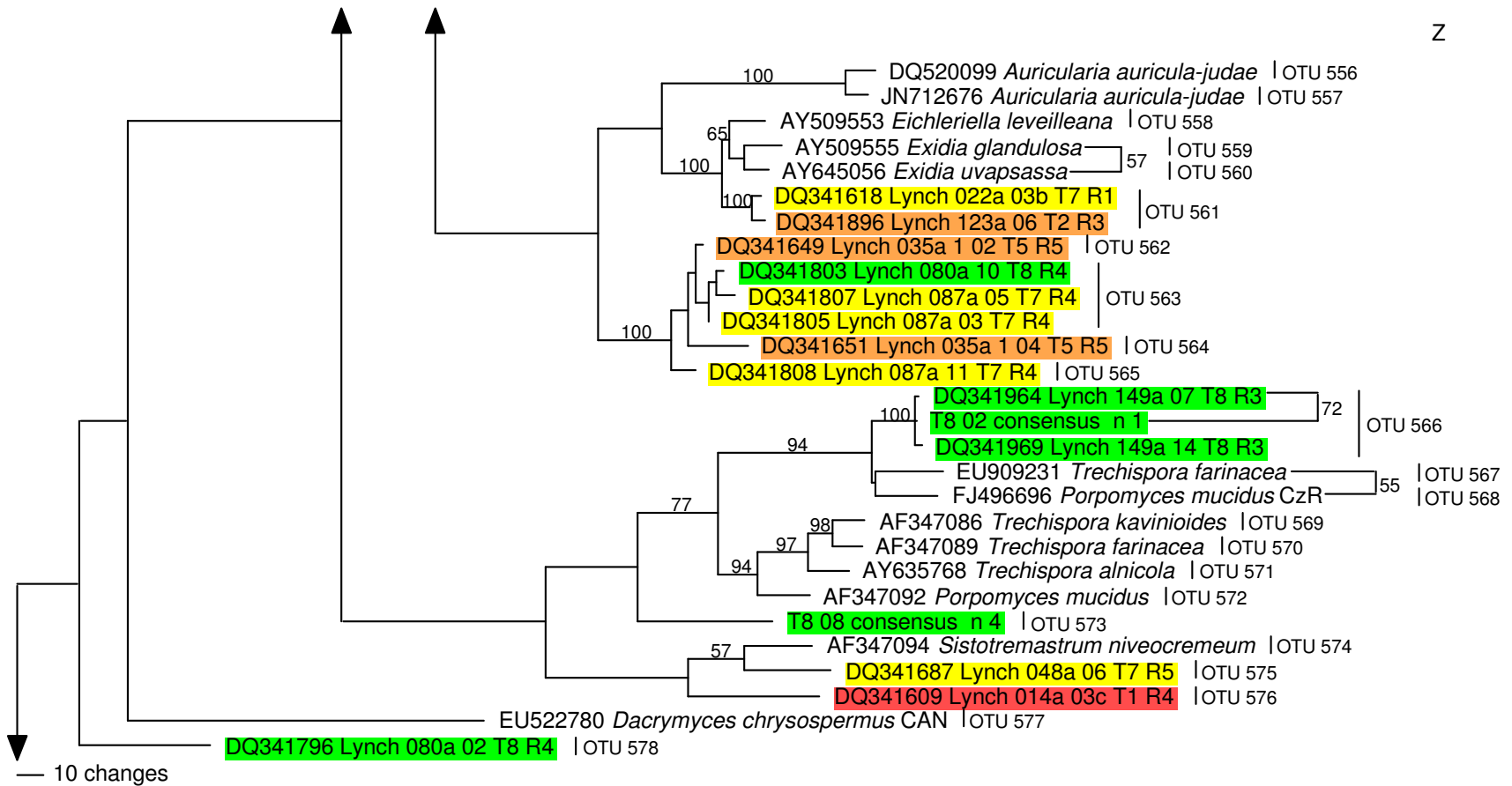












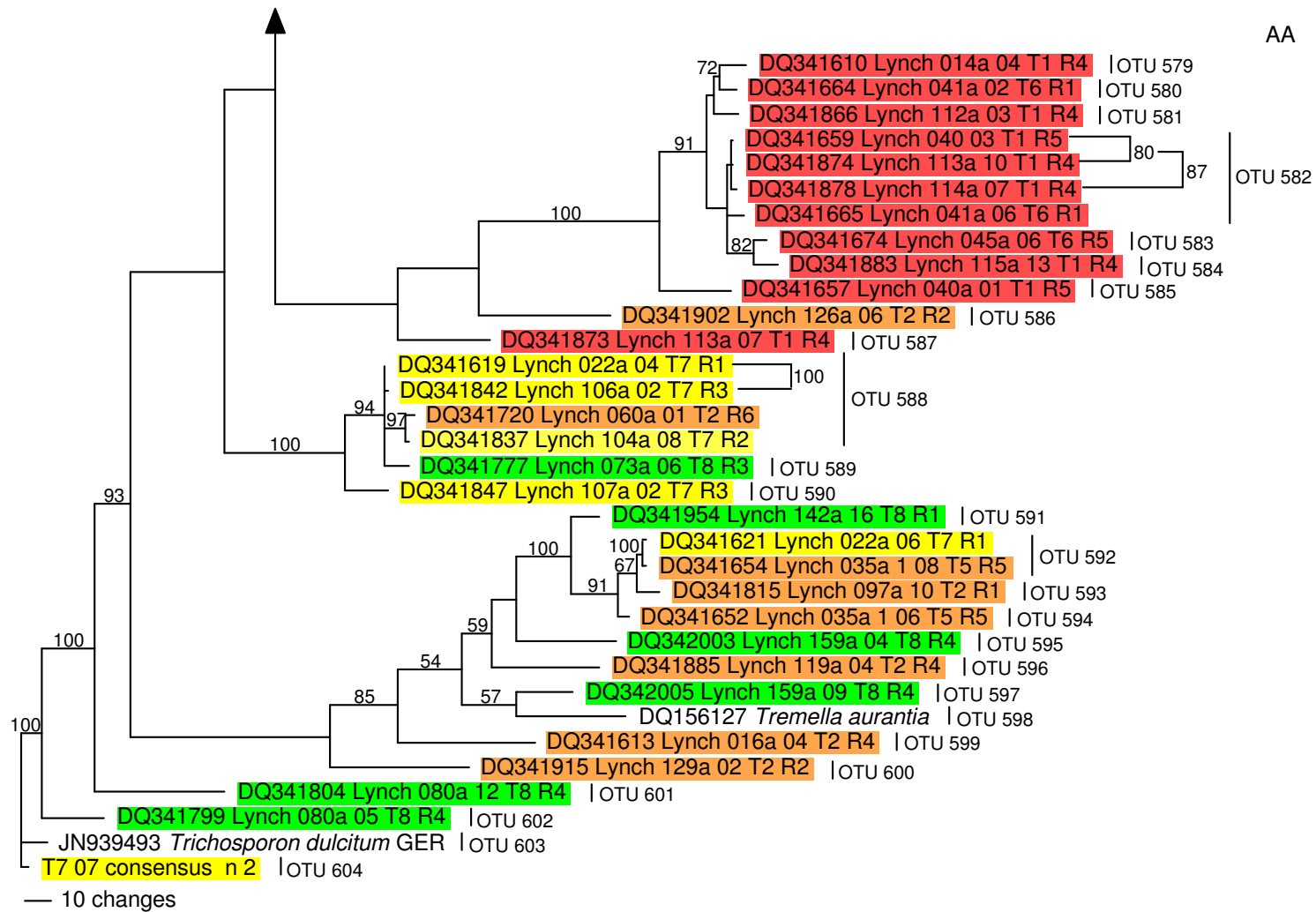




Table 3.1 Environmental distance P-Values for each environment tested individually

Environment	P-Value
CT T1R1	0.73
CT T1R2	1.0
CT T1R3	1.0
CT T1R4	1.0
CT T1R5	0.23
CT T1R6	0.27
NT T2R1	0.34
NT T2R2	0.02
NT T2R3	0.06
NT T2R4	1.0
NT T2R5	$\leq 0.01$
NT T2R6	0.24
HTS T7R1	0.13
HTS T7R2	0.49
HTS T7R3	0.42
HTS T7R4	$\leq 0.01$
HTS T7R5	$\leq 0.01$
HTS T7R6	0.29
NTS T8R1	$\leq 0.01$
NTS T8R2	$\leq 0.01$
NTS T8R3	$\leq 0.01$
NTS T8R4	$\leq 0.01$

When sequences from all environments were tested together to determine if the different environments in the tree were significantly different from each other, the UniFrac P-Value was reported as  $\ll 0.001$ , indicating highly significant differences among the different sites.

The P test (Martin 2002) significance value for all environments tested together was  $\ll 0.001$ , again indicating highly significant differences among the different sites.

### **3.3 Jackknife environment cluster**

When a statistical resampling technique called jackknife environment cluster was performed, all sites were strongly clustered together, >99.9% at the furthest node (Figure 3.2). However at the second furthest node, all environments were clustered together with 50-70% support, excluding NT T2R4 and NT T2R5. At the remaining nodes, environments were clustered together with <50% support.

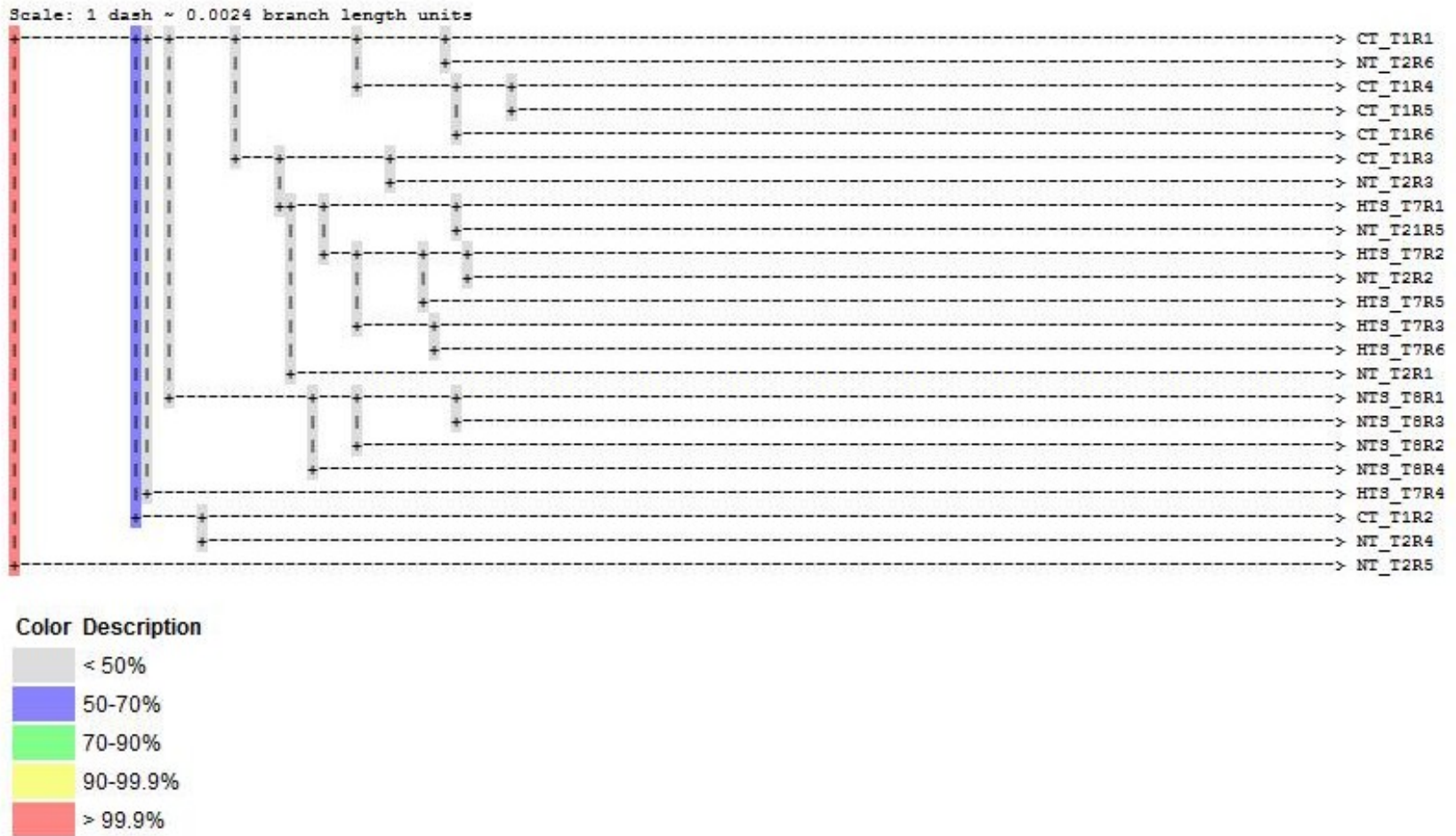
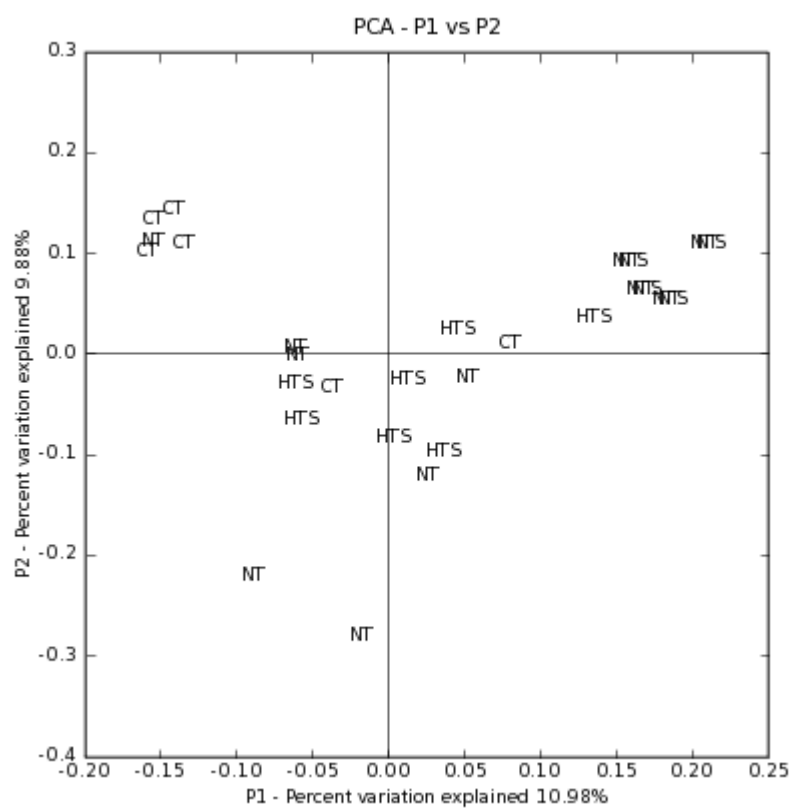


Figure 3.2 Jackknife environment cluster for all environments

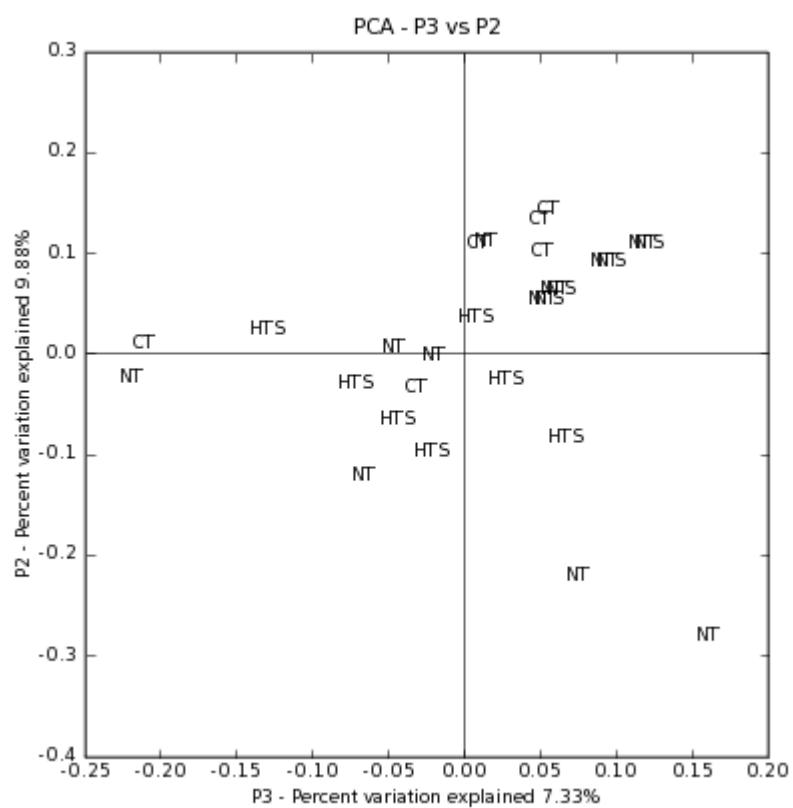
### **3.4 Principal coordinates analysis (PCoA)**

Principal coordinates analysis of the data distinguished NTS from the other sites, CT, NT, and HTS with NTS having the greatest distance from agricultural treatments, CT and NT (Figure 3.3A-C). NTS was furthermore distinguished as NTS sites were grouped closely together. CT was generally distinct from the rest of the sites as 4 of the 6 CT sample sites were grouped closely together and relatively away from the other sites, in the top left corner (Figure 3.3 A). Two NT sites near the bottom right corner were relatively distinct from the other sites as the distance between them and the other sites was comparatively greater (Figure 3.3 A). Finally, HTS sites are not in close proximity to one another, not being tightly clustered, and the juxtaposition of HTS sites with different treatments suggested that the lineages of Agaricomycetes found in HTS are not distinct from those of sites within CT, NT, and NTS. Principal components 1 (P1), 2 (P2), and 3 (P3) explain 10.98%, 9.88%, and 7.33% of the variation, respectively.

A)



B)



C)

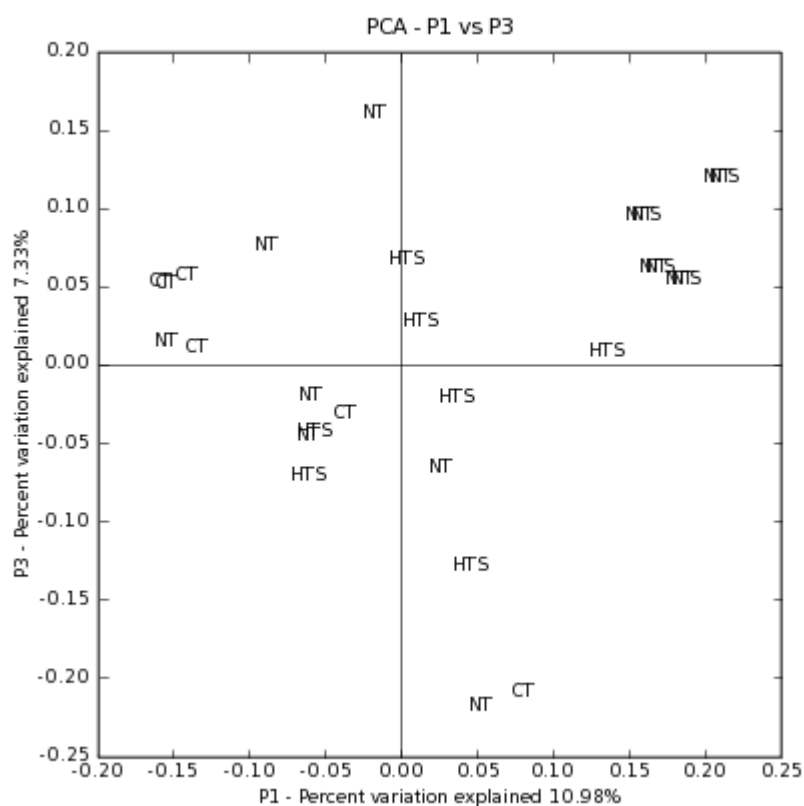


Figure 3.3 Principal coordinates analysis of KBS LTER sites based on UniFrac values from KBS and reference sequences. (A) PCoA for principal component 1 (P1) measured against principal component 2 (P2). (B) PCoA for principal component 3 (P3) measured against P2. (C) PCoA for P1 measured against P3.

### 3.5 Taxonomic representation in treatments studied

The recovered sequences from KBS LTER represented taxa from 13 of the 19 major clades of Agaricomycetes identified by Hibbett (2006). Table 3.2 provides a summary of the major clades and which treatments they were found in; more detailed description of the major clades can be found in Discussion. The majority of the sequences

were within the Agaricales clade, similar to findings of Bahnmann (2009). The Cantharellales, Hymenochaetales, Polyporales, and Agaricales were found across all treatments. The Auriculariales and Tulasnellales were found in NT, HTS, and NTS treatments but were absent from the CT treatment. The Sebaciniales, Geastrales, and Thelephorales were found in only HTS and NTS treatments. Trechisporales was found in CT, HTS, and NTS treatments and was absent in the NT treatment. Gomphoid/Phalloid was present in NT and HTS treatments. Corticiales was present in CT and NT treatments and Russulales was found only in the HTS treatment.

Table 3.2 Major clade distribution in CT, NT, HTS, and NTS treatments at KBS LTER

CLADE	Treatment found	Role in samples from KBS
Tulasnellales	NT, HTS, NTS	ECM, saprotroph, root pathogen of crops
Cantharellales	All	Saprotroph
Polyporales	All	Saprotroph
Agaricales	All	ECM, saprotroph
Thelephorales	HTS, NTS	ECM
Hymenochaetales	All	Saprotroph
Corticiales	CT, NT	Root pathogen of corn
Geastrales	HTS, NTS	Saprotroph
Gomphoid/Phalloid	NT, HTS	Saprotroph
Sebaciniales	HTS, NTS	ECM, saprotroph
Russulales	HTS	Saprotroph
Auriculariales	NT, HTS, NTS	Saprotroph
Trechisporales	CT, HTS, NTS	Saprotroph

As can be seen in Figure 3.1, similar OTUs are not strictly clustered together and separated by treatment alone. Though clusters in the tree where related OTUs may only be found in plots that are similar, like HTS and NTS, there are groups of OTUs that are shown to be related to taxa in completely different plots. This is not surprising as all the



major clades, except for Russulales found in only HTS, are detected in at least 2 different treatments, due to the relatively diverse nutritional roles of many of the major clades.

## CHAPTER 4: DISCUSSION

### 4.1 Broad phylogenetic placement

Broad phylogenetic placement by the Neighbour-Joining method produced a large tree that is not easily comparable to the results of previous studies from the same sites, simply due to the larger data set. Constraint of the backbone of the tree forced reference sequences to group in phylogenetically appropriate clades, whilst allowing unknown sequences to fall where appropriate. Bootstrap support for pairs or groups of sequences was very high (>95%) for over 150 pairs or groups. The topology of the tree for these groups is stable and nearly all the characters informative for those groupings are adequate to validate the topology (Berry and Gascuel 1996, Bremer 1988). High bootstrap values can be considered to be statistically significant and indicate uniform support for a clade (Felsenstein 1985, Berry and Gascuel 1996, Soltis and Soltis 2003).

Analysis of 816 sequences (including reference sequences) representing 604 OTUs (including reference sequences), found that the largest proportion of sequences (13.8% of all OTUs) fell into the minor clade Clavarioid of Agaricales. The Agaricoid clade of Agaricales accounted for the second greatest proportion of sequences (11.4% of all OTUs).

### 4.2 Tulasnellales (Figure 3.1. A)

The Tulasnellales often form mycorrhizal associations with terrestrial plants, including orchids and liverworts (Langer 1994, Kottke et al. 2003). Other members of this clade are root pathogens of crops or are saprotrophic and found in soil (Langer 1994, Kottke et al. 2003). This clade contains 21 (4.3%) of the OTUs found at the KBS LTER

sites. Bootstrap support for most inner nodes is >82% with the exception of one node with 62% support. The outer-most nodes of the Tulasnellales clade have 90% and 94% bootstrap support. Fourteen of 21 OTUs were from HTS plots, and the rest were from NTS and NT plots. The absence of OTUs from CT plots suggests that members of this clade may be particularly sensitive to soil disruption by tillage yet persist well in plots that are not tilled.

### 4.3 Cantharellales (Figure 3.1. B, C)

The Cantharellales contain fungi with a variety of morphologies as well as ecological roles: ectomycorrhizal, saprotrophic, and even pathogenic (Hibbett and Thorn 2001, Moncalvo et al. 2006). However, some fungi within this order form mutually beneficial associations with trees, shrubs, and other plants (Moncalvo et al. 2006). The Cantharellales are represented by 39 OTUs that are allied to *Minimedusa* and *Burgoa*. The species of *Minimedusa* that are allied with these OTUs are usually found on fresh leaves (Kuthubutheen and Muid 1984, Matsushima 1995, Peláez et al. 2001). However, most other *Minimedusa* sp. have been found growing over corticolous lichens and *Burgoa* is generally lichenicolous (Diederich and Lawrey 2007, Humphrey et al. 2002). These OTUs are widely distributed across the different treatments but most prominent (~40% of OTUs) in the CT plots and the least in NTS plots. It is likely that members of this clade detected at KBS are saprotrophic. The variety of substrates that members of this clade can utilize may contribute to their presence in all treatments sampled.

#### 4.4 Polyporales (Figure 3.1. D-F)

The Polyporales play ecologically important roles as wood-decayers, timber pathogens, and fungi that produce white-rot (Binder et al. 2005). However, there are no documented mycorrhizal species (Binder et al. 2005). This clade contains 23 OTUs, representing 3.8% of all OTUs from KBS LTER. Bootstrap support for inner nodes is varied in this clade, with support as low as 54% yet as high as 100%. The clade with the third most OTUs, the Polyporales, seem to be well sampled in all treatments, with roughly 12-15 recovered sequences in each treatment. High sampling frequency of the Polyporales in all four sites suggests that this clade is fairly persistent where there is appropriate substrate and furthermore it does not seem to be negatively affected by tillage. However, their presence in the HTS and NTS plots can be explained by their affinity to lignin-rich substrate, such as coarse-textured herbaceous plants like *Solidago*, dominant in non-agricultural sites at KBS.

#### 4.5 Agaricales (Figure 3.1. G-U)

The Agaricales is the largest clade of mushroom-forming fungi and includes more than half of all known species of the Agaricomycetes (Hibbett et al. 1997, Hibbett and Thorn 2001). Many of the fungi in Agaricales have fruiting bodies with stem and umbrella-like caps, but others are resupinate and jelly fungi (Hibbett 2006). Species in this major clade are primarily ectomycorrhizal or saprotrophic, causing white or brown-rot (Hibbett 2006). In addition, *Clavaria*, *Hygrocybe*, and *Camarophylloopsis* are indicators of grassland health (Newton et al. 2003). As previously mentioned, this major clade accounted for 330 (~70%) of all OTUs from KBS LTER. Bahnmann (2009) found

69% of all of her OTUs were Agaricales. By far, this is the most prominent clade in this study as well; it can be further subdivided into 9 minor clades: Clavariaceae, Stephanosporaceae, Agaricoid, Tricholomoid, *Schizophyllum-Lachnella*, Marasmioid, Pluteoid, Hygrophoroid, and Pterulaceae.

#### **4.5.1 Clavarioid (Figure 3.1. G-J)**

The Clavariaceae or Clavarioid clade accounted for 65 OTUs or 19.7% of Agaricales. Most (>94%) of the OTUs in this minor clade were found in HTS and NTS sites, with the exception of 3 OTUs from two NT plots. Bootstrap support for the OTUs in this clade is varied, with some inner nodes weakly supported at 51% whereas some inner nodes are strongly supported at 100%. Members of this clade are saprotrophic and terrestrial in soil or among leaf litter in grasslands or in hardwood forests (Breitenbach and Kränzlin 1986, Matheny et al. 2006) and some are mycorrhizal associates of Ericaceae (Petersen and Litten 1989). Some species are also grassland fungi which may explain an ecological role that they may play in HTS; as with species of *Hygrocybe*, they have been suggested as indicators of grassland health (Keizer 1993, McHugh et al. 2001, Newton et al. 2003). Also, Clavarioids may simply be more sensitive to soil disturbance and may establish and persist best in plots with suitable substrate at the soil surface. This clade may act as a particularly good indicator of non-agricultural sites.

#### **4.5.2 Stephanosporaceae (Figure 3.1. J)**

This minor clade contains only 4 OTUs, of which 2 are reference sequences and 2 were from a single HTS plot. The absence of this clade from NTS, NT, and CT plots and

low numbers of OTUs recovered may be due to inefficient sampling due to the rarity of this clade in KBS LTER sites.

#### **4.5.3 Agaricoid clade (Figure 3.1. K-N)**

Members of this clade are diverse - some are ectomycorrhizal (*Cortinarius* and *Hebeloma*) and others are saprotrophic; some taxa produce the hallucinogenic compound psilocybin (*Inocybe* and *Psilocybe*) and some members of this clade, namely the Agaricaceae, have a symbiotic relationship with attine or fungus gardening ants (Matheny et al. 2006, Vellinga 2004, Watling and Gregory 1987, Chapela et al. 1994, Mueller et al. 1998). The Agaricoid clade accounts for 54 recovered OTUs, which are represented almost equally in CT, NT, HTS, and NTS. Bootstrap support for this clade is varied with inner nodes ranging from 51% to 100% support. Like some of the previously mentioned clades, the Agaricoid presence in all 4 treatments indicates that members of this clade have diverse ecologies and can perhaps colonize a wide variety of substrates and may also be quite resistant to soil disturbance.

#### **4.5.4 Tricholomoid clade (Figure 3.1. O, P, T)**

This clade is represented by 32 OTUs, including species found in the minor clade, Entolomatoid. Bootstrap support for this clade is varied, showing as weak support as 52% to as strong support as 100%. OTUs of sequences recovered from KBS soils were found in all treatments. This could indicate that this clade is fairly persistent in soils subject to different degrees of disturbance by tillage, from no disturbance to high disturbance. Furthermore, the members of this clade may not require substrate at the soil

surface to persist, as the Tricholomoids can be found in CT plots where crop residue would be tilled into the soil. Members of this clade are typically saprotrophic on litter and in soil in grassland ecosystems and have a broad distribution, which can help explain their distribution across all four treatments at KBS (Noordeloos 1988). Five OTUs were closely allied to *Nolanea*, a member of the Entolomatoid subclade which is often used as an indicator of undisturbed grassland habitats (Newton et al. 2003).

#### **4.5.5 *Schizophyllum-Lachnella* clade (Figure 3.1. Q)**

This minor clade is represented by 8 OTUs from KBS LTER. Bootstrap support is moderate for one group (72%), while the rest of the groups are strongly supported at >93%. OTUs from this clade were from NTS, NT, and CT plots. OTUs grouping around reference sequences of *Lachnella* are mainly from NT and CT plots, which is surprising as *Lachnella* are generally saprotrophic on wood (Unterseher et al. 2005). However, *Lachnella* may also be saprotrophic on herbaceous stems and Lynch (2004) considered it is likely that species here were on stems of corn, soybean or wheat.

#### **4.5.6 Marasmioid clade (Figure 3.1. Q)**

The Marasmioid clade has 4 OTUs recovered from KBS soils and 4 OTUs that are reference sequences. One OTU grouping in this clade has moderate bootstrap support (75%) whereas the other OTU groupings have >99% support. Recovered sequences that are represented as OTUs were found mainly in the NTS plots with one OTU being found in the HTS plot, indicating that this clade may be associated with aboveground successional communities and is not persistent in soils that are regularly disturbed by

tillage. OTUs were allied closely with *Mycena purpureofusca*, a white-rot fungus and *Armillaria gemina*, a root-inhabiting fungus (Sun et al. 2012, Bérubé and Dessureault 1989).

#### **4.5.7 Pluteoid clade (Figure 3.1. R, S)**

The Pluteoid clade consists of 43 OTUs, allied with *Pluteus* sp., *Volvariella*, *Amanita*, and *Pleurotus*, with varied bootstrap support for inner nodes, from 51%-100%. Most of the taxa in this clade are decomposers except for *Amanita* which is ectomycorrhizal (Matheny et al. 2006). Members of *Pleurotus* can even attack nematodes (Thorn et al. 2000). Most of the OTUs were recovered from HTS and NT plots. Only 5.4% of the OTUs in this group were from NTS while 20.9% of the OTUs from this clade were from CT plots. While most of the OTUs were from two very different treatments, HTS and NT, this clade's presence in all plots indicates that it is fairly persistent and resistant to different types of agricultural disturbance, whether that disturbance may be soil disturbance by tillage or aboveground plant community disturbance by mowing. This is the clade referred to as "Sister clade to *Volvariella*" by Lynch (2004) and Bahnmann (2009). The members are now resolved as part of the Pluteoid clade but still do not have named reference sequences in GenBank.

#### **4.5.8 Hygrophoroid clade (Figure 3.1. T)**

This minor clade has 14 OTUs from KBS LTER. All but one of the 9 OTUs that clustered with *Hygrocybe* were from NTS, and the other was from HTS. The five other OTUs in this clade from KBS LTER clustered around *Typhula* and *Camaprophyllus* and



were found in HTS, NT, and CT plots. The proportion of Hygrophoroids found in NTS plots suggests that this clade typically does best in plots where there is no tillage and a diverse assemblage of plants. Indeed, species in the Hygrophoroid clade have been thought to be mainly terrestrial litter decomposers and *Hygrocybe* sp. are classically associated with high diversity grasslands (Tanesaka et al. 1993, McHugh et al. 2001). However, recent evidence from stable isotope analyses suggests they may be associated with bryophytes or algae and it is unlikely that they are decomposers (Seitzman et al. 2011).

#### **4.5.9 Pterulaceae clade (Figure 3.1. U)**

This minor clade contains 11 OTUs, only one of which is a reference sequence, *Pterula echo*, a wood-inhabiting fungus (Munkacsi et al. 2004). Unfortunately, there is no bootstrap support for the monophyly of the OTUs from KBS LTER with the reference sequence *Pterula echo*. The 10 OTUs in this clade were found in each of NTS plots but in no other treatments, indicating that this clade may be well-associated with established aboveground plant communities not subject to disturbance by tillage.

#### **4.6 Thelephorales (Figure 3.1. V)**

The Thelephorales is the sister group to Polyporales, which is surprising as all species in the Thelephorales are mycorrhizal whereas the species found within the Polyporales are all saprotrophic (Hibbett 2006). The Thelephorales clade is represented by only 3 OTUs recovered from KBS soils. These OTUs are linked most closely with *Thelephora* sp. and *Tomentella botryoides* with low bootstrap support and were recovered

from HTS and NTS soils. Detection by Lynch (2004) and not by Bahnmann (2009) may suggest that these rare OTUs from Thelephorales may be detected only with a larger sampling effort.

#### **4.7 Hymenochaetales (Figure 3.1. V, W, Y)**

Species in this clade are mainly saprotrophic (Larsson et al. 2006). The majority of these species are primary decomposers and cause white-rot (Larsson et al. 2006). The Hymenochaetales also contains species that exhibit many different life strategies; some colonize living trees, blurring the distinction between saprotrophic and parasitic strategies (Larsson et al. 2006). Some, including *Rickenella* (OTUs 493-507) are capable of fruiting on or in association with Bryophytes (Larsson et al. 2006). *Coltricia perennis* forms an ectomycorrhizal association with *Pinus banksiana* (jack pine) (Larsson et al. 2006). One of the most interesting groups in the Hymenochaetales is *Hyphoderma*, which has nematode-capturing abilities (Tzean and Liou 1993). Specialized cells on the hyphae called stephanocysts and echinocysts are covered by an adhesive mucilage and attach easily to the nematode cuticle; captured nematodes are killed and the bodies penetrated by hyphae (Tzean and Liou 1993).

The Hymenochaetales is represented by 29 OTUs, accounting for 5% of OTUs. Many of the sequences have grouped with different reference sequences representing different genera of Hymenochaetales, indicating the variety of Hymenochaetales found in KBS soils. Hymenochaetales is found equally in CT, NT, and HTS, but less in NTS. This could mean that the clade Hymenchaetales is resistant to disturbance or may even persist because of disturbance. Furthermore, the variety of substrates and life strategies

exhibited by species in this clade may have allowed for a higher sampling frequency than other clades. Indeed, suitable saprobic substrates as well as bryophytes were available in all sites (Lynch 2004).

#### **4.8 Corticiales (Figure 3.1. V)**

Species in this order have diverse nutritional roles but most have resupinate fruiting bodies (Lawrey et al. 2008). Some nutritional ecologies include mutualistic and pathogenic forms (*Waitea* and *Laetisaria*) as well as lignicolous saprobes (Diederich et al. 2003, Binder et al. 2005, DePriest et al. 2005, Lawrey et al. 2007, Stalpers and Loerakker 1982). Internal nodes show >95% bootstrap support. Corticiales is represented by only 2 OTUs recovered from CT and NT that are allied with *Waitea*, root pathogens of many different plants including corn, rice, and turfgrass (Leiner and Carling 1994).

#### **4.9 Geastrales (Figure 3.1. W)**

Commonly known as “earthstars”, this order is named after the star-like fruiting bodies (Hosaka et al. 2006). Members of this clade are typically saprotrophic, on rotting wood or soil, and are common in horticultural gardens with wood-chip mulch (Flegler 1984, Sunhede 1989, Pegler et al. 1995, Geml et al. 2005). This clade contains only 4 OTUs from KBS LTER, 3 from NTS plots, and 1 from HTS. This could indicate that this particular clade is sensitive to soil disturbance by tillage or that its presence is facilitated by a diverse assemblage of aboveground plant communities, as are present in the NTS and HTS plots. Mainly, Geastrales may be present in NTS and HTS plots as there may be more suitable substrate available for decomposition, compared to CT or NT

plots.

#### **4.10 Gomphoid/Phalloid (Figure 3.1. W)**

The Gomphoid/Phalloid clade contains of a group of fungi that is both morphologically and ecologically diverse (Hosaka et al. 2006). Fruiting body morphologies include, stink-horns, coral fungi, club fungi, gilled mushrooms, resupinate fungi, and false truffles (Hosaka et al. 2006). Both ectomycorrhizal and saprobic taxa are represented by this clade (Hosaka et al. 2006). One of the less represented clades in the phylogeny, the Gomphoid/Phalloid clade accounts for only 3 OTUs from NT and HTS plots. Poor representation of this clade may also be due to undersampling or the preference of these fungi for richer or less disturbed sites (Pegler et al. 1995). Phalloids, represented by OTUs 509-511 are allied with *Mutinus elegans*, usually found on rotting wood and *Phallus hadriani*, often found in sandy soils (Pegler et al. 1995).

#### **4.11 Sebacinales (Figure 3.1. X)**

This clade is made up of fungi that are mainly terrestrial and form mycorrhizal associations with plants (Weiss et al. 2004). Mycorrhizal taxa of *Sebacinaceae* include mycobionts of ectomycorrhizas, orchid mycorrhizas, ericoid mycorrhizas, and jungermannioid mycorrhizas (Weiss et al. 2004, Duckett et al. 2006). Sebacinales is divided into two distinct clades, A and B, which differ in their ecology (Weiss et al. 2004). Clade A represents Sebacinales that form mycorrhizal associations with the achlorophyllous orchids, *Neottia nidus-avis* and *Hexalectris spicata*, and other related photosynthetic orchids (Julou et al. 2005, McKendrick et al. 2002, Selosse et al. 2002,

Selosse et al. 2004, Taylor et al. 2003). At the same time, many of the Sebacinoids in clade A form ectomycorrhizal associations with tree and plant roots (Selosse et al. 2002, Urban et al. 2003, Walker and Parrent 2004, Moyersoen 2006). Sebacinoids in clade B have a wider array of associations, some even associating with liverwort thalli (Kottke et al. 2003). Clade B contains the important, nonspecific root endophyte, *Piriformospora indica* (Verma et al. 1998). Sebacinoidales have been widely shown to form mycorrhizal associations with the Ericaceae (Weiss et al. 2004). The Sebacinoidales clade was represented by 9 separate OTUs with >98% bootstrap support. OTU 518 has 98% bootstrap support with *Piriformospora indica*, a non-specific root endophyte, and related species (Verma et al. 1998). The 3 remaining unknown KBS sequences share 100% bootstrap support with *Craterocolla cerasi*, which is saprotrophic on dead wood (Breitenbach and Kränzlin 1986). OTUs from this clade are found only in HTS and NTS plots, so although *Piriformospora* can form associations with crop plants such as wheat and maize (Varma et al. 1999), it was not found in agricultural plots in this study.

#### **4.12 Russulales (Figure 3.1. X)**

The Russulales are morphologically diverse, containing a variety of fruiting body forms including resupinate, discoid, effused-reflexed, clavarioid, pileate, and gasteroid (Miller et al. 2006). Some species in this clade are saprotrophic, causing white-rot and some act as timber pathogens (Hibbett 2006, Miller et al. 2006). However there are some species in this clade that are ectomycorrhizal, root parasites, and even insect symbionts (Miller et al. 2006). This clade contains only 2 OTUs from KBS, unknown sequences that are linked closely with *Peniophora* sp (a white-rotting saprotroph). Both unknown OTUs

were found in HTS plots, presumably where there would be appropriate woody or coarse herbaceous substrate that would be absent from NT and CT plots. Again, poor representation of this clade may be due to undersampling.

#### **4.13 Auriculariales (Figure 3.1. Z)**

The Auriculariales clade consists of fungi that are saprotrophic, growing mainly on dead wood (Weiss and Oberwinkler 2001). The phylogenetic placement of Auriculariales is close to Sebaciniales and Trechisporales (Hibbett et al. 2007). Five OTUs, detected by Lynch (2004), were found in this clade with bootstrap support of 100%. The OTUs derived here all came from NT, HTS, and NTS plots. In Lynch's (2004) study, clusters of OTUs around *Exidia* and *Exidiopsis*, both reference sequences for Auriculariales, occurred in all other treatments except CT. These plots differ in their plant community composition but none of these treatments is tilled suggesting that members of this clade may persist in soils that are not disturbed by tillage regardless of the aboveground community composition. Since members of this clade are found on decaying woody materials, their distribution in predominantly non-agricultural treatments is consistent with the ecological description of Auriculariales as there is more litter available on the surface of these plots than is available on CT plots.

#### **4.14 Trechisporales (Figure 3.1. Z)**

The Trechisporales are composed of mainly resupinate species that give the impression of being soil-dwelling saprotrophs, but there is no indication of a mycorrhizal habit (Larsson et al. 2004, Liberta 1973). Trechisporales represent 4 OTUs with strong

(>98%) bootstrap support for groupings with the exception of one pair with weak 57% support. Of the OTUs recovered from KBS soils, two are from NTS plots and one each from HTS and CT plots. No sequences were recovered from NT plots. Although the OTU detected in the CT plot has 57% bootstrap support to *Sistotremastrum niveocreameum*, a wood-decaying fungus (Larsson et al. 2004), the occurrence of this clade in plots that did not contain woody substrate suggests that these saprotrophs are not limited to wood.

#### **4.15 Non-represented clades**

Although Agaricomycetes are generally important saprotrophs and ectomycorrhizal fungi, not all the clades in the Agaricomycetes are represented in this study. While reference sequences were given for each clade, no sequences from Hysterangiales, Gloeophyllales, Jaapiales, Atheliales, Boletales, or Amylocorticiales were recovered from KBS soils. Ectomycorrhizal fungi (e.g. Hysterangiales and most Boletales) were not expected in KBS plots since no suitable hosts are present in CT or NT and few or none are in plots of HTS or NTS. Gloeophyllales is an order of brown-rot fungi, and these along with the saprotrophic, brown-rot members of Boletales are associated with conifers, not present in the KBS plots sampled. As far as they are known at present, the Jaapiales, Atheliales, and Amylocorticiales are small groups with narrow ecological niches (Binder et al. 2005, Larsson et al. 2004), thus their apparent absence from KBS soils is not surprising.

#### 4.16 Representation in CT plots

Of the 13 major clades detected, representatives from 6 clades, Cantharellales, Trechisporales, Hymenochaetales, Corticiales, Polyporales, and Agaricales, were found in CT plots. The clades with the greatest presence were the Cantharellales clade with 17 OTUs and the Agaricales with 37 OTUs. The Cantharellales found were allied to taxa that are saprobic on leaves. The Agaricales clade contains a wide variety of minor clades that have diverse ecological roles. The diversity of niches to be exploited by members of this major clade allow for the dominating presence of Agaricales, especially as decomposers (Matheny et al. 2006). The remaining four clades all contained less than 4 OTUs in CT plots. Of all the treatments, CT contains the fewest major clades. This is likely due to the effect of tillage on fungal hyphae as well as appropriate substrates for decomposers being turned into the soil rather than being left on the surface. Furthermore, CT plots are planted in corn, soybean, or wheat during the growing year. The lack of plant diversity and the transience of corn, soybean, or wheat crops are likely causes of the lack of fungal diversity when compared to NTS and HTS plots. The reduction in plant diversity may affect the availability of growth-limiting resources for fungi (Tilman 1982, 1987). UniFrac p-test significance for all environments tested together and P-test significance both gave values of  $\ll 0.001$ , isolating each site from the others as significantly different. PCoA of the data show that 4 of the 6 CT replicate plots are clustered tightly together and away from the rest of the treatments, with the exception of one NT site found in the middle of the CT cluster (Figure 3.3A). The remaining two CT plots are near the middle of the ordination space. It appears that 4 of 6 CT plots are phylogenetically distinct from the other sites, while 2 of the CT plots appear to contain



lineages of Agaricomycetes that are similar to those in HTS plots. This is not surprising as the 6 major clades that were detected from CT plots are also detected in HTS plots, with the exception of Corticiales.

#### **4.17 Representation in NT plots**

Eight major clades, the Auriculariales, Tulasnellales, Cantharellales, Gomphoid/Phalloid, Hymenochaetales, Corticiales, Polyporales, and Agaricales were present to some degree in NT plots. Similar to the findings in the CT plots, most of the OTUs in NT plots belong to the Agaricales. Naturally, as the largest clade with the most OTUs overall, this is not unexpected, with Agaricales contributing to ~55% of the OTUs in NT plots. Again, Agaricales may be present due to many minor clades whose main nutritional roles are that of a mycorrhizal symbiont to higher plants and decomposer of litter (Matheny et al. 2006). The increase in number and diversity of clades found in NT plots compared to CT plots may be attributed to the absence of tillage. All clades found in CT plots were also found in NT plots, with the exception of Trechisporales (which was recovered once each in CT and HTS plots and twice in NTS). PCoA analysis (Figure 3.3) shows no particular trend in the ordination of NT sites. Two particular NT plots are clustered very closely together, suggesting that these two plots contain a very similar assemblage of closely related species. The remaining NT sites are scattered throughout the ordination space indicating that species found in NT sites are not unique to NT sites.

#### 4.18 Representation in HTS plots

All of the 13 major clades detected in this study were detected in HTS plots with the exception of one, the Corticiales. Two OTUs in Corticiales detected in KBS LTER were linked only with *Waitea*, a pathogen of grasses including the crops wheat and maize, present only in CT and NT plots.

As in the CT and NT plots, the highest number of OTUs in HTS came from the Agaricales clade, accounting for ~55% of all OTUs detected in these plots. The second highest number of OTUs in HTS came from Cantharellales and Tulasnellales clades, while the remaining clades accounted for less than 6% of the OTUs. OTUs 517-518 from HTS are strongly linked with the root associates *Sebacina* and *Piriformospora* but OTUs 514 and 520-522 from NTS are not strongly linked with any reference taxon in the Sebaciniales; the latter three are weakly linked with the saprotrophic *Craterocolla*. All Cantharellales recovered from KBS LTER are phylogenetically close to saprotrophic species of *Minimedusa*; isolates T-791 (NTS), X-14 (NT), and X-44 (from soil of a nearby deciduous forest) grew vigorously in culture and are clearly saprotrophic (Thorn, unpublished). PCoA of the data show HTS plots to be not as closely clustered together as NTS plots and the distance between HTS sites in the ordination suggest that HTS contains a wide array of different clades of Agaricomycetes and indeed HTS plots contain the greatest number of clades of Agaricomycetes. The plant communities in HTS plots are diverse and not uniform between replicate plots (e.g. one plot had developed a considerable stand of saplings of black locust (*Robinia pseudoacacia*) by 2005 (<http://lter.kbs.msu.edu/datatables/237>), so it is not surprising to find a diverse and heterogeneous community of Agaricomycetes in these plots as well. Also, the diversity of

fungi found here may be attributed to the lack of tillage, as these plots are not disrupted by soil homogenization.

#### **4.19 Representation in NTS plots**

Somewhat fewer clades were found in the NTS plots compared to the HTS plots; 10 of 19 major clades found were Auriculariales, Sebaciniales, Tulasnellales, Cantharellales, Trechisporales, Geastrales, Hymenochaetales, Polyporales, Thelephorales, and Agaricales. Gomphoid/Phalloid, Corticiales, and Russulales were not found in NTS plots yet were found in HTS plots; this could indicate that these clades may be associated with non-agricultural sites with mild disturbance in the past. As in the previous treatments, Agaricales dominated with ~75% of the OTUs in NTS belonging to Agaricales. The remaining clades contained OTUs that each contributed to less than 5% of OTUs found in NTS plots. Finding large numbers of OTUs of Agaricales can be explained by their overall large presence within the Agaricomycetes and the many functional roles they play, as previously discussed. NTS is characterized by a distinct group of Agaricomycetes: Auriculariales, Sebaciniales, Tulasnellales, Geastrales, Thelephorales, and all minor clades of Agaricales. Almost half of all detected clades in these plots are not detected in CT plots. This suggests that these particular clades may be more sensitive to tillage than clades that are present in both NTS and CT plots. Furthermore, the detection of these clades in NTS plots and not in CT plots may be due to the greater diversity of plants. PCoA of the data suggest that Agaricomycetes found in the NTS plots are significantly different from fungi found in other treatments; also, the extremely close clustering of NTS sites in Figure 3.3 suggests that fungi in the NTS plots

are phylogenetically closely related to one another. UniFrac P-Values of  $\leq 0.01$  for all NTS plots indicate that these plots differ significantly from the rest of the tree in the lineages they contain.

#### **4.20 Conservation of fungi through sustainable practices**

Major clades of Agaricomycetes provide ecosystem services that are beneficial to soil fertility and play an essential role in nutrient cycling (Ananyeva et al. 1999). The goal of sustainable agriculture is to enhance natural ecosystem services while maintaining viable agricultural production. The overarching goal is to ensure that future generations are well-supported and that our actions today are facilitating this goal. The conservation of fungi in general is overlooked as a goal to sustainable agriculture but it is evident that by the ecological descriptions and results of this and previous studies that some agricultural practices are detrimental to (at least to some members of) Agaricomycete communities.

Though successional communities, HTS and NTS, had the greatest diversity of Agaricomycetes, these communities are not present in most large-scale agricultural regimes. NT plots had 25% more clades of Agaricomycetes than did CT plots. The species of fungi found in NT plots have diverse ecological roles as decomposers and mycorrhizal fungi that contribute to the free ecological services mentioned above. Therefore, it is suggested that, in order to enhance or maintain diversity of fungal communities in agricultural regimes, reduction in soil disturbance and cropping rotations are adopted (Stromberger 2005). Adopting conservative agricultural practices is not only beneficial to maintaining diverse fungal communities but also contributes to ecosystem

health – objectives of sustainable agriculture (Doran 2002). These practices are also of immediate value to farmers as their crops are dependent on soil health.

Conversion from conventional tillage to no tillage management or reduced-tillage will reduce soil physical disturbance. Fungal hyphae will escape being broken up and this should increase the length of fungal hyphae and increase the proportion of fungal biomass, since organic matter would remain available at the surface for degradation (Stromberger 2005). Beare et al. (1997) reported lengths of fungal hyphae in surface soil under no till agricultural treatments were 1.3-1.5 times longer than fungal hyphal lengths under conventional tillage. Cropping rotations that incorporate different crops will increase in the heterogeneity of niches available for Agaricomycetes via more diversified substrate resources (Stomberger 2005, Bossio et al. 1998, Schutter et al. 2001) and the increase in plant diversity will likely promote ecosystem functioning (Reich et al. 2012). Maintaining a diversity of Agaricomycetes could be further incorporated as a solution for sustainable agriculture by planting varied habitats surrounding large agricultural fields or even the preserving similar surrounding habitats, like non-tilled land or natural vegetation. In this way, a greater diversity of taxa of Agaricomycetes and likely other fungi, such as vesicular arbuscular mycorrhizal fungi, may be made available to planted crops.

## CHAPTER 5: CONCLUSION

Sustainable agricultural practices are beginning to be adopted to help conserve soil as a resource and protect it from further degradation. However, the degree to which certain agricultural practices, like tillage, affect soil Agaricomycetes is only beginning to be understood. In general, soil fungi are affected by conventional tillage, likely through soil homogenization and damage to fungal hyphae. CT plots had the lowest Agaricomycete species diversity of all the plots. NT plots had more clades of Agaricomycetes when compared to CT plots. The non-agricultural HTS and NTS plots had the greatest diversity of Agaricomycetes. These results support previous studies on tillage affecting soil Agaricomycetes, from the same area.

### 5.1 Direction of future research

Lynch (2004) and Bahnmann (2009) found similar overall patterns of minor clade distribution among treatments; however, numerous unknown species were detected in multiple plots within each study year. Lynch (2004) found many situations in which entire clades had no relevant ingroups to aid in phylogenetic resolution; this was a function of poor database coverage in GenBank. However, with improved GenBank database coverage, an increase in the number of GenBank reference sequences, and the placement of previously unknown taxa of Agaricomycetes based on published (Hibbett et al. 2007, Matheny et al. 2007) and unpublished data (Thorn and Hibbett), these clades are now more resolved and better supported, and previously unknown species have been identified accordingly. Even with improvements in GenBank reference sequences, the clade referred to as “Sister clade to *Volvariella*” by Lynch (2004) and Bahnmann (2009)

is now resolved as part of the Pluteoid clade but still does not have named reference sequences in GenBank. In addition, PCoA showed how different plots were separated by the phylogenetic lineages of Agaricomycetes they contained, which was not shown in previous work.

Lynch's (2004) and Bahnmann's (2009) findings on the effects of niche heterogeneity on Agaricomycete diversity are mirrored in this study. Even with a larger data set, the general trend remains the same: as niche heterogeneity (e.g. plant diversity) increased there was an increase in diversity of Agaricomycetes. However, these results are limited to KBS LTER in Michigan. It may be necessary to test the research questions put forth by this study to other comparable locations. It would be interesting to see if the trends exhibited here are supported in other locations as well. This will be central to advancing our knowledge on Agaricomycete presence in agricultural and successional soils.

Next generation sequencing (NGS), a sensitive sequencing technique that can produce millions of sequences from the same volume of samples and can provide a more comprehensive data set to work with. Using single DNA molecules and skipping traditional DNA amplification, short reads of sequences (now approximately 250 to 400 base pairs) can be produced (ten Bosch and Grody 2008, Voelkerding et al. 2009, Tucker et al. 2009, Fullwood et al. 2009, Morozova and Marra 2008, Petterson et al. 2009). NGS would help to provide a more quantitative data set that may reveal trends not detectable at this level of study. Rare taxa may be better represented due to an increase in sequences and may be more easily resolved. Furthermore, NGS may obtain a more quantitative measure of the relative abundance of predominant taxa. Obtaining combined sequence

data for both plants and fungi could allow for identification of plant community members associated with particular clades or OTUs of fungi, greatly improving the speculations made herein about the possible ecological relations of the fungi detected. Sustainable agriculture will require changes in many agricultural practices, yet further investigation is necessary to determine which of the interacting agricultural practices has the greatest impact on Agaricomycetes in the Michigan region and other agriculturally relevant areas of North America.

This study demonstrates that tillage affects the community assemblage of Agaricomycetes and that, in general, the diversity of Agaricomycetes in soils that are conventionally tilled is less than in soils under no till management, but each of these has less fungal diversity than areas with a diverse plant community, such as in the successional treatments at KBS LTER. However the effects of conventional tillage on community structure of Agaricomycetes may indeed be synergistic with the effects of other traditionally unsustainable agricultural practices such as mono-cropping, chemical fertilizer inputs, and herbicide and pesticide usage. Niche heterogeneity, aboveground plant diversity, and chemical inputs are factors potentially affecting community assembly of Agaricomycetes that should be examined in future studies.



## References

- Alguacil, M. M., E. Lumini, A. Roldan, J. R. Salinas-Garcia, P. Bonfante and V. Bianciotto. The impact of tillage practices on arbuscular mycorrhizal fungal diversity in subtropical crops. *Ecological Applications* **18**:527-536.
- Ananyeva, N. D., T. S. Demkina, W. J. Jones, M. L. Cabrera and W. C. Steen. 1999. Microbial biomass in soils of Russia under long-term management practices. *Biology and Fertility of Soils* **29**:291-299.
- Bahmann, B. D. 2009. Identity and diversity of Agaricomycetes (Fungi: Basidiomycota) in temperate agricultural soils. MSc. thesis, University of Western Ontario, London, Ontario, Canada.
- Bartnicki-Garcia, S. and E. Lippman. 1969. Fungal morphogenesis: cell wall construction in *Mucor rouxii*. *Science* **165**:302-304.
- Beare, M. H., S. Hu, D. C. Coleman and P. F. Hendrix. 1997. Influence of mycelia fungi on soil aggregation and organic matter storage in conventional and no tillage soils. *Applied Soil Ecology* **5**:211-219.
- Bending, G. D. and D. J. Read. 1995. The structure and function of the vegetative mycelium of ectomycorrhizal plants. VI. activities of nutrient mobilizing enzymes in birch litter colonized by *Paxillus involutus* (Fr.) Fr. *New Phytologist* **130**:411-417.
- Berry, V. and O. Gascuel. 1996. On the interpretation of bootstrap trees: appropriate threshold of clade selection and induced gain. *Molecular Biology and Evolution* **13**:999-1011.
- Bérubé, J. A. and M. Dessureault. 1989. Morphological studies of the *Armillaria mellea* complex: two new species, *A. gemina* and *A. calvescens*. *Mycologia* **81**:216-225.
- Binder, M., D. S. Hibbett, K-H. Larsson, E. Larsson, E. Langer and G. Langer. 2005. The phylogenetic distribution of resupinate forms across the major clades of mushroom-forming fungi (Homobasidiomycetes). *Systematics and Biodiversity* **3**:113-157.
- Blanchette, R. A. 1991. Delignification by wood-decaying fungi. *Annual Review of Phytopathology* **29**:381-398.
- Bossio, D. A., K. M. Skow, N. Gunapala and K. J. Graham. 1998. Determinants of soil microbial communities: effects of agricultural management, season, and soil type of phospholipids fatty acid profiles. *Microbial Ecology* **36**:1-2.

- Breitenbach, J. and F. Kränzlin. 1986. Fungi of Switerland. Vol 2: non-gilled fungi. Verlag Mycologia, Lucerne, Switzerland.
- Bremer, K. 1988. The limits of amino acid sequence data in angiosperm phylogenetic reconstruction. *Evolution* **42**:795-803.
- Broughton, L. C. and K. L. Gross. 2000. Patterns of diversity in plant and soil microbial communities along a productivity gradient in a Michigan old field. *Oecologia* **125**: 420-427.
- Brundrett, M. C. 2002. Coevolution of roots and mycorrhizas of land plants. *New Phytologist* **154**:275-304.
- Brundrett, M. C. 2004. Diversity and classification of mycorrhizal associations. *Biological Reviews* **79**:473-495.
- Brundrett, M. C. 2009. Mycorrhizal associations and other means of nutrition of vascular plants: understanding global diversity of host plants by resolving conflicting information and developing reliable means of diagnosis. *Plant and Soil* **320**:37-77.
- Brussaard, L., V. M. Beham-Pelletier, D. E. Bignell, V. K. Brown, W. Didden, P. Folgarait, C. Fragoso, D. Wall Freckman, V. V. S. R. Gupta, T. Hattori, D. L. Hawksworth, C. Klopatek, P. Lavelle, D. W. Malloch, J. Rusek, B. Soderstrom, J. M. Tiedje and R. A. Virginia. 1997. Biodiversity and ecosystem functioning in soil. *Ambio* **26**:563-570.
- Carlile, M. J., S. C. Watkinson and G. W. Gooday. 2001. Saprotrophs and ecosystems. Pages 297-362. *The Fungi*, 2<sup>nd</sup> edition. Academic Press, London.
- Chapela, I. H., S. A. Rehner, T. R. Schultz and U. G. Mueller. 1994. Evolutionary history of the symbiosis between fungus-growing ants and their fungi. *Science* **266**:1691-1694.
- Coleman, D.C., C. P. P. Reid and C. V. Cole. 1983. Biological strategies of nutrient cycling in soil systems. *Advances in Ecological Research* **13**:1-55.
- Costanza, R. 1992. Toward an operational definition of ecosystem health. Pages 239-256. *Ecosystem Health: New Goals for Environmental Management*. Island Press, Washington.
- Dai, Y-C. and B-K. Cui. 2011. *Fomitiporia ellipsoidea* has the largest fruiting body among the fungi. *Fungal Biology* **115**:813-814.

- DePriest, P. T., M. Sikaroodi, J. D. Lawrey and P. Diederich. 2005. *Marchandiomyces lignicola* sp. nov. shows recent and repeated transition between a lignicolous and a lichenicolous habit. *Mycological Research* **109**:57-70.
- Diederich, P. and J. D. Lawrey. 2007. New lichenicolous, muscicolous, corticolous and lignicolous taxa of *Burgoa* s. l. and *Marchandiomyces* s. l. (anamorphic Basidiomycota), a new genus for *Omphalina foliacea*, and a catalogue and key to the non-lichenized, bulbiferous basidiomycetes. *Mycological Progress* **6**:61-80.
- Diederich, P., B. Schulteis and M. Blackwell. 2003. *Marchandiobasidium aurantiacum* gen. sp. nov., the teleomorph of *Marchandiomyces aurantiacus* (Basidiomycota, Ceratobasidiales). *Mycological Research* **107**:523-527.
- DNA Baser Sequence Assembler v3.x. 2011. Heracle BioSoft SRL. Retrieved March 2012 from <http://www.DnaBaser.com>
- Doran, J. W. 2002. Soil health and global sustainability: translating science into practice. *Agriculture, Ecosystems and Environment* **88**:119-127.
- Doran, J. W. and T. B. Parkin. 1994. Pages 20-120 in J. W. Doran, D. C. Coleman, D.F. Bezdicsek and B. A. Stewart, eds. *Defining Soil Quality for a Sustainable Environment*. American Society of Agronomy Special Publication No. 35. Madison, Wisconsin
- Doran, J. W. and M. R. Zeiss. 2000. Soil health and sustainability: managing the biotic component of soil quality. *Applied Soil Ecology* **15**:3-11.
- Duckett, J. G., J. Russell and R. Ligrone. 2006. Basidiomycetous endophytes in jungermannialean (leafy) liverworts have novel cytology and species-specific host ranges: a cytological and experimental study. *Canadian Journal of Botany* **84**:1075-1093.
- Edgar, R. C. 2004. MUSCLE: multiple sequence alignment with high accuracy and high throughput. *Nucleic Acids Research* **32**:1792-1797.
- Felsenstein, J. 1985. Confidence limits on phylogenies: an approach to using the bootstrap. *Evolution* **39**:783-791.
- Fenchel, T. 1992. What can ecologists learn from microbes: Life beneath a square centimeter of sediment surface. *Functional Ecology* **6**:499-507.

- Flegler, S. L. 1984. An improved method for production of *Sphaerobolus* fruit bodies in culture. *Mycologia* **76**:944-946.
- Freckman, D. W., T. H. Blackburn, L. Brussaard, P. Hutchings, M. A. Palmer and P. V. R. Snelgrove. 1997. Linking biodiversity and ecosystem functioning of soils and sediments. *Ambio* **26**:556-562.
- Fullwood, M. J., C. L. Wei, E. T. Liu and Y. Ruan. 2009. Next-generation DNA sequencing of paired-end tags (PET) for transcriptome and genome analyses. *Genome Research* **19**:521-532.
- Galvez, L., D. D. Douds, Jr., L. E. Drinkwater and P. Wagoner. Effect of tillage and farming system upon VAM fungus populations and mycorrhizas and nutrient uptake of maize. *Plant and Soil* **228**:299-308.
- Gardes, M. and T. D. Bruns. 1993. ITS primers with enhanced specificity for basidiomycetes – application to the identification of mycorrhizae and rusts. *Molecular Ecology* **2**:113-118.
- Gascuel, O. 1997. BIONJ: an improved version of the NJ algorithm based on a simple model of sequence data. *Molecular Biology and Evolution* **14**:685-695.
- Geml, J., D. D. Davis and G. M. Geiser. 2005. Phylogenetic analyses reveal deeply divergent species lineages in the genus *Sphaerobolus* (Phallales: Basidiomycota). *Molecular Phylogenetics and Evolution* **35**:313-322.
- Gooday, G. W. 1971. An autoradiographic study of hyphal growth of some fungi. *Journal of General Microbiology* **67**:413-441.
- Gouy, M., S. Guindon and O. Gascuel. 2010. SeaView version 4: a multiplatform graphical user interface for sequence alignment and phylogenetic tree building. *Molecular Biology and Evolution* **27**:221-224.
- Gregory, T. R. 2008. Understanding evolutionary trees. *Evolution: Education and Outreach* **1**:121-137.
- Hajibabaei, M., G. A. C. Singer, P. D. N. Hebert and D. A. Hickey. 2007. DNA barcoding: how it complements taxonomy, molecular phylogenetics and population genetics. *TRENDS in Genetics* **23**:167-172.
- Hayman, D. S. 1980. VA mycorrhiza and crop production. *Nature* **287**:487-488.

- Harinikumar, K. M. and D. J. Bagyaraj. 1989. Effect of cropping sequence, fertilizers and farmyard manure on vesicular-arbuscular mycorrhizal fungi in different crops over three consecutive seasons. *Biology and Fertility of Soils* **7**:173-175.
- Hatakka, A. 1994. Lignin-modifying enzymes from selected white-rot fungi: production and role from in lignin degradation. *FEMS Microbiology Reviews* **13**:125-135.
- Hawksworth, D. L. 2001. The magnitude of fungal diversity: the 1.5 million species estimate revisited. *Mycological Research* **105**:1422-1432.
- Hawksworth, D. L. and S. Lagreca. 2007. New bottles for old wine: fruit body types, phylogeny, and classification. *Mycological Research* **111**:999-1000.
- Hibbett, D. S. 2006. A phylogenetic overview of the Agaricomycotina. *Mycologia* **98**:917-925.
- Hibbett, D. S., M. Binder, J. F. Bischoff, M. Blackwell, P. F. Cannon, O. E. Eriksson, S. Huhndorf, T. James, P. M. Kirk, R. Lücking, H. Thorsten Lumbsch, F. Lutzoni, P. B. Matheny, D. J. McLaughlin, M. J. Powell, S. Redhead, C. L. Schoch, J. W. Spatafora, J. A. Stalpers, R. Vilgalys, M. C. Aime, A. Aptroot, R. Bauer, D. Begerow, G. L. Benny, L. A. Castlebury, P. W. Crous, Y. Dai, W. Gams, D. M. Geiser, G. W. Griffith, C. Gueidan, D. L. Hawksworth, G. Hestmark, K. Hosaka, R. A. Humber, K. D. Hyde, J. E. Ironside, U. Koljalg, C. P. Kurtzman, K. Larsson, R. Lichtwardt, J. Longcore, J. Miadlikowska, A. Miller, J. Moncalvo, S. Mozley-Standridge, F. Oberwinkler, E. Parmasto, V. Reeb, J. D. Rogers, C. Roux, L. Ryvarden, J. Sampaio, A. Schubler, J. Sugiyama, R. G. Thorn, L. Tibell, W. A. Untereiner, C. Walker, Z. Wang, A. Weir, M. Weiss, M. M. White, K. Winka, Y. Yao and N. Zhang. 2007. A higher-level phylogenetic classification of the *Fungi*. *Mycological Research* **111**:509-547.
- Hibbett, D. S. and R. G. Thorn. 2001. Pages 212-268 in D. J. McLaughlin, E. G. McLaughlin and P. A. Lemke, eds. *Basidiomycota: Homobasidiomycetes. The Mycota. VIIB. Systematics and Evolution*. Springer-Verlag, Berlin.
- Hosaka, K., S. T. Bates, R. E. Beever, M. A. Castellano, W. Colgan 3<sup>rd</sup>, L. S. Dominquez, E. R. Nouhra, J. Geml, A. J. Giachini, S. R. Kenney, N. B. Simpson, J. W. Spatafora and J. M. Trappe. 2006. Molecular phylogenetics of the gomphoid-phalloid fungi with an establishment of the new subclass Phallomycetidae and two new orders. *Mycologia* **98**:949-959.
- Humphrey, J. W., S. Davey, A. J. Peace, R. Ferris and K. Harding. 2002. Lichens and bryophyte communities of planted and semi-natural forests in Britain: the influence of site type, stand structure and deadwood. *Biological Conservation* **107**:165-180.

- Johansson, J. F., L. R. Paul and R. D. Finlay. 1994. Microbial interactions in the mycorrhizosphere and their significance for sustainable agriculture. *FEMS Microbiology Ecology* **48**:1-13.
- Julou, T., B. Burghardt, G. Gebauer, D. Berveiller, C. Damesin and M-A. Selosse. 2005. Evolution of mixotrophy in orchids: insight from a comparative study of green and achlorophyllous *Cephalanthera damasonium*. *New Phytologist* **166**:639-653.
- Karlen, D. L., M. J. Mausbach, J. W. Doran, R. G. Cline, R. F. Harris and G. E. Schumann. 1997. Soil quality: a concept, definition, and framework for evaluation. *Soil Science Society of America Journal* **61**:4-10.
- Kennedy, A. C. and R. I. Papendick. 1995. Microbial characteristics of soil quality. *Journal of Soil and Water Conservation* **50**:243-248.
- Kennedy, A. C. and K. L. Smith. 1995. Soil microbial diversity and the sustainability of agricultural soils. *Plant and Soil* **170**:75-86.
- Keizer, P. J. 1993. The influence of nature management on the management of macromycete flora. Pages 251-269 in D. N. Peyley, L. Boddy, B. Ing and P. M. Kirk, eds. *Fungi of Europe: investigation, recording and mapping*. Royal Botanic Gardens, Kew, England.
- Kibblewhite, M. G., K. Ritz and M. J. Swift. 2008. Soil health in agricultural systems. *Philosophical Transactions of the Royal Society Biological Sciences* **363**:685-701.
- Kirk, P. M., P. F. Cannon, D. W. Minter and J. A. Stalpers, eds. 2008. *Dictionary of the Fungi*. CAB International, Wallingford.
- Kottke, I., A. Beiter, M-A. Weiss, I. Haug, F. Oberwinkler and M. Nebel. 2003. Heterobasidiomycetes form symbiotic associations with hepatics: Jungermanniales have sebacinoid mycobionts while *Aneura pinguis* (Metzgeriales) is associated with a *Tulasnella* species. *Mycological Research* **107**:957-968.
- Kuthubutheen, A. J. and S. Muid. 1984. *Pneumatospora obcoronata* gen. et sp.nov. from Malaysia. *Transactions of the British Mycological Society* **83**:423-429.
- Landeweert, R., E. Hoffland, R. D. Findlay, T. W. Kuyper, N. van Breemen. 2001. Linking plants to rocks: ectomycorrhizal fungi mobilize nutrients from minerals. *Trends in Ecology and Evolution* **16**:248-254.

- Langer, G. 1994. Die Gattung *Botryobasidium* Donk (Corticaceae, Basidiomycetes). *Bibliotheca Mycologica* **158**:1-459
- Larsson, K-H., E. Larsson and U. Kõljalg. 2004. High phylogenetic diversity among corticoid homobasidiomycetes. *Mycological Research* **108**:983-1002.
- Larsson, K-H., E. Parmasto, M. Fischer, E. Langer, K. K. Nakasone and S. A. Redhead. 2006. Hymenochaetales: a molecular phylogeny for the hymenochaetoid clade. *Mycologia* **98**:926-936.
- Lavelle, P., C. Gilot, C. Fragoso and B. Pashanasi. 1994. Pages 291-308 in D. J. Greenland and I. Szabolcs, eds. *Soil resilience and sustainable land use*. CAB International, United Kingdom.
- Lawrey, J. D., M. Binder, P. Diederich, M. C. Molina, M. Sikaroodi and D. Ertz. 2007. Phylogenetic diversity of lichen-associated homobasidiomycetes. *Molecular Phylogenetics and Evolution* **44**:778-789.
- Lawrey, J. D., P. Diederich, M. Sikaroodi and P. M. Gillevet. 2008. Remarkable nutritional diversity of Basidiomycetes in the Corticiales, including a new foliicolous species of *Marchandiomyces* (anamorphic Basidiomycota, Corticiaceae) from Australia. *American Journal of Botany* **95**:816-823.
- Leiner, R. H. and D. E. Carling. 1994. Characterization of *Waitea circinata* (Rhizoctonia) isolated from agricultural soils in Alaska. *Plant Disease* **78**:385-388.
- Liberta, A. E. 1973. The genus *Trechispora* (Basidiomycetes, Corticiaceae). *Canadian Journal of Botany* **51**:1871-1892.
- Lozupone, C. and R. Knight. 2005. UniFrac: a new phylogenetic method for comparing microbial communities. *Applied and Environmental Microbiology* **71**:8228-8235.
- Lozupone, C., M. Hamady and R. Knight. 2006. UniFrac-an online tool for comparing microbial community diversity in a phylogenetic context. *BMC Bioinformatics* **7**: 371-385.
- Lozupone, C. M., E. Lladser, D. Knights, J. Stombaugh and R. Knight. 2011. UniFrac: an effective distance metric for microbial community comparison. *The International Society for Microbial Ecology Journal* **5**:169-172.
- Lynch, M. D. J. 2004. Species richness and composition of soil Basidiomycota. MSc. thesis, University of Western Ontario, London, Ontario, Canada.
- Lynch, M. D. J. and R. G. Thorn. 2006. Diversity of basidiomycetes in Michigan agricultural soils. *Applied and Environmental Microbiology* **72**:7050-7056.

- Lynch, J. M. and E. Bragg. 1985. Microorganisms and soil aggregate stability. *Advances in Soil Sciences* **2**:133-171.
- Mannering, J. V. and C. R. Fenster. 1983. What is conservation tillage? *Journal of Soil and Water Conservation* **38**:140-143.
- Martin, A. P. 2002. Phylogenetic approaches for describing and comparing the diversity of microbial communities. *Applied and Environmental Microbiology* **69**:3673-3692.
- Matheny, P. B., J. M. Curtis, V. Hofstetter, M. C. Aime, J-M. Moncalvo, Z-W. Ge, Z-L. Yang, J. C. Slot, J. F. Ammirati, T. J. Baroni, N. L. Bougher, K. W. Hughes, D. J. Lodge, R. W. Kerrigan, M. T. Seidl, D. K. Aanen, M. DeNitis, G. M. Daniele, D. E. Desjardin, B. R. Kropp, L. L. Norvell, A. Parker, E. C. Vellinga, R. Vilgalys and D. S. Hibbett. 2006. Major clades of Agaricales: a multilocus phylogenetic overview. *Mycologia* **98**:982-995.
- Matson, P. A., W. J. Parton, A. G. Power and M. J. Swift. 1997. Agricultural intensification and ecosystem properties. *Science* **277**:504-509.
- Matsushima, M. 1995. *Matsushima Mycological Memoirs*. **8**. – Published by the author, Kobe.
- McGonigle, T. P. and M. H. Miller. 1996. Development of fungi below ground in association with plants growing in disturbed and undisturbed soils. *Soil Biology and Biochemistry* **28**:263-269.
- McGonigle, T. P. and M. H. Miller. 1996a. Mycorrhizae, phosphorus absorption, and yield of maize in response to tillage. *Soil Science Society of America Journal* **60**:1856-1861.
- McHugh, R., D. Mitchell, M. Wright and R. Anderson. 2001. The fungi of Irish grasslands and their value for conservation. *Proceedings of the Royal Irish Academy* **101**:255-242.
- McKendrick, S. L., J. R. Leake, D. L. Taylor and D. J. Read. 2002. Symbiotic germination and development of the myco-heterotrophic orchid *Neottia nidus-avis* in nature and its requirement for locally distributed *Sebacina* spp. *New Phytologist* **154**:233-247.



- Mueller, U. G., S. A. Rehner and T. R. Schultz. 1998. The evolution of agriculture in ants. *Science* **281**:2034-2038.
- Miller, R. M. and J. D. Jastrow. 1990. Hierarchy of root and mycorrhizal fungal interactions with soil aggregation. *Soil Biology and Biochemistry* **22**:579-584.
- Miller, S. L., E. Larsson, K-H. Larsson, A. Verbeken and J. Nuytinck. 2006. Perspectives in the new Russulales. *Mycologia* **98**:960-970.
- Moncalvo, J-M., F. M. Lutzoni, S. A. Rehner, J. Johnson and R. Vilgalys. 2000. Phylogenetic relationships of agaric fungi based on nuclear large subunit ribosomal DNA sequences. *Systematic Biology* **49**:278-305.
- Moncalvo, J-M., R. H. Nilsson, B. Koster, S. M. Dunham, T. Bernauer, P. B. Matheny, T. M. Porter, M. Weiss, S. Garnica, E. Danell, G. Langer, E. Langer, E. Larsson, K-H. Larsson and R. Vilgalys. 2006. The cantharelloid clade: dealing with incongruent gene trees and phylogenetic reconstruction methods. *Mycologia* **98**:937-948.
- Moncalvo, J-M., R. Vilgalys, S. A. Redhead, J. E. Johnson, T. Y. James, M. C. Aime, V. Hofstetter, S. J. W. Verduin, E. Larsson, T. J. Baroni, R. G. Thorn, S. Jacobsson, H. Clemencon and O. K. Miller. 2002. One hundred and seventeen clades of eugarics. *Molecular Phylogenetics and Evolution* **23**:357-400.
- Morgenstern, I., S. Klopman and D. S. Hibbett. 2008. Molecular evolution and diversity of lignin degrading heme peroxidases in the Agaricomycetes. *Journal of Molecular Evolution* **66**:243-257.
- Morozova, O. and M. A. Marra. 2008. Applications of next-generation sequencing technologies in functional genomics. *Genomics* **92**:255-264.
- Moyersoen, B. 2006. *Pakaraimaea dipterocarpacea* is ectomycorrhizal, indicating an ancient Gondwanaland origin for the ectomycorrhizal habit in Dipterocarpaceae. *New Phytologist* **172**:753-762.
- Munkasci, A. B., J. J. Pan, P. Villesen, U. G. Mueller, M. Blackwell and D. J. McLaughlin. 2004. Convergent coevolution in the domestication of coral mushrooms by fungus-growing ants. *Proceedings of the Royal Society London* **271**:1777-1782.

- Nei, M. and S. Kumar. 2000. Pages 73-100. Molecular evolution and phylogenetics. Oxford University Press, New York.
- Newton, A. C., L. M. Davy, E. Holden, A. Silverside, R. Watling and S. D. Ward. 2003. Status, distribution, and definition of mycologically important grasslands in Scotland. *Biological Conservation* **11**:11-23.
- Noordeloos, M. E. 1988. Entolomataceae Kotl. & P. Pages 77-177 in C. Bas, T. H. W. Kuyper, M. E. Noordeloos and E. C. Vellinga, eds. *Flora Agaricina Neerlandica*, vol. 1. A. A. Balkema, Rotterdam.
- Oberwinkler, F. and E. Horak. 1979. Stephanosporaceae-a new family of Basidiomycota with aphyllorphoid and gastroid carpospores. *Plant Systematics and Evolution* **131**:157-164.
- Ohkuma, M., Y. Maeda, T. Johjima and T. Kudo. 2001. Lignin degradation and roles of white rot fungi: study on an efficient symbiotic system in fungus-growing termites and its application to bioremediation. *Riken Review* **42**:39-42.
- Oldeman, L. R. 1994. The global extent of soil degradation. Pages 99-118 in D. J. Greenland and I. Szabolcs, editors. *Soil Resilience and Sustainable Land Use*. CAB International, Wallingford.
- O'Sullivan, A. C. 1997. Cellulose: the structure slowly unravels. *Cellulose* **4**:173-207.
- Parkin, T. B. 1993. Spatial variability of microbial processes in soil – a review. *Journal of Environmental Quality* **22**:409-417.
- Parkinson, D. and D. C. Coleman. 1991. Microbial communities, activity and biomass. *Agriculture, Ecosystems and Environment* **34**:3-33.
- Pegler, D. N. 1993. False truffles: Basidiomycotina. Pages 137-204 in D. N. Pegler, B. M. Spooner and T. W. K. Young, eds. *British truffles: a revision of British hypogeous fungi*. Royal Botanic Gardens, Kew, England
- Pegler, D. N., T. Lassøe, B. M. Spooner. 1995. Pages 176, 255. *British puffballs, earthstars and stinkhorns*. Royal Botanic Gardens, Kew, England.
- Peláez, F., J. Collado, G. Platas, T. Diez, A. Basilio, A. González, F. Vincente, P. Dai, G. Harris, M. Roxenbach, S. Dreikorn and J. Thompson. 2001. Studies on *Tricellulortus pepformis*, a tropic mitosporic fungus. *Nova Hedwigia* **72**:217-230.
- Petersen, R. H. and W. Litten. 1989. A new species of *Clavaria* fruiting with *Vaccinium*. *Mycologia* **81**:325-327.

- Pettersson, E., J. Lundeberg and A. Ahmadian. 2009. Generations of sequencing technologies. *Genomics* **93**:105-111.
- Pruesse, E., C. Quast, K. Knittel, B. Fuchs, W. Ludwig, J. Peplies and F. O. Glöckner. 2007. SILVA: a comprehensive online resource for quality checked and aligned ribosomal RNA sequence data compatible with ARB. *Nucleic Acids Research* **35**:7188-7196.
- Ramette, A. 2007. Multivariate analyses in microbial ecology. *FEMS Microbiology Ecology* **62**:142-160.
- Ranjard, L., D. P. H. Lejon, C. Mougel, L. Schehrer, D. Merdinoglu and R. Chaussode. 2003. Sampling strategy in molecular microbial ecology: influence of soil sample size on DNA fingerprinting analysis of fungal and bacterial communities. *Environmental Microbiology* **5**:1111-1120.
- Redhead, S. A., R. Vilgalys, J-M. Moncalvo, J. Johnson and J. S. Hopple Jr. 2001. *Coprinus* Pers. and the disposition of *Coprinus* species *sensu lato*. *Taxon* **50**:203-241.
- Reich, P. B., D. Tilman, F. Isbell, K. Mueller, S. E. Hobbie, D. F. B. Flynn and N. Eisenhauer. 2012. Impacts of biodiversity loss escalate through time as redundancy fades. *Science* **336**:589-592.
- Rillig, M. C. 2004. Arbuscular mycorrhizae, glomalin, and soil aggregation. *Canadian Journal of Soil Science* **84**:355-363.
- Roldan, A., J. R. Salinas-Garcia, M. M. Alguacil and F. Caravaca. 2007. Soil sustainability indicators following conservation tillage practices under subtropical maize and bean crops. *Soil and Tillage Research* **93**:273-282.
- Schaeffer, D. J., E. E. Herricks and H. W. Kerster. 1988. Ecosystem health. 1. Measuring ecosystem health. *Environmental Management* **12**:445-455.
- Schmit, J. P. and D. J. Lodge. 2005. Classical methods and modern analysis of studying fungal diversity. Pages 193-214 in J. Dighton, J. F. White and P. Oudemans, eds. *The fungal community*. CRC Press, Taylor and Francis Group, Florida.

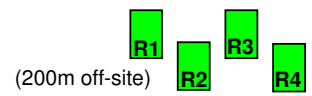
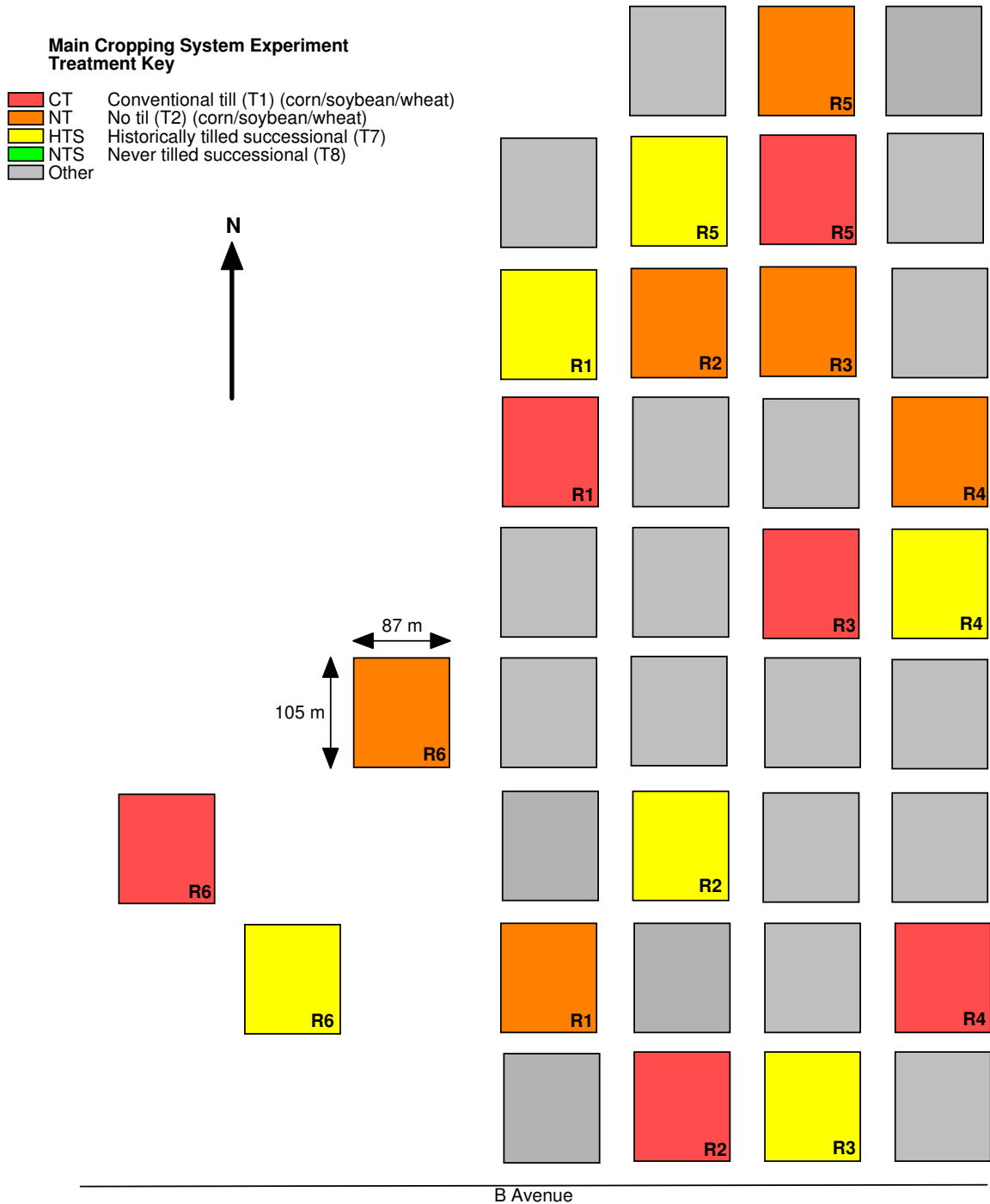
- Schutter, M. E., J. M. Sandeno and R. P. Dick. 2001. Seasonal, soil type, and alternative management influences on microbial communities of vegetable cropping systems. *Biology and Fertility of Soils* **34**:397-410.
- Selosse, M. A., A. Faccio, G. Scappaticci and P. Bonfante. 2004. Chlorophyllous and achlorophyllous specimens of *Epipactis microphylla* (Neottieae, Orchidaceae) are associated with ectomycorrhizal septomycetes, including truffles. *Microbial Ecology* **47**:416-426.
- Selosse, M. A., M-A. Weiss, J. L. Jany and A. Tillier. 2002. Communities and populations of sebacinoid basidiomycetes associated with the achlorophyllous orchid *Neottia nidus-avis* (L.) L.C.M. Rich and neighbouring tree ectomycorrhizae. *Molecular Ecology* **11**:1831-1844.
- Smith, M. L., J. N. Bruhn and J. B. Anderson. 1992. The fungus *Armillaria bulbosa* is among the largest and oldest living organisms. *Nature* **356**:428-431.
- Smith, S. E. and D. J. Read. 2008. *Mycorrhizal symbiosis*, 3<sup>rd</sup> ed. Academic, London.
- Soltis, P. S. and D. E. Soltis. 2003. Applying the bootstrap in phylogeny reconstruction. *Statistical Science* **18**:256-267.
- Stalpers, J. A. and W. M. Loerakker. 1982. *Laetisaria* and *Limonomyces* species (Corticaceae) causing pink diseases in turf grass. *Canadian Journal of Botany* **60**:529-537
- Steele, G. C. and A. P. J. Trinci. 1975. The extension zone of mycelia hyphae. *New Phytologist* **75**:583-587.
- Straatsma, G., F. Ayer and S. Egli. 2001. Species richness, abundance, and phenology of fungal fruiting bodies over 21 years in a Swiss forest plot. *Mycological Research* **105**:515-523.
- Stromberger, M. E. 2005. Fungal communities of Agroecosystems. Pages 813-832 in J. Dighton, J. F. White and P. Oudemans, eds. *The fungal community: its organization and role in the ecosystem*, 3<sup>rd</sup> ed. CRC Press, Taylor and Francis Group, Florida.

- Sun, S., L. Ruan, Y. Zhang, Y. Tao and K. Hu. 2012. Decolourization characterizations of crude enzymes from a novel basidiomycete *Mycena purpureofusca*. African Journal of Microbiology Research **6**:3501-3509.
- Sunhede, S. 1989. Geastraceae (Basidiomycotina). Morphology, ecology, and systematics with special emphasis on the North European species. Synopsis fungorum, vol 1. Fungiflora, Oslo.
- Swofford, D. L. 2002. PAUP\*: Phylogenetic Analysis Using Parsimony (and Other Methods) 4.0 Beta. Sinauer, Sunderland, MA.
- Tamura, K., M. Nei and S. Kumar. 2004. Prospects for inferring very large phylogenies by using the neighbor-joining method. Proceedings of the National Academy of Sciences **101**:11030-11035.
- Tamura, K., D. Peterson, N. Peterson, G. Stecher, M. Nei and S. Kumar. 2011. MEGA5: molecular evolutionary genetics analysis using likelihood, distance and parsimony methods. Molecular Biology and Evolution **28**:2731-2739.
- Tanesaka, E., H. Masuda and K. Kinugawa. 1993. Wood degrading ability of Basidiomycota that are wood decomposers, litter decomposers, or mycorrhizal symbionts. Mycologia **85**:347-354.
- Taylor, D. L., T. D. Bruns, T. S. Szaro and S. A. Hodges. 2003. Divergence in mycorrhizal specialization within *Hexalectris spicata* (Orchidaceae), a non-photosynthetic desert orchid. American Journal of Botany **90**:1168-1179.
- ten Bosch, J. R. and W. W. Grody. 2008. Keeping up with the next generation: massively parallel sequencing in clinical diagnostics. The Journal of Molecular Diagnostics **10**:482-494.
- Thorn, R. G., J-M. Moncalvo, C. A. Reddy and R. Vilgalys. 2000. Phylogenetic analysis and the distribution of nematophagy support a monophyletic Pleurotaceae within the polyphyletic pleurotooid-lentinoid fungi. Mycologia **92**:241-252.
- Thorn, R. G., C. A. Reddy, D. Harris and E. A. Paul. 1996. Isolation of saprophytic basidiomycetes from soil. Applied and Environmental Microbiology **62**:4288-4292.

- Tibbett, M. and F. E. Sanders. 2002. Ectomycorrhizal symbiosis can enhance plant nutrition through improved access to discrete organic nutrient patches of high resource quality. *Annals of Botany* **89**:783-789.
- Tilman, D. 1982. Resource competition and community structure. Princeton University Press, Princeton, New Jersey.
- Tilman, D. 1987. Secondary succession and the pattern of plant dominance along experimental nitrogen gradients. *Ecological Monographs* **57**:189-214.
- Tilman, D., K. G. Cassman, P. A. Matson, R. Naylor and S. Polasky. 2002. Agricultural sustainability and intensive production practices. *Nature* **418**:671-677.
- Tisdall, J. M. 1991. Fungal hyphae and structural stability of soil. *Australian Journal of Soil Research* **29**:729-743.
- Tucker, T., M. Marra and J. M. Friedman. 2009. Massively parallel sequencing: the next big thing in genetic medicine. *The American Journal of Human Genetics* **85**:142-154.
- Tzean, S. S. and J. Y. Liou. 1993. Nematophagous resupinate basidiomycetous fungi. *Phytopathology* **83**:1015-1020.
- Unterseher, M., P. Otto and W. Morawetz. 2005. Species richness and substrate specificity of lignicolous fungi in the canopy of a temperate, mixed deciduous forest. *Mycological Progress* **4**:117-132.
- Urban A., M-A. Weiss and R. Bauer. 2003. Ectoymycorrhizae involving sebacinoïd mycobionts. *Mycological Research* **107**:3-14.
- Varma, A., S. Verma, N. S. Sudha, B. Bütchorn and P. Franken. 1999. *Piriformospora indica*, a cultivable plant-growth-promoting root endophyte. *Applied and Environmental Microbiology* **65**:2741-2744.
- Vellinga, E. C. 2004. Ecology and distribution of lepiotaceous fungi (Agaricaceae)-a review. *Nova Hedwigia* **78**:273-299.
- Verma, S., A. Varma, K. H. Rexer, A. Hassel, G. Kost, A. Sarbhoy, P. Bisen, B. Bütchorn and P. Franken. 1998. *Piriformospora indica*, gen. et sp. nov., a new root-colonizing fungus. *Mycologia* **90**:896-903.

- Vilgalys, R. and M. Hester. 1990. Rapid genetic identification and mapping of enzymatically amplified ribosomal DNA from several *Cryptococcus* species. *Journal of Bacteriology* **172**:4238-4246.
- Voelkerding, K. V., S. A., Dames and J. D. Durtschi. 2009. Next-generation sequencing: from basic research to diagnostics. *Clinical Chemistry* **55**:641-658
- Walker, G. M. 1998. Page 12. *Yeast physiology and biotechnology*. John Wiley & Sons, West Sussex, England.
- Walker, J. F and J. L. Parrent. 2004. Molecular phylogenetic evidence for the mycorrhizal status of *Tremellodendron* (Sebacinaceae). *Memoirs of the New York Botanical Garden* **89**:291-296.
- Wardle, D. A. and K. E. Giller. 1996. The quest for a contemporary ecological dimension to soil biology. *Soil Biology and Biochemistry* **12**:1549-1554.
- Wardle, D. A., R. D. Bardgett, J. N. Klironomos, H. Setälä, W. H. van der Putten and D. H. Wall. 2004. Ecological linkages between aboveground and belowground biota. *Science* **304**:1629-1633.
- Watling, R. and N. M. Gregory. 1987. Pages 1-121 *in* N. M. Gregory and R. Watling, eds, *Strophariaceae and Coprinaceae pp. British fungus flora: agarics and boleti*. Royal Botanic Garden, Edinburgh.
- Weil, R. R. 1990. Defining and using the concept of sustainable agriculture. *Journal of Agronomic Education* **19**:126-130.
- Weiss, M-A. and F. Oberwinkler. 2001. Phylogenetic relationships in Auriculariales and related groups – hypotheses derived from nuclear ribosomal DNA sequences. *Mycological Research* **105**:403-415.
- Weiss, M-A., M. Selosse, K. Rexer, A. Urban and F. Oberwinkler. 2004. Sebacinales: a hitherto overlooked cosm of heterobasidiomycetes with a broad mycorrhizal potential. *Mycological Research* **108**:1003-1010.

**Appendix 1.** Main Cropping System Experiment (MCSE) at Kellogg Biological Station Long Term Ecological Research (KBS LTER) site. Numbers preceded by “R” are replicate plots within treatments. Distances between plots are not to scale.





**Appendix 2.** GenBank accession numbers for reference sequences used in phylogenetic analysis. Exemplar sequences shown in **bold** and closest BLAST matches shown in regular font.

<b>Species</b>	<b>Accession Number</b>	<b>Clade</b>
<b><i>Agaricus campestris</i></b>	FJ755230	Agaricoid
<i>Agrocybe smithii</i>	DQ110873	Agaricoid
<b><i>Alloclavaria purpurea</i></b>	DQ457657	Hymenochaetales
<i>Alnicola inculta</i>	JN938855	Agaricoid
<b><i>Alpova diplophloeus</i></b>	DQ384577	Boletales
<b><i>Amanita citrina</i></b>	EU522722	Pluteoid
<b><i>Amaurodon aquicoeruleus</i></b>	AM490944	Thelephorales
<i>Amaurodon viridis</i>	AM490942	Thelephorales
<i>Amaurodon viridis</i>	AY586625	Thelephorales
<b><i>Amylocorticium cebennense</i></b>	GU187561	Amylocorticiales
<b><i>Anomoporia bombycina</i></b>	GU187564	Amylocorticiales
<b><i>Antrodia albida</i></b>	EU232272	Antrodia clade
<i>Armillaria gemina</i>	DQ338543	Marasmioid
<b><i>Astraeus hygrometricus</i></b>	DQ682996	Boletales
<b><i>Athelia epiphylla</i></b>	GU187558	Atheliales
<b><i>Auricularia auricula-judae</i></b>	DQ520099	Auriculariales
<i>Auricularia auricula-judae</i>	JN712676	Auriculariales
<b><i>Boletus edulis</i></b>	DQ071747	Boletales
<b><i>Bondarzewia montana</i></b>	DQ234539	Russulales
<b><i>Botryobasidium botryosum</i></b>	DQ089013	Cantharellales
<b><i>Burgoa moriformis</i></b>	DQ915477	Cantharellales
<i>Camarophylloopsis hymenocephala</i>	DQ457679	Clavarioid
<i>Camarophylloopsis hymenocephala</i>	EF561628	Clavarioid
<i>Camarophylloopsis schulzeri</i>	AM946415	Clavarioid
<b><i>Camarophyllus pratensis</i></b>	AF261457	Hygrophoroid
<i>Ceraceomyces fouquieriae</i>	GU187608	Phlebia clade
<b><i>Ceratobasidium ramicola</i></b>	HQ424243	Tulasnellales
<b><i>Chondrostereum purpureum</i></b>	AY586644	Marasmioid
<i>Clavaria alboglobospora</i>	HQ877682	Clavarioid
<i>Clavaria argillacea</i>	HQ877683	Clavarioid
<i>Clavaria acuta</i>	AY228353	Clavarioid
<i>Clavaria acuta</i>	EF535278	Clavarioid
<i>Clavaria acuta</i>	HQ877679	Clavarioid
<i>Clavaria citrinorubra</i>	HQ877686	Clavarioid
<b><i>Clavaria falcata</i></b>	Thorn, unpublished	Clavarioid

<b>Species</b>	<b>Accession Number</b>	<b>Clade</b>
<i>Clavaria fragilis</i>	HQ877687	Clavarioid
<i>Clavaria fuscata</i>	HQ877691	Clavarioid
<i>Clavaria rosea</i>	HQ877694	Clavarioid
<i>Clavaria straminea</i>	EF535267	Clavarioid
<i>Clavaria subacuta</i>	HQ877699	Clavarioid
<b><i>Clavariadelphus ligula</i></b>	AF347099	Gomphales
<i>Clavicorona taxophila</i>	AF115333	Clavarioid
<i>Clavicorona taxophila</i>	HQ877701	Clavarioid
<i>Clavulinopsis helvola</i>	AY586647	Clavarioid
<b><i>Clavulinopsis helvola</i></b>	EU118617	Clavarioid
<i>Clavulinopsis laeticolor</i>	EU118618	Clavarioid
<i>Clitocybe subditopoda</i>	FJ755224	Tricholomoid
<i>Clitopilus giovanellae</i>	EF413026	Tricholomoid
<b><i>Clitopilus prunulus</i></b>	AY228348	Tricholomoid
<b><i>Coltricia perennis</i></b>	AF287854	Hymenochaetales
<b><i>Coniophora olivacea</i></b>	GU187572	Boletales
<b><i>Coprinellus micaceus</i></b>	AY207182	Agaricoid
<i>Coprinellus micaceus</i>	HQ604762	Agaricoid
<i>Coprinellus radians</i>	FJ185160	Agaricoid
<i>Coprinellus xanthothrix</i>	FJ755223	Agaricoid
<i>Coprinus cordisporus</i>	DQ389723	Agaricoid
<i>Coprinus friesii</i>	AF041503	Agaricoid
<i>Cortinarius austroduracinus</i>	AY669653	Agaricoid
<i>Cortinarius austroturmalis</i>	AF539730	Agaricoid
<i>Cortinarius badiovinaceus</i>	FJ717583	Agaricoid
<i>Cortinarius brunneus</i>	FJ039682	Agaricoid
<i>Cortinarius carneolus</i>	AF539712	Agaricoid
<i>Cortinarius junghuhnii</i>	HQ604675	Agaricoid
<i>Cortinarius lustrates</i>	AY174853	Agaricoid
<i>Cortinarius malicorius</i>	AY669583	Agaricoid
<i>Cortinarius obtusus</i>	HQ604670	Agaricoid
<i>Cortinarius suaveolens</i>	AY669574	Agaricoid
<i>Cortinarius vaginatus</i>	AY669609	Agaricoid
<b><i>Craterocolla cerasi</i></b>	DQ520103	Sebacinales
<b><i>Cyathus striatus</i></b>	AF336247	Agaricoid
<b><i>Cyphella digitalis</i></b>	AY635771	Cyphellaceae
<b><i>Cyphellopsis anomala</i></b>	AY570999	Schizophyllaceae
<b><i>Dacrymyces chrysospermus</i></b>	EU522780	Dacrymycetales

<b>Species</b>	<b>Accession Number</b>	<b>Clade</b>
<i>Daedalea quercina</i>	AF518613	Antrodia clade
<i>Dictyonema glabratum</i>	EU825960	Hygrophoroid
<i>Eichleriella leveilleana</i>	AY509553	Auriculariales
<i>Epithele typhae</i>	DQ457665	CorePolypores
<i>Erythricium laetum</i>	GU590878	Corticiales
<i>Exidia glandulosa</i>	AY509555	Auriculariales
<i>Exidia uvapsassa</i>	AY645056	Auriculariales
<i>Gautieria otthii</i>	AF393058	Gomphales
<i>Geastrum rufescens</i>	AF336251	Geastrales
<i>Gloeocystidiellum aculeatum</i>	AF265546	Russulales
<i>Gloeocystidiellum aculeatum</i>	AF506433	Russulales
<i>Gloeophyllum sepiarium</i>	HM536061	Gloeophyllales
<i>Gloeoporus taxicola</i>	AY586656	Phlebia clade
<i>Grandinia barba-jovis</i>	HQ604827	Cantharellales
<i>Hebeloma eburneum</i>	JN939973	Agaricoid
<i>Hydnochaete duportii</i>	AY635770	Hymenochaetales
<i>Hydnomerulius pinastri</i>	GU187580	Boletales
<i>Hygrocybe coccinea</i>	EU435146	Hygrophoroid
<i>Hygrocybe conica</i>	AF261450	Hygrophoroid
<i>Hygrocybe conica</i>	AY684167	Hygrophoroid
<i>Hygrophoropsis aurantiaca</i>	AY684156	Boletales
<i>Hymenochaete rubiginosa</i>	AY586665	Hymenochaetales
<i>Hymenochaete semistupposa</i>	EU599573	Hymenochaetales
<i>Hygrophoropsis aurantiaca</i>	AY684156	Boletales
<i>Hyphoderma medioburiense</i>	DQ677497	Hymenochaetales
<i>Hyphoderma obtusum</i>	AY586670	Hymenochaetales
<i>Hyphoderma roseocremeum</i>	AY586672	Hymenochaetales
<i>Hyphoderma setigerum</i>	AY586673	Hymenochaetales
<i>Hyphodontia alutaria</i>	DQ873603	Hymenochaetales
<i>Hyphodontia alutaria</i>	EU118631	Hymenochaetales
<i>Hyphodontia arguta</i>	DQ873605	Hymenochaetales
<i>Hyphodontia borealis</i>	AY586677	Hymenochaetales
<i>Hyphodontia nespori</i>	DQ873622	Hymenochaetales
<i>Hyphodontia paradoxa</i>	FN907912	Hymenochaetales
<i>Hypochnicium detriticum</i>	DQ677507	Residual Polypores
<i>Hypochnicium geogenium</i>	JN939576	Residual Polypores
<i>Inocybe abjecta</i>	HQ604751	Agaricoid
<i>Inonotus linteus</i>	AY839832	Hymenochaetales

<b>Species</b>	<b>Accession Number</b>	<b>Clade</b>
<i>Irpex lacteus</i>	EU522839	Phlebia clade
<i>Jaapia argillacea</i>	EU118636	Jaapiales
<i>Lachnella alboviolascens</i>	AY571012	Schizophyllaceae
<i>Lachnella villosa</i>	AY571013	Schizophyllaceae
<i>Laetisaria fuciformis</i>	EU118639	Corticiales
<i>Leccinum aurantiacum</i>	AF139689	Boletales
<i>Lentaria albovinacea</i>	AJ406552	Gomphales
<i>Lentinellus cochleatus</i>	AF506417	Russulales
<i>Lentinus crinitus</i>	AY615980	Core Polypores
<i>Lepista nebularis</i>	DQ457658	Tricholomoid
<i>Lepista nuda</i>	AF139963	Tricholomoid
<i>Lindtneria trachyspora</i>	EU118646	Stephanosporaceae
<i>Lyophyllum boudieri</i>	AF223206	Tricholomoid
<i>Lyophyllum boudieri</i>	DQ825430	Tricholomoid
<i>Lyophyllum decastes</i>	AY207228	Tricholomoid
<i>Lyophyllum tylicolor</i>	AF139964	Tricholomoid
<i>Meruliopsis taxicola</i>	EU118648	Phlebia clade
<i>Meruliopsis taxicola</i>	GQ470633	Phlebia clade
<i>Minimedusa obcoronata</i>	GQ303309	Cantharellales
<i>Minimedusa polyspora</i>	DQ915476	Cantharellales
<i>Mutinus elegans</i>	AY574643	Phallales
<i>Mycena purpureofusca</i>	HQ604764	Marasmioid
<i>Naucoria salicis</i>	FJ904180	Agaricoid
<i>Neolentinus lepideus</i>	HM536075	Gloeophyllales
<i>Nolanea verna</i>	EU669337	Tricholomoid
<i>Nolanea sericea</i>	AF223170	Tricholomoid
<i>Omphalina antarctica</i>	GQ483368	Hygrophoroid
<i>Omphalina farinolens</i>	EF413028	Hygrophoroid
<i>Peniophora incarnata</i>	AF506425	Russulales
<i>Peniophora pini</i>	EU118651	Russulales
<i>Phallus hadriani</i>	DQ218514	Phallales
<i>Phanaerochaete chrysosporium</i>	AF139966	Phlebia clade
<i>Phanaerocheate ginnsii</i>	GQ470645	Phlebia clade
<i>Phanaerocheate stereoides</i>	GQ470661	Phlebia clade
<i>Phlebia radiata</i>	AF287885	Phlebia clade
<i>Piloderma fallax</i>	DQ469285	Atheliales
<i>Piriformospora indica</i>	AY505557	Sebacinales
<i>Pleurotus tuber-regium</i>	EU908174	Pluteoid

<b>Species</b>	<b>Accession Number</b>	<b>Clade</b>
<i>Plicaturopsis crispa</i>	DQ470820	Amylocorticiales
<i>Pluteus cervinus</i>	EU486448	Pluteoid
<i>Pluteus cervinus</i>	HQ604793	Pluteoid
<b><i>Polyporus tuberaster</i></b>	AF261544	Core Polypores
<b><i>Podoscypha multizonata</i></b>	EU118663	Residual Polypores
<b><i>Porotheleum fimbriatum</i></b>	DQ457673	Schizophyllaceae
<i>Porpomyces mucidus</i>	AF347092	Trechisporales
<b><i>Porpomyces mucidus</i></b>	FJ496696	Trechisporales
<i>Psathyrella artemisiae</i>	AM712248	Agaricoid
<i>Psathyrella fibrillosa</i>	DQ389686	Agaricoid
<i>Psathyrella longicauda</i>	DQ389676	Agaricoid
<i>Psathyrella multipedata</i>	AM712279	Agaricoid
<i>Psathyrella sphaerocystis</i>	DQ389707	Agaricoid
<i>Psathyrella tenuicula</i>	DQ389704	Agaricoid
<i>Psilocybe cyanescens</i>	HM035076	Agaricoid
<i>Psilocybe inquilina</i>	AF261598	Agaricoid
<i>Psilocybe pratensis</i>	AF261600	Agaricoid
<i>Psilocybe schoeneti</i>	AF261602	Agaricoid
<i>Psilocybe xeroderma</i>	AF261601	Agaricoid
<b><i>Pterula echo</i></b>	AY629315	Pterulaceae
<i>Pycnoporus cinnibarinus</i>	AF393074	Core Polypores
<i>Pycnoporus cinnibarinus</i>	AY586703	Core Polypores
<b><i>Radulomyces notabilis</i></b>	EU118664	Pterulaceae
<b><i>Ramaria rubella</i></b>	AY645057	Gomphales
<i>Ramariopsis aurantio-olivaea</i>	HQ877711	Clavarioid
<i>Rhizochaete americana</i>	AY219391	Phlebia clade
<i>Rhizochaete fouquieriae</i>	AY219390	Phlebia clade
<i>Rhizoctonia zeae</i>	GQ221862	Tulasnellales
<i>Rhizoctonia zeae</i>	JN189718	Tulasnellales
<b><i>Rickenella fibula</i></b>	AY700195	Hymenochaetales
<b><i>Russula emetica</i></b>	DQ421997	Russulales
<i>Sarcomyxa serotina</i>	EU365678	Hygrophoroid
<b><i>Schizophyllum commune</i></b>	FJ372712	Schizophyllaceae
<i>Schizopora paradoxa</i>	AJ406463	Hymenochaetales
<b><i>Scleroderma citrinum</i></b>	EU718151	Boletales
<b><i>Scytinostroma portentosum</i></b>	AF506470	Russulales
<i>Sebacina vermifera</i>	DQ520096	Sebacinales
<b><i>Sebacina vermifera</i></b>	DQ520103	Sebacinales

Species	Accession Number	Clade
<i>Sebacina vermifera</i>	DQ983815	Sebacinales
<b><i>Serpula himantioides</i></b>	GU187602	Boletales
<b><i>Sistotrema confluens</i></b>	AY647214	Cantharellales
<b><i>Sistotremastrum niveocremeum</i></b>	AF347094	Trechisporales
<b><i>Sphaerobolus ingoldii</i></b>	AF139975	Geastrales
<i>Sphaerobolus stellatus</i>	HQ604795	Geastrales
<b><i>Steccherinum fimbriatum</i></b>	EU118668	Phlebia clade
<b><i>Stephanospora caroticolor</i></b>	AF518652	Stephanosporaceae
<b><i>Stropharia rugosoannulata</i></b>	AF518654	Agaricoid
<b><i>Suillus luteus</i></b>	AY586715	Boletales
<i>Thanatephorus cucumeris</i>	AF354076	Tulasnellales
<i>Thanatephorus cucumeris</i>	DQ917658	Tulasnellales
<i>Thelephora sp</i>	AF287890	Thelephorales
<i>Tomentella botryoides</i>	AY586717	Thelephorales
<i>Tomentellopsis bresadoliana</i>	EU118674	Thelephorales
<i>Tomentellopsis echinospora</i>	AY586718	Thelephorales
<i>Trametes versicolor</i>	AF139961	Core Polypores
<i>Trametes versicolor</i>	AF347107	Core Polypores
<b><i>Trametes versicolor</i></b>	AY684159	Core Polypores
<i>Trametes versicolor</i>	HM595617	Core Polypores
<i>Trechispora farinacea</i>	AF347089	Trechisporales
<i>Trechispora farinacea</i>	EU909231	Trechisporales
<b><i>Trechispora alnicola</i></b>	AY635768	Trechisporales
<i>Trechispora kavinioides</i>	AF347086	Trechisporales
<b><i>Tremella aurantia</i></b>	DQ156127	Tremellales
<i>Tricholoma apium</i>	AY586721	Tricholomoid
<i>Tricholoma apium</i>	DQ389736	Tricholomoid
<i>Tricholoma orirubens</i>	DQ389734	Tricholomoid
<i>Tricholomella constricta</i>	AF223187	Tricholomoid
<i>Trichosporon dulcitum</i>	JN939493	Tremellales
<b><i>Tulasnella violea</i></b>	DQ520097	Tulasnellales
<i>Tulostoma kotlabae</i>	DQ112629	Agaricoid
<b><i>Typhula phacorrhiza</i></b>	AF261374	Hygrophoroid
<b><i>Volvariella bombycina</i></b>	HM562256	Pluteoid
<b><i>Waitea circinata</i></b>	AY885164	Corticiales

**CURRICULUM VITAE**

Name: Jessie Rachel Wong

Education: University of Western Ontario  
London, Ontario, Canada  
2006-2010, B.Sc. in Biology and Environmental Science

University of Western Ontario  
London, Ontario, Canada  
2010-2012, M.Sc. in Biology with Environment and Sustainability

Awards: Western Graduate Research Scholarship  
2010-2012

Relevant Work Experience: Research Associate  
Dr. R. G. Thorn  
The University of Western Ontario  
Sept 2010-April 2010

Teaching Assistant  
The University of Western Ontario  
2010-2012