EFFECT OF SOIL AND MICROBIOTA COMPOSITION IN BLACK MOREL CONTINUOUS AND NON-CONTINUOUS CULTURES

ZHANG YAN

UNIVERSITI SAINS MALAYSIA

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EFFECT OF SOIL AND MICROBIOTA COMPOSITION IN BLACK MOREL CONTINUOUS AND NON-CONTINUOUS CULTURES

by

ZHANG YAN

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TABLE OF CONTENTS

ACKNOWLEDGEMENT ..... **ii**

TABLE OF CONTENTS ..... **iii**

LIST OF TABLES ..... **vii**

LIST OF FIGURES ..... **viii**

LIST OF SYMBOLS ..... **xi**

LIST OF ABBREVIATIONS ..... **xii**

LIST OF APPENDICES ..... **xiii**

ABSTRAK ..... **xiv**

ABSTRACT ..... **xvi**

CHAPTER 1 INTRODUCTION **1**

1.1 Introduction 1

1.2 Rationale and significance of study 3

1.3 Problem statement 4

1.4 Objectives of the study 6

CHAPTER 2 LITERATURE REVIEW **7**

2.1 General characteristics of morels 7

2.1.1 The life cycle of morels 7

2.1.2 Diversity of the morel species 11

2.1.3 Nutritional values of morels 14

2.1.4 Heterothallism and mating type gene in morels 15

2.2 Continuous cropping obstacles of morels 17

2.2.1 Morel cultivation 17

2.2.2 Morel cultivation techniques 19

2.2.3 Continuous cropping obstacles 22

2.2.1(a) Changes in soil physicochemical characteristics 24

2.2.1(b) Soil microorganisms and continuous cropping obstacles 29

2.3 Standards for soil physicochemical analysis in agriculture of China 33

2.4 Statistical analyses method R language 35

CHAPTER 3 INVESTIGATING PRIMORDIA YIELD DECLINE IN CONTINUOUS MOREL CULTIVATION THROUGH A CONTROLLED COMPARATIVE EXPERIMENT 37

3.1 Introduction 37

3.1.1 Objective 39

3.2 Description of the experimental site 39

3.3 Materials and Methods 41

3.3.1 Spawn production 41

3.3.1(a) Starter cultures preparation of *Morchella sextelata* 41

3.3.1(b) Mating type genes detection 42

3.3.1(c) Mother spawn preparation 43

3.3.1(d) Final spawn preparation 43

3.3.2 Field cultivation 44

3.3.1(a) Inoculation 44

3.3.1(b) Cultivation and management 45

3.3.1(c) Harvesting of morel fruiting bodies 46

3.3.3 Indoor controlled cultivation 46

3.4 Results 53

3.4.1 Spawn production 53

3.4.1(a) Starter cultures preparation of *Morchella sextelata* 53

3.4.1(b) Mating type genes detection 53

3.4.1(c) Mother spawn and final spawn 56

3.4.2 Field cultivation 58

3.4.3 Continuous cropping soils and non-continuous cropping soils 64

3.4.4 Primordium yield under different cropping regimes 64

3.5 Discussion 66

3.5.1 Spawn production 66

3.5.2 Field cultivation 67

3.5.3 Indoor controlled cultivation 73

3.6 Conclusion 75

CHAPTER 4 THE RELATIONSHIP BETWEEN SOIL PHYSICOCHEMICAL FACTORS AND MOREL PRODUCTION IN TWO CROPPING SYSTEMS AND THE CO-OCCURRENCE OF CROP-SENSITIVE OTUS 76

4.1 Introduction 76

4.1.1 Objectives 77

4.2 Materials and methods 78

4.2.1 Soil sampling 78

4.2.2 Soil physicochemical analysis 78

4.2.3 DNA extraction, PCR amplification and sequencing 79

4.2.4 Statistical analysis 82

4.3 Results 84

4.3.1 Relationships between primordium yield and soil physicochemical characteristics 84

4.3.2 Changes in fungal richness and community composition 87

4.3.3 Cropping-sensitive OTU identification, co-occurrence networks and key taxa 95

4.4 Discussion 105

4.4.1 Soil physicochemical characteristics as bioindicators of soil health status 105

4.4.2 Cropping regimes impact the fungal community composition 107

4.4.3 Cropping-sensitive fungi 108

4.5 Conclusion 112

CHAPTER 5 COMMUNITY ASSEMBLAGE AND ECOLOGICAL TRAITS OF SOIL BACTERIOME AT DIFFERENT STAGES UNDER TWO CROPPING REGIMES 114

5.1 Introduction 114

5.1.1 Objectives 115

5.2 Materials and methods 115

5.2.1 Experiment description and sampling 115

5.2.2 DNA extraction, PCR amplification and sequencing 116

5.2.3 Statistical analysis 119

5.3 Results 121

5.3.1 Impact of the cultivation procedure on primordium yields, soil morel mycelium abundance, and soil properties 121

5.3.2 Impact of cropping regime on the diversity, community composition and structure of the bacterial communities across the three developmental stages of *M. sextelata* 125

5.3.3 Evidence of the processes explaining the bacterial community assembly in each cropping regime 131

5.3.4 Soil properties as deterministic factors explaining the change of bacterial community 132

5.3.5 Identification of indicators of stage of developments and of production 137

5.4 Discussion 145

5.4.1 Underlying mechanisms of bacterial community assembly under NCC and CC cropping regime 145

5.4.2 Trophic types of abundant indicator OTUs 149

5.4.3 Bacterial-fungal interaction 153

5.5 Conclusion 156

CHAPTER 6 GENERAL CONCLUSION AND FUTURE RECOMMENDATIONS 157

REFERENCES 159

APPENDICES

LIST OF PUBLICATIONS

LIST OF TABLES

Page

Table 4.1 PCR cycling conditions used to generate the 16S and ITS amplicons for high-throughput sequencing. 81

Table 4.2 The value of soil physiochemical variables in every treatment. 89

Table 4.3 The number of high-quality sequences in every replicate. 91

Table 4.4 Results of PERMANOVA testing the effects of *Cropping regime* (NCC, CC and CCi) and *Stage* (BS, CD and PD) and *Isolate* (HX13 and YN05) on fungal communities. 92

Table 4.5 Properties of fungal co-occurrence networks..... 100

Table 4.6 Keystone OTUs identified in soil fungi communities documented with taxonomy assignments, stage, Modules, OTU IDs, degree of co-occurrence values, eignevector_centrality, group, whether or not csOTU and top50. 104

Table 5.1 The value of soil physiochemical variables in every treatments. 123

Table 5.2 Number of high-quality sequences in each replicate. 126

Table 5.3 The value of alpha diversity in every treatment..... 127

Table 5.4 Spearman's correlations between bacterial community and soil physicochemical characteristics, morel abundance and yield based on Mantel tests. NCC, non-continuous cropping regime; CC, continuous cropping regime. 135

Table 5.5 Abundant indicator OTUs of NCC and CC. 140

Table 5.6 Spearman's correlations between abundant taxa and soil morel relative abundance..... 141

LIST OF FIGURES

Page

Figure 2.1 A generalized overview of the lifecycle of morel. 8

Figure 2.2 Powdery mildew and conidia in the outdoor cultivation of *Morchella importuna* (in this laboratory).. 10

Figure 2.3 Maximum likelihood (ML) phylogenetic analysis of 75 species in the genus *Morchella* based on a four-gene dataset comprising ITS, EF1-a, RPB1, and RPB2, with Verpa and Disciotis serving as outgroups. 13

Figure 2.4 The industry of morels in China. 18

Figure 2.5 Schematic diagram of the two successful cultivation technologies of morels. 21

Figure 3.1 Experimental design for investigating the effects of continuous cropping on morel yield. 38

Figure 3.2 The map of Hu County, the study area extracted from the map of Shaanxi province. 40

Figure 3.3 Composition of the indoor controlled cultivation frame for morel, illustrating the structural design. 49

Figure 3.4 Cropping regimes for indoor controlled cultivation. 50

Figure 3.5 Schematic diagram of the indoor controlled cultivation. 52

Figure 3.6 Starter cultures of *M. sextelata* isolates. 54

Figure 3.7 The electrophoretogram of agarose gel for the isolate HX13 and YN05 mating type genes MAT-1-1-1 and MAT-1-2-1. 55

Figure 3.8 The mother spawn and final spawn of *M. sextelata.* 57

Figure 3.9 Greenhouse structure diagram and ridges cross-section view. 59

Figure 3.10 Inside and outside of the greenhouse for morel cultivation. 60

Figure 3.11 Sowing final spawn of *M. sextelata* and adding exogenous nutrition bags. 61

Figure 3.12 Harvested fruit bodies of *M. sextelata*.. 63

Figure 3.13 Primordium yield under different cropping regimes..... 65

Figure 4.1 Line charts of physicochemical characteristics significantly related to morel primordial yield during black morel cultivation..... 85

Figure 4.2 Physicochemical characteristics and their Pearson's correlation with primordium yield. 86

Figure 4.3 Temporal dynamics of diversity and distribution patterns of crop-associated fungi in NCC, CC and CCi systems at BS, CD and PD stage. 90

Figure 4.4 PCoA analysis of soil microbial community associated with *M. sextelata* based on the Bray distance metric. 93

Figure 4.5 Niche breadth and generalists and specialists in soil fungal communities. 95

Figure 4.6 Co-occurrence patterns of cropping sensitive OTUs. 97

Figure 4.7 Cumulative relative abundance of the cropping sensitive modules in NCC, CC and CCi soil networks (y-axis reported cumulative relative abundance) at BS, CD and PD stage. NCC (forestgreen), CC (red), CCi (blue). 98

Figure 4.8 Spearman's correlations between modules and environmental variables in NCC, CC and CCi. Only significant correlations ($P < 0.05$) are shown. 99

Figure 4.9 Mean relative abundances of cropping-sensitive OTUs identified by indicator species analysis and edgeR. 102

Figure 5.1 Schematic of the experimental design for indoor controlled cultivation. 118

Figure 5.2 Soil morel mycelium abundance. 122

Figure 5.3 Soil properties. Ratio line chart of soil physicochemical characteristics over time.....124

Figure 5.4 Temporal dynamics of diversity and distribution patterns of crop-associated bacteriomes under NCC and CC regimes at stages BS, CD, and PD. 128

Figure 5.5 Top 10 relative abundances of bacteria at the phylum level.....130

Figure 5.6 Top 10 relative abundances of bacteria at the family level.....130

Figure 5.7 Relative influence of the ecological assembly process in the NCC and CC cropping systems.....133

Figure 5.8 Assembly processes of different communities and cropping systems using the null model.....134

Figure 5.9 Correlations between bacterial communities and soil physicochemical characteristics, relative abundance of morel soil, and primordial yield in the NCC and CC regimes.....136

Figure 5.10 Taxonomic composition of bacterial indicator OTUs present at > 3 % relative abundance in all treatments.....139

Figure 5.11 Heatmap of trophic types and N-cycling functions of the abundant indicator OTUs.....143

Figure 5.12 Responses of bacterial community pattern, assembly, and ecological function in non-continuous cropping (NCC) and continuous cropping (CC) regimes during morel cultivation.....144

LIST OF SYMBOLS

α	Alpha
β	Beta
cm	Centimetre
mm	Millimetre
h	Hour
min	Minutes
g	Gram
L	Litre
µL	Microlitre
mL	Millilitre
°C	Degrees celsius

LIST OF ABBREVIATIONS

OM	Content of organic matter
TN	Total nitrogen
AN	Alkali-hydrolysable nitrogen
TP	Total phosphor
AP	Available phosphor
TK	Total potassium
ITS	Internal transcribed spacer
16S rDNA	16S ribosomal RNA
PCoA	Principal coordinate analysis
ENB	Exogenous nutrition bags
WBPDA	Potato dextrose agar with wheat bran
NCC	Non-continuous cropping
CC	Continuous cropping
CCi	Continuous cropping soil sowed with isolate YN05
BS	Bare soil
CD	Conidial
PD	Primordial

LIST OF APPENDICES

Appendix A The ITS sequences of HX13 isolates

Appendix B The ITS sequences of YN05 isolates

Appendix C Ratio line chart of soil physicochemical characteristics over time

Appendix D Changes of top10 abundant fungal families

KESAN KOMPOSISI TANAH DAN MIKROBIOTA KE ATAS KULTUR MOREL HITAM BERTERUSAN DAN TIDAK BERTERUSAN

ABSTRAK

Morel hitam *Morchella sextelata* (Morchellaceae, Pezizales) adalah cendawan berharga boleh dimakan yang ditanam dalam skala besar, tetapi penurunan hasil yang teruk berlaku semasa penanaman berterusan. Kesan penanaman jangka panjang terhadap sifat tanah, ketidakseimbangan mikrobiom, dan kesannya terhadap hasil morel masih belum jelas. Untuk membantu mengisi jurang pengetahuan ini, kajian ini menggunakan eksperimen dalaman untuk menilai bagaimana rejim penanaman mempengaruhi sifat tanah, komuniti mikrobial, dan penghasilan morel. Kajian ini menilai kesan sistem penanaman tidak berterusan (NCC) dan penanaman berterusan (CC) pada tiga peringkat pembangunan *M. sextelata* (tanah pukal, peringkat konidial, dan primordial) dengan menggunakan metapengekodan palang rDNA dan analisis rangkaian. CC mengurangkan hasil morel primordia dengan ketara pada tahun ketiga berbanding NCC, dengan yang pertama menunjukkan pengurangan sebanyak 99.7%. Hasil kajian menunjukkan bahawa semasa tahun pertama, miselium *M. sextelata* mengatasi komuniti kulat tanah dengan mengurangkan kepelbagaian alfa dan keluasan nic corak kulat tanah pada kandungan yang lebih banyak berbanding rejim penanaman berterusan, yang menyebabkan hasil tanaman yang tinggi, tetapi mikobiom tanah yang kurang kompleks. Beg nutrisi eksogen dan benih miselia morel ditambah secara berturut-turut ke dalam tanah untuk mengekalkan penanaman berterusan. Penambahan nutrien tambahan merangsang pertumbuhan penguraian saprotrofik kulat. Aktiviti pengurangan saprotrof tanah, termasuk *M. sextelata*, menyebabkan peningkatan ketara dalam kandungan nutrien tanah. Ini membawa kepada kesan perencatan terhadap

pembentukan morel primordia, yang mengakibatkan penurunan mendadak dalam hasil morel di bawah keadaan penanaman berterusan. Inokulasi dengan miselia morel mempunyai kesan yang ketara terhadap kepelbagaian bakteria tanah. Ia mengurangkan kepelbagaian bakteria dalam rejim penanaman tidak berterusan (NCC). Sebaliknya, ia meningkatkan bilangan ahli bakteria umum dalam rejim penanaman berterusan (CC). Penanaman berterusan juga membawa kepada pengumpulan nutrien di dalam tanah, menyebabkan peralihan dari proses stokastik ke proses deterministik dalam komposisi komuniti bakteria. Tambahan lagi, inokulasi miselia memberi kesan positif kepada kelimpahan potensi bakteria kopiotrofik/denitrifikasi dan pengikat nitrogen manakala mengurangkan kelimpahan bakteria oligotrofik/nitrifikasi. Menariknya, kesan ini lebih ketara di NCC daripada di CC. Hasil ini menunjukkan bahawa peningkatan potensi bakteria oligotrofik/nitrifikasi dan pengikat nitrogen yang memudahkan penguraian nutrien dalam beg nutrien eksogen oleh morel, dengan itu dapat mengekalkan keseimbangan nitrogen di dalam tanah. Penemuan ini memberikan panduan untuk mengurangkan kesan buruk penanaman berterusan terhadap hasil morel dan meningkatkan pemahaman interaksi miselia morel dengan mikrobiom tanah.

EFFECT OF SOIL AND MICROBIOTA COMPOSITION IN BLACK MOREL CONTINUOUS AND NON-CONTINUOUS CULTURES

ABSTRACT

The black morel *Morchella sextelata* (Morchellaceae, Pezizales) is a valuable edible mushroom cultivated on a large scale, but severe yield decline occurs during continuous cropping. The effects of long-term cropping on soil properties, microbiome imbalances, and their impact on morel yield remain unclear. To help fill this knowledge gap, this study employed an indoor experiment to assess how cropping regimes affect soil properties, microbial communities, and morel production. This study assessed the impact of non-continuous cropping (NCC) and continuous cropping (CC) systems at three developing stages of *M. sextelata* development (bulk soil, conidial, and primordial stage) by using rDNA metabarcoding and network analysis. CC significantly reduced morel primordia yield in the third year compared to NCC, with the former showing a 99.7% reduction. The results showed that during the first year, *M. sextelata* mycelium overwhelmed the resident soil fungal community by reducing the alpha diversity and niche breadth of soil fungal patterns to a greater content compared to the continuous cropping regime, leading to high crop yield, but less complex soil mycobiome. To sustain continuous cropping, exogenous nutrition bags and morel mycelial spawn were consecutively added to the soil. The additional nutrient input stimulated the growth of fungal saprotrophic decomposers. The degrading activity of soil saprotrophs, including *M. sextelata*, caused a significant increase in soil nutrient content. This led to an inhibitory effect on the formation of morel primordia, resulting in a sharp decline in morel yield under continuous cropping conditions. Inoculation with morel mycelia had a notable impact on soil bacterial diversity. It decreased bacterial diversity in the non-

continuous cropping (NCC) regime. In contrast, it increased the number of generalist bacterial members in the continuous cropping (CC) regime. Continuous cropping also led to the accumulation of nutrients in the soil beds, causing a shift from a stochastic to a deterministic process in the composition of the bacterial community. Additionally, mycelial inoculation had a positive effect on the abundance of potential copiotrophic/denitrifying and N-fixing bacteria while decreasing the abundance of oligotrophic/nitrifying bacteria. Interestingly, this effect was more pronounced in the NCC than in the CC. These results suggest that the increase in potential copiotrophic/nitrifying and N-fixing bacteria facilitated the decomposition of nutrients in exogenous nutrient bags by morels, thereby maintaining nitrogen balance in the soil. These findings provide insights to mitigate the adverse effects of continuous cropping on morel yield and improve understanding of morel mycelia interactions with soil microbiomes.

CHAPTER 1

INTRODUCTION

1.1 Introduction

Morchella species, commonly known as morels, are highly sought-after edible fungi valued for their culinary appeal and nutritional value. Morels are rich in various polysaccharides, contributing to their nutritional and medicinal value. The main polysaccharides found in morels include β-glucans, mannans, and galactomannans. These compounds are known for their potential health benefits, such as boosting the immune system, reducing inflammation, and providing antioxidant properties. Their presence, along with essential amino acids and trace elements, enhances the overall nutritional profile of morels, making them a sought-after delicacy for both their taste and health benefits (Xie et al., 2024; Qiu et al., 2024). The historical use of morels for medicinal purposes is well documented and dates back to the Ming Dynasty. Li Shizhen described the use of morels to treat various stomach diseases in his famous pharmaceutical text "Compendium of Materia Medica" (1578 AD) (Liu et al., 2018). Today, morels, often referred to as "Guchhi", have significant economic and cultural importance in regions such as the northwestern Himalayas in India (Kumar et al., 2024).

Scientific research has revealed a number of potential health benefits associated with the consumption of morels. Studies have demonstrated their hepatoprotective properties (Nitha et al., 2013), their antioxidant capacity (Fu et al., 2013), their neuroprotective effects (Xiong et al., 2016) and even their antitumour and

immunomodulatory activities (Liu et al., 2016; Li et al., 2017). This unique combination of exquisite flavour and potential therapeutic benefits has made morels a highly valued commodity both in the culinary world and in the field of medicine (Meng et al., 2019; Badshah et al., 2021; Deng et al., 2024).

The increasing global appetite for morels has strongly promoted the cultivation of morels, especially in China (Liu et al., 2023). As wild morel populations are unable to meet the increasing demand, the country has seen a remarkable expansion in the cultivation of black morel varieties such as *Morchella sextelata*, *Morchella exima* and *Morchella importuna*. Recent estimates suggest that these cultivation efforts now cover around 10,000 hectares and yield an average of over 3,000 kg per hectare (Zhang et al., 2023; Deng et al., 2024). However, this rapid growth in morel cultivation is not without its challenges. Intensive cultivation methods have led to an increase in disease and pest infestations, posing a significant threat to yields and the long-term sustainability of this emerging industry (Liu et al., 2022).

Morchella spp. or morel has a prestigious position in culinary tradition due to their unique flavour profile and striking appearance (Xie et al., 2024). This esteemed status has led to a growing market demand that far exceeds the availability of wild morels (Liu et al., 2018), making cultivation a necessity. To maximise yields, many growers have adopted continuous cropping systems where the same morel species or even isolates are grown on the same soil beds without interruption (Chen et al., 2023). However, this intensive practise often results in decreasing yields over time (Liu et al.,

2022). Similar challenges, including yield decline and quality degradation, have been documented in the cultivation of other edible mushrooms such as *Agaricus bisporus* and *Ganoderma lucidum* in continuous cropping systems (Yuan et al., 2019; Fu et al., 2021).

Although morel cultivation has increased significantly, the long-term ecological consequences of permanent cultivation are still largely unexplored. In particular, the effects of repeated cultivation on the delicate balance of the soil microbiome and ultimately on the sustainable yield of morels need to be further investigated. This knowledge gap is particularly critical as the interactions between fungi and bacteria play an essential role in shaping soil health and function. Understanding the intricate interplay between *M. sextelata* mycelia and their associated soil microbial communities is critical for optimising large-scale cultivation. While there is some research on morel cultivation, these studies have mainly focused on maximising fruiting body production. The complex dynamics of fungal community development during the critical early growth stages of *M. sextelata* – mycelial expansion, conidia development and primordia formation, especially in continuous cropping systems - remain poorly characterised.

1.2 Rationale and significance of study

This research provides valuable new insights into the role of the soil mycobiome in relation to the continuous cultivation of *M. sextelata*. The results provide a deeper understanding of the complex interplay between morel mycelia, soil fungi and bacteria, especially during the critical early stages of morel development. This knowledge can be

used to develop targeted strategies to mitigate the harmful effects of soil-borne pathogens and to manipulate fungal and bacterial communities to increase morel production yields. By understanding the factors that contribute to decline and quality degradation in these systems, strategies can be developed to improve the sustainability and productivity of morel cultivation. It would be of significance for understanding the obstacles in the permanent cultivation of morels caused by the fluctuation of the mycobiome and the variations in the physicochemical properties of the soil. The study of the fungal and bacterial communities in the soil during the cultivation of morels is of crucial importance. This approach provides a dynamic understanding of the ecosystem and helps to identify both beneficial and harmful microbial species within the morel mycobiome.

1.3 Problem statement

The term "continuous cropping" refers to the practice of cultivating the same crop in a specific location for more than one year. In China, maximising yields often requires growing the same crop repeatedly on the same plot of land without any breaks, a practice that frequently poses challenges associated with continuous cropping. This approach is also common among farmers who grow morel isolates consecutively on the same farm beds to maintain high yields. However, this method of intensive cultivation tends to cause a gradual decline in yield over time. Unlike most edible fungi, which are typically grown in sterilised substrate packages, morels are cultivated directly in soil beds, where their mycelial growth and fruiting are influenced by interactions with

resident soil fungal communities. Previous studies have shown that morel yield is positively correlated with the alpha-diversity of soil fungal communities (Tan et al., 2021), while certain fungal species act as soil-borne pathogens that can negatively affect black morels (Yu et al., 2022).

The current research primarily focuses on the production of the morel fruiting bodies (Liu et al., 2022). However, little is understood about the fungal community dynamics during the early stages of the morel life cycle, such as mycelial and conidial development (Du & Yang, 2021). Although the role of bacteria in continuous morel cultivation remains unclear, research has demonstrated the presence of a complex microbiota associated with morels in both natural environments and production systems (Orlofsky et al., 2021). Alterations in bacterial community structure and composition have been observed during morel development, suggesting that morels can influence the soil microbiota. These changes appear to be associated with successful morel fruiting, irrespective of substrate type, cultivation method, or environment, whether it is a greenhouse or a natural habitat (Orlofsky et al., 2021; Tan et al., 2021; Liu et al., 2022; Zhang et al., 2023). Nevertheless, the bacterial community dynamics throughout continuous morel cultivation cycles remain largely unknown. Functional inference analyses have highlighted the importance of specific bacterial communities involved in nitrogen fixation, nitrification, and nutrient mobilisation during morel cultivation (Yu et al., 2022; Orlofsky et al., 2021; Pion et al., 2013). Therefore, understanding the mechanisms governing soil microbial communities and their properties during morel cultivation is crucial for managing the diversity, functions, and succession of these

communities. By gaining insights into these microbial dynamics, strategies can be developed to optimise morel cultivation and sustain long-term production systems.

1.4 Objectives of the study

1. To investigate the influence of continuous cultivation of the same morel isolate on primordia yield by conducting an indoor controlled comparative experiment.

2. To examine the relationship between the soil physicochemical factors and morel production under continuous and non-continuous cropping regimes.

3. To determine the fungal community pattern as well as to explore co-occurrence patterns of cropping-sensitive OTUs under continuous and non-continuous cropping regimes.

4. To determine community assemblage and ecological traits of soil bacteriome at different stages under continuous and non-continuous cropping regimes.

CHAPTER 2

LITERATURE REVIEW

2.1 General characteristics of morels

2.1.1 The life cycle of morels

The morel belongs to the phylum Ascomycota (order Pezizales), which also includes the genus Tuber (truffle). It comprises a large number of species that are widely distributed in the temperate zones of the world, with descriptions from the northern hemisphere (China, East Asia, Europe, USA) being particularly numerous (Du & Yang, 2021; Kumar et al., 2024). Its occurrence has also been described in some Mediterranean and subtropical regions (Pilz et al., 2007; Kuo et al., 2012). In the past, many morel species were described based on morphological characters, but due to the enormous phenotypic polymorphism, identification based on these traditional taxonomic criteria alone was neither easy nor precise (Pilz et al., 2007). Various environmental, biotic and abiotic factors can influence the growth, development and all morphological characteristics of the fungus, making identification even more complex (Kuo et al., 2012).

The ascocarps are the reproductive structures of morels, which serve to produce and spread the spores. After spore germination, a new life cycle begins with the growth of hyphae, which anastomose and form mycelial masses (Figure 2.1) (Du & Yang, 2021).

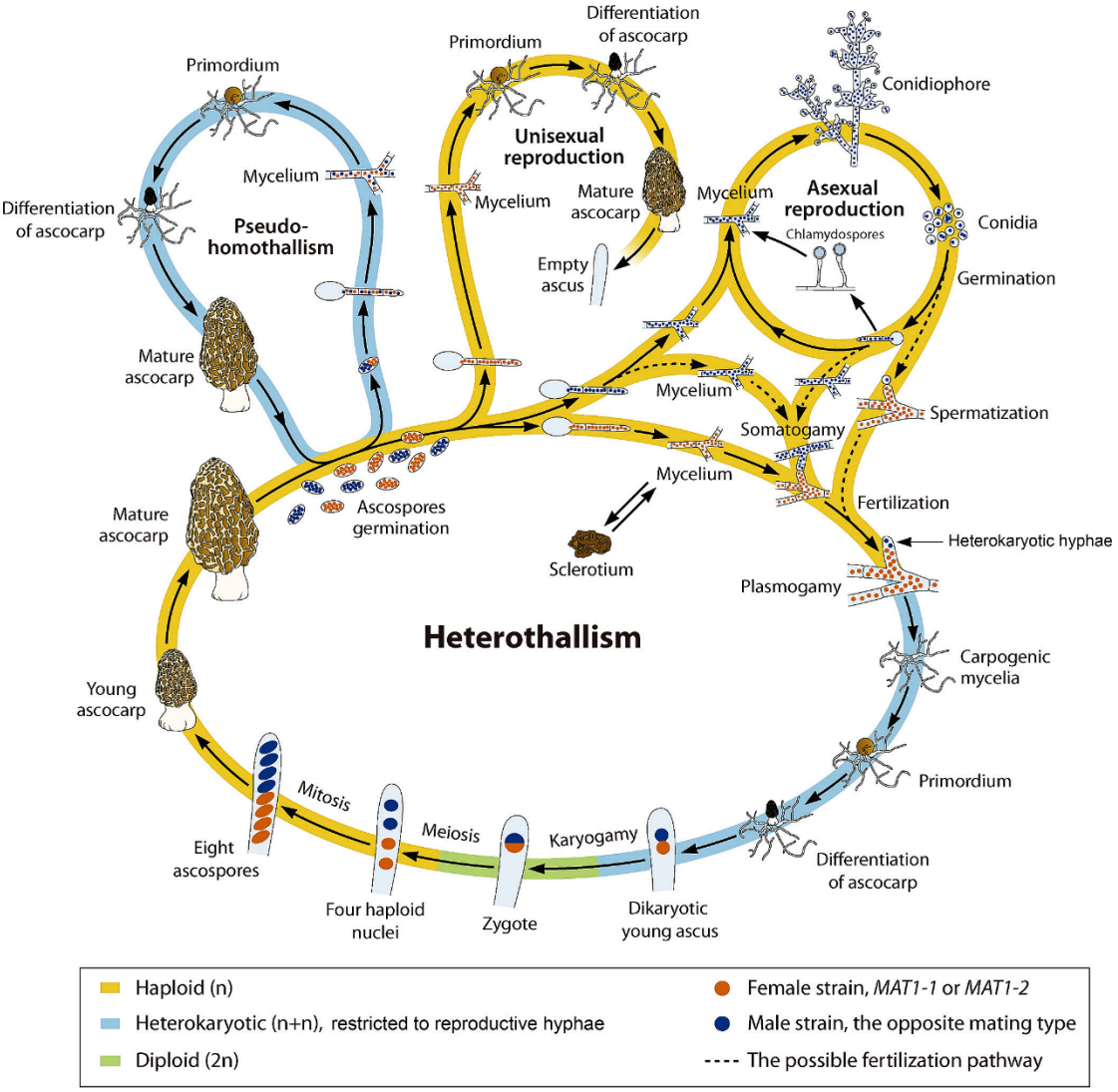

Figure 2.1 A generalized overview of the lifecycle of morel (Du & Yang 2021).

From there, the primordia are formed, which have the potential to produce a new ascocarp or fruiting body (Du et al., 2017). The life cycle described here is complex and briefly outlined as it does not include the asexual reproduction phase and many aspects are not yet fully understood. A life cycle has been proposed in which heterothallism has been identified as the predominant sexual mode of reproduction for morels (Du & Yang, 2021). This discovery greatly improves the understanding of the reproductive strategies of morels, which are highly valued for their edible and medicinal properties.

The hyphae form a number of structures, including conidia (mitospores), chlamydospores and sclerotia. The conidia (Figure 2.2) are thought to serve as reproductive organs as part of the asexual stage of the morel (Du & Yang, 2021; Liu et al., 2023). Chlamydospores and conidia are structures that are resistant to unfavourable environmental conditions, but the greatest interest lies in their possible association with the formation of ascocarps in artificial cultures (Liu et al., 2018). The sclerotia are dense mycelial masses with thick-walled hyphae, which are not found in other edible fungi and can reach sizes visible to the naked eye (5 cm) (Liu et al., 2018).

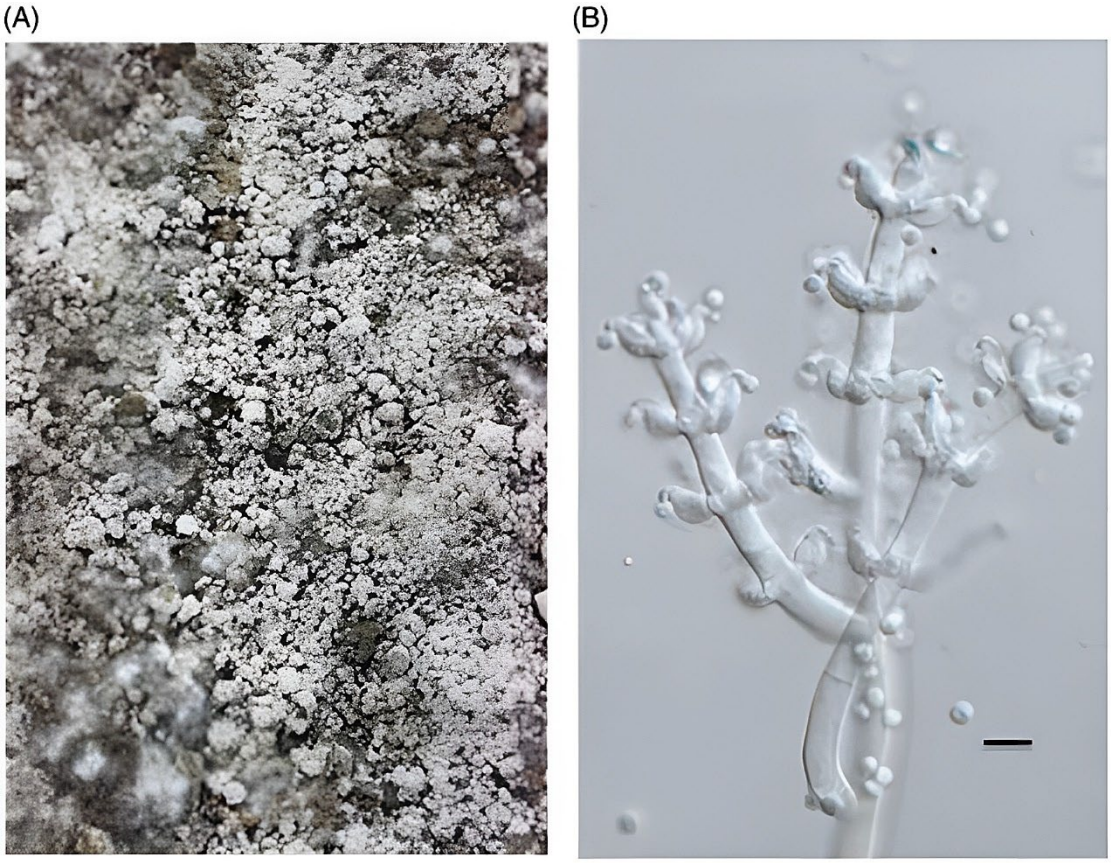

Figure 2.2 Powdery mildew and conidia in the outdoor cultivation of *Morchella importuna* (in this laboratory). (A) Powdery mildew in the soil; (B) Conidia (bar =10 μm) (Liu et al., 2018).

The sclerotia serve as nutrient storage structures that allow the fungus to survive under unfavourable conditions (drought, extreme temperatures, fire, snow, etc.), from which a new mycelium and even ascocarps can emerge under favourable conditions (Du & Yang, 2021; Liu et al., 2023). However, the specific conditions that induce the sclerotia to form either vegetative or carpogenic mycelia, which can later bear fruit, are still unknown.

2.1.2 Diversity of the morel species

The identification of morel species is crucial for understanding their biology and for assessing their growth and fruiting habits (Liu et al., 2018). Traditionally, the fungus has been identified based on macro- and micromorphological taxonomic characters, with more than 100 species described using dichotomous keys and binomial names. However, these methods are not very accurate as the morel has a large phenotypic polymorphism. For this reason, a taxonomic reclassification was carried out in 2011 (O'Donnell et al., 2011) using sections of conserved genes (Internal Transcribed Spacer (ITS), RNA Polymerase II Largest Subunit (RPB1), RNA Polymerase II Second Largest Subunit (RPB2), Elongation Factor 1-Alpha (EF1-α)) to accurately distinguish species (Du et al., 2016).

This revised phylogenetic framework, based on molecular data, established three distinct clades within the *Morchella* genus: the Elata Clade (black morels), the Esculenta Clade (yellow morels), and the Rufobrunnea Clade (Du et al., 2012; Kuo et al., 2012). This phylogenetic tree now serves as the foundation for the current

taxonomic classification of morels. To date, 78 phylospecies have been described globally, with many exhibiting a high degree of endemism, often restricted to specific geographic regions or continents (Du & Yang, 2021; Liu et al., 2023). A comprehensive maximum likelihood (ML) phylogenetic analysis, incorporating a four-gene dataset (ITS, RPB1, RPB2, and EF1-α) and utilizing Verpa and Disciotis as outgroups, was conducted on 75 *Morchella* species (Figure 2.3) (Du & Yang, 2021). However, it is important to note that *M. castaneae*, *M. vulgaris*, and *Morchella* sp. Mes-28 were excluded from this analysis due to the limited availability of genetic data, with only ITS sequences present in GenBank.

M. sextelata (Figure 2.3) belongs to the Elata Clade in the phylogenetic tree, which is grouped with *M. exuberans, M. importuna, M. oweri* and *M. eximia*. This indicates that the *M. sextelata* is evolutionarily relatively closely related to these species.

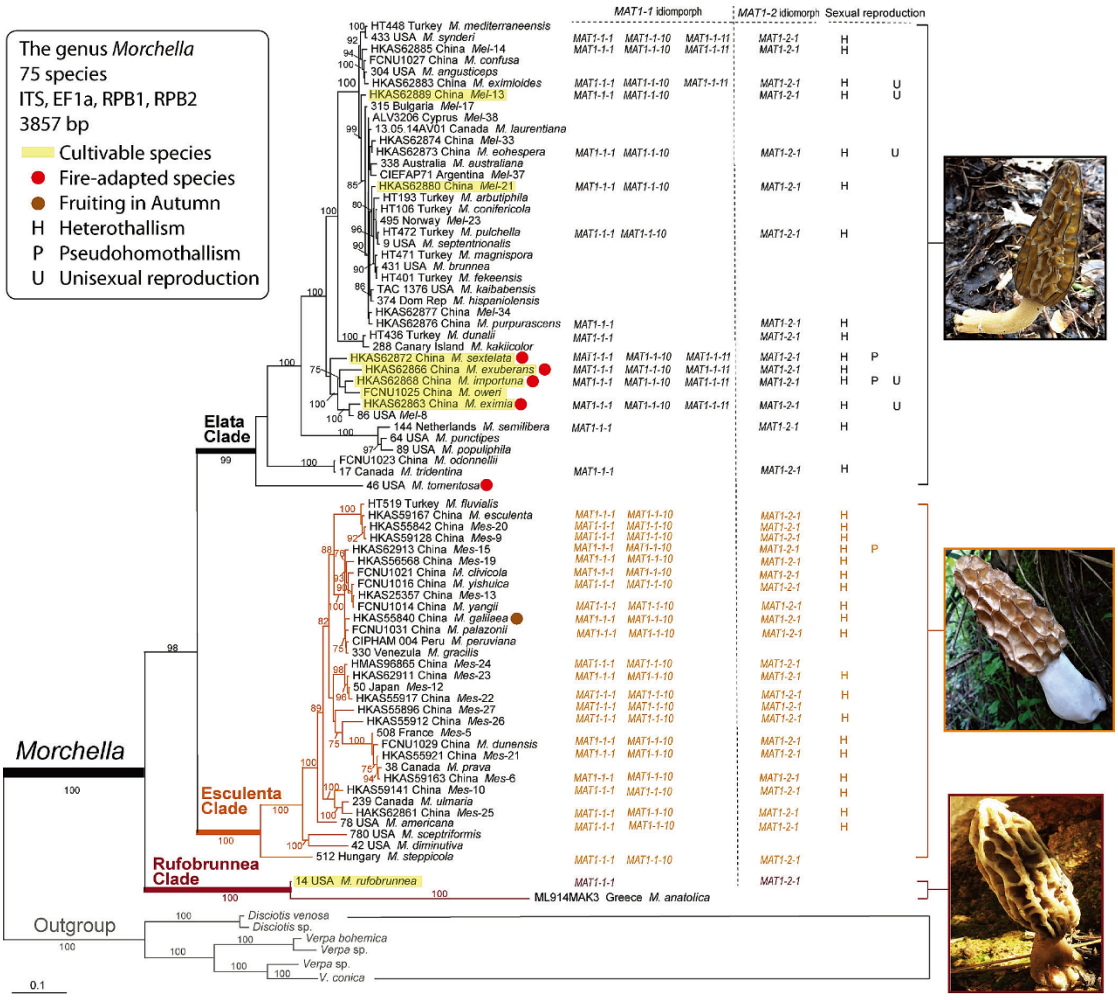

Figure 2.3 Maximum likelihood (ML) phylogenetic analysis of 75 species in the genus *Morchella* based on a four-gene dataset comprising ITS, EF1-a, RPB1, and RPB2, with Verpa and Disciotis serving as outgroups. The species *Morchella castaneae*, *M. vulgaris*, and *Morchella* sp. Mes-28 were excluded from this analysis due to the availability of only ITS sequences in GenBank. The ML analyses were performed using PhyML 3.0 with the GTR1I1G model of molecular evolution. Numbers adjacent to the nodes indicate branch support at the 75% level. Mating-type genes identified and known sexual reproduction modes for *Morchella* species are listed on the right (Du & Yang 2021).

2.1.3 Nutritional values of morels

As the "king of mushrooms", morels are not only known for their special flavour and high nutritional value, but also contain a variety of biological activities, such as hypoglycemic, anti-viral, and tumour-inhibiting properties (Cai et al., 2013; Yan et al., 2013; Li, 2018; Sun et al., 2019; Zhang et al., 2021), which play an important role in both Chinese and Western medicine. Morels can effectively scavenge free radicals and improve physiological function as well as learning and memory functions in rats with Alzheimer's disease (Liu et al., 2019). The biological activity of morels is related to their polysaccharides, amino acids, fatty acids, trace elements and other active components such as antioxidants, which have good antioxidant, antitumour and protective effects on the body (Qiu et al., 2024).

Mushrooms are low-fat, low-protein and low-calorie natural foods favoured by the majority of consumers (Li et al., 2022). Morels are rich in proteins, amino acids and unsaturated fatty acids, which are beneficial to the body (Nan et al., 2021). At the same time, the high content of polysaccharides and polyphenols coupled with cellulose, minerals and some functional components such as steroids and flavonoids contribute to their immune-boosting, antiviral and anti-fatigue properties (Gu et al., 2018).

Morel is a nutrient-rich food source with a higher crude protein content than many common mushroom varieties (Gu et al., 2018). Analysis of morel fruiting bodies has revealed the presence of 19 amino acids, including all 8 essential amino acids required for human health (Li et al., 2022). Furthermore, morels are rich in essential

macro and trace minerals, including potassium, calcium, magnesium, sodium and iron, as well as smaller amounts of zinc, manganese, copper, chromium, cobalt and nickel (Li et al., 2022). In addition to their mineral content, morels are a good source of various vitamins, such as B1, B2, B12 and biotin, which often exceed the levels of other mushrooms (Gu et al., 2018). These nutritional properties emphasise the value of morels as a high-quality food source for human consumption.

2.1.4 Heterothallism and mating type gene in morels

Heterothallism, the predominant mating system in *Morchella* species, requires the interaction of two distinct mating types, MAT1-1 and MAT1-2, for sexual reproduction. This bipolar heterothallic system promotes genetic diversity within populations, helping to prevent inbreeding and enhance genetic recombination (Du & Yang, 2021). Genetic studies of black morel species have demonstrated that these species engage in both sexual reproduction through heterothallism and asexual reproduction, allowing for adaptability under varying environmental conditions. This mixed reproductive strategy helps introduce genetic variation during favourable periods while also enabling asexual propagation in less suitable conditions (Du et al., 2017).

Genomic analysis of *M. importuna*, Mel-20, and *M. sextelata* has shown that these species are heterothallic, with significant genetic diversity at the MAT locus. This diversity underscores the role of heterothallism as a crucial reproductive system in these species (Chai et al., 2017). Additionally, de novo sequencing and comparative analysis

of opposite polarity monospores in *M. importuna* further support the centrality of heterothallism in its life cycle (Liu et al., 2018).

In studies of 22 yellow morel species, hybridization events were observed between different species. This suggests that heterothallism not only promotes sexual reproduction but may also drive interspecies hybridization, thereby increasing genetic diversity and shaping the evolutionary dynamics of *Morchella* species. (Du et al., 2020). Heterothallism, therefore, not only ensures sexual reproduction but also contributes to the complex reproductive strategies that allow *Morchella* species to adapt and evolve in diverse environments.

MAT genes are important regulatory loci that control sexual reproduction and development in fungi (Du & Yang, 2021). The two primary MAT genes, MAT1-1-1 and MAT1-2-1, are essential for successful sexual reproduction in morel species (Du & Yang, 2021). MAT 1-1-1 gene is typically associated with the MAT1-1 locus in heterothallic fungi. MAT 1-2-1 gene is found in the MAT1-2 locus and controls similar reproductive processes but operates in a complementary way to MAT1-1-1. To prevent a decrease in yield or even the risk of morel extinction, screening and identifying MATs through molecular markers is a crucial step in morel cultivation.

2.2 Continuous cropping obstacles of morels

2.2.1 Morel cultivation

As the current supply is based almost exclusively on wild collection, morels are an extremely valuable and often overexploited resource in their natural forest ecosystems. However, a significant breakthrough occurred about a decade ago with the successful development of commercial-scale artificial cultivation techniques for certain morel species in China (Liu et al., 2018; Xu et al., 2022; Xie et al., 2024). This technology has since been adopted in other countries with varying degrees of success (Kumar et al., 2024). Since its introduction in 2012, commercial morel cultivation in China has experienced explosive growth, expanding from a modest 200 hectares to an astonishing 16,466 hectares by 2021-2022 (Liu et al., 2023). This remarkable 80-fold increase within a decade illustrates the rapid adoption of this technology, with acreage now spanning the entire country (Figure 2.4).

M. sextelata species, found in the Sichuan, Shaanxi, and Yunnan provinces of China (Du & Yang, 2021), exhibits a unique ecological association with forest fires, often appearing in abundance in the years immediately following such events (Liu et al., 2018). Significantly, *M. sextelata* is among the few morel species that have been successfully cultivated through artificial means.

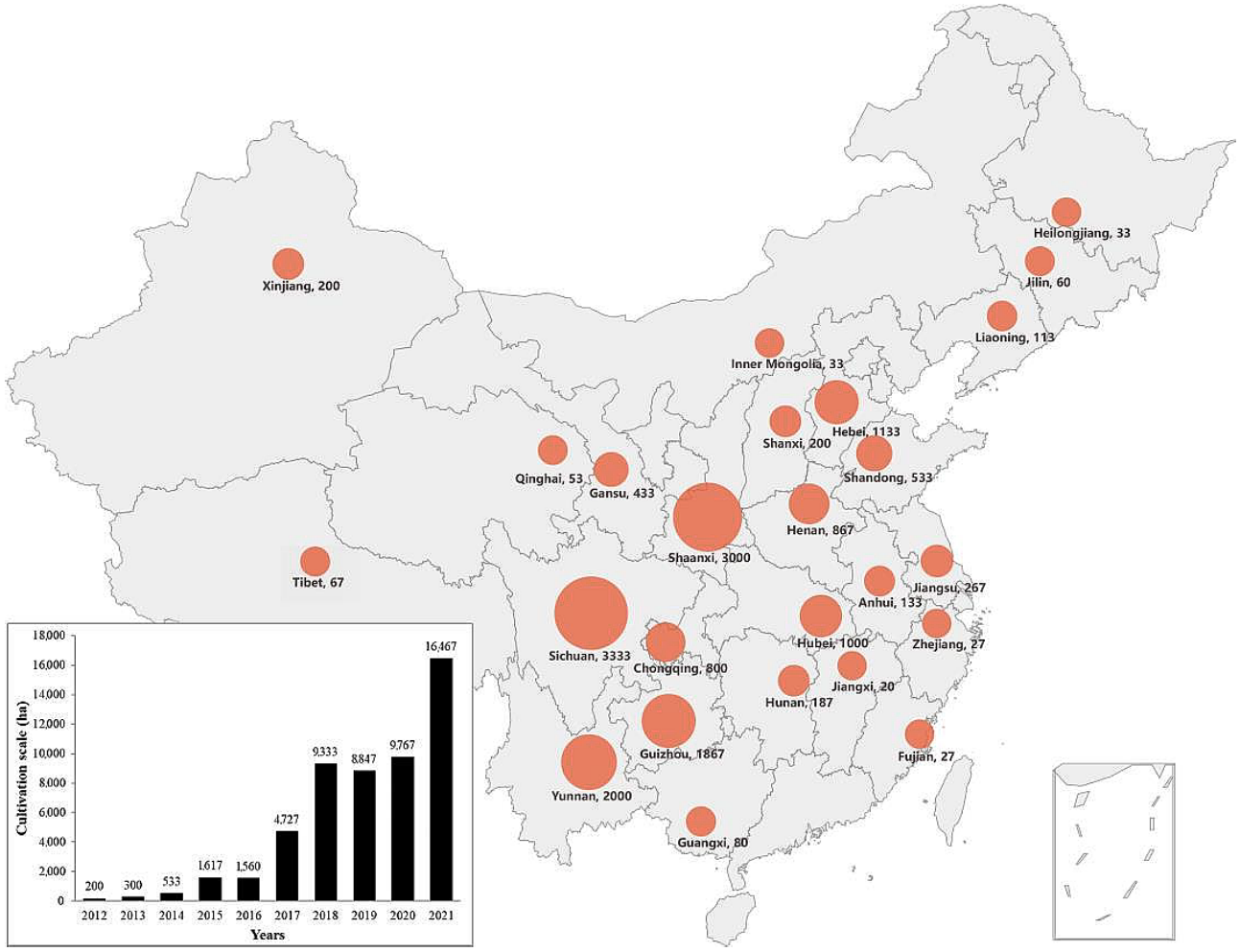

Figure 2.4 The industry of morels in China (Liu et al., 2023). The graph shows the annual change trend of morel cultivated area in China from 2012 to 2021, and the map shows the current areas in the country's provinces from 2021 to 2022, expressed in hectares after the province names and correlated with the size of the salmon-colored circles for each province.

2.2.2 Morel cultivation techniques

The cultivation of morels is complex and closely linked to their unique biology, in contrast to the relatively simple process for other edible mushrooms such as *Pleurotus ostreatus* and *Lentinula edodes*, which often use lignocellulosic substrates for fruiting. Morel can indeed grow on lignocellulose as a substrate, but they cannot complete their entire life cycle under these conditions alone. The presence of soil and possibly plants is essential for the completion of their life cycle. This suggests that morels have specific environmental requirements that they do not share with other mushroom such as *P. ostreatus* or *L. edodes*, which can fruit on lignocellulosic substrates without the need for soil or plants.

In China, France, Denmark and other countries, there are artificial morel production systems (indoor or outdoor) in which the mushroom is sown directly into the soil (greenhouse) or in agroforestry systems (Liu et al., 2018; Xu et al., 2022). Successful morel cultivation techniques have been developed in China since 2012, achieving yields of up to 3000 kg/ha (Xu et al., 2022; Zhang et al., 2023). The rapid expansion in China, with around 10,000 ha under cultivation, is due to the domestication of some morel species and the selection of suitable genotypes for soil inoculation, as well as the application of exogenous nutrient sources based on cereal grains and lignocellulosic residues (Liu et al., 2018; Xu et al., 2022). To date, the main species described as cultivable are *M. sextelata, M. eximia* and *M. importuna* (Xu et al., 2022),

which belong to the Elata group, are naturally associated with greenhouse environments and have a likely saprotrophic behaviour as common traits that enable their cultivation.

Two primary cultivation systems have emerged in the domestication and cultivation of morels: indoor cultivation (Figure 2.5 A) and outdoor cultivation (field cultivation) (Figure 2.5 B). The American scientist Ower (Ower, 1982; Liu et al., 2023) pioneered indoor cultivation in the 1980s. Ower's method focused on the production and utilisation of sclerotia, hardened mushroom masses that serve as "seeds". These sclerotia were cultivated and then germinated to eventually produce ascomata, the fruiting bodies of morels. His innovative approach was to provide the germination substrate with plastic bags containing moist rags or pieces of paper. These bags were enriched with a urea-rich nutrient solution to support the development of the fruiting bodies. Despite the pioneering nature of Ower's work, he did not succeed in spreading and implementing his method for indoor cultivation worldwide.

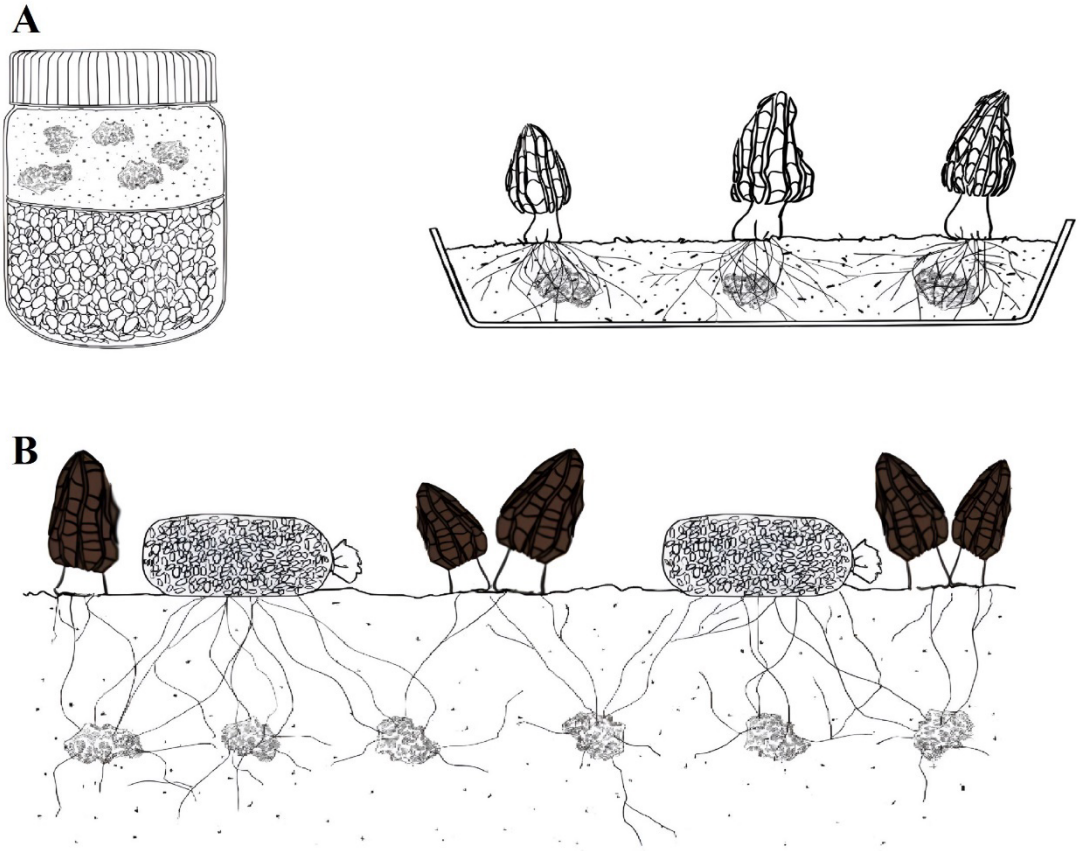

Figure 2.5 Schematic diagram of the two successful cultivation technologies of morels. (A) Ower's indoor method, using sclerotia as "seeds", sown in shallow trays, (B) Field cultivation method, where the addition of external nutrition bags boosts the ascomata formation. (Liu et al., 2023).

Despite the importance of vegetative morel cultures as a source of "starter cultures" for artificial outdoor cultivation, few studies have focused on their physiology and forms of preservation, an important topic as they appear to lose vigour easily and age faster than other edible mushrooms (Du & Yang, 2021). Once the 'spawn' is directly seeded into the soil, vegetative growth occurs, culminating in the production of reproductive structures such as conidiophores that disperse viable microconidia (Liu et al., 2018). At this stage, exogenous nutrient bags containing lignocellulose are applied, which are removed after some time, resulting in stress due to sudden nutrient limitation, which must lead to fruiting (Tan et al., 2021; Liu et al., 2023).

2.2.3 Continuous cropping obstacles

Due to various geographical constraints, many farmers tend to favour continuous planting. As a result, persistent problems associated with continuous cropping have emerged, resulting in lower yields or even complete failure of the morel crop, significant loss of farmers' income and considerable waste of land resources (Gao et al., 2021).

The practise of repeatedly cultivating the same or closely related plant species on the same soil, known as continuous cropping, can lead to a harmful syndrome known as "continuous cropping obstacle". This phenomenon manifests itself in declining plant vigour, which manifests itself in reduced growth, lower yields and quality, and increased susceptibility to pests and diseases (Wang et al., 2020; Chen et al., 2020). This problem is also referred to by various other names, including "soil sickness",

"replant disease", "field fatigue" and "monoculture" (Wang et al., 2015). The obstacle of continuous cropping affects a variety of plant species, both annual and perennial, and has been observed to varying degrees in cereal crops, crops, fruit trees, flowers, medicinal plants and edible mushrooms (Wang et al, 2020; Huang et al, 2021; Gao et al, 2021; Yuan et al, 2021; Chen et al, 2023).

Barriers to continuous cropping and their detrimental effects on plant health have been extensively studied, often using high-throughput amplicon sequencing techniques. For example, research on sugar beet has shown that continuous cropping can lead to a decline in beneficial soil microorganisms, an increase in pathogenic microbes and the accumulation of allelopathic compounds that negatively affect plant growth (Huang et al., 2019; Huang et al., 2021).

These obstacles are not limited to plants, but also have a significant impact on the cultivation of various edible fungi that grow in arable soils. Species such as *Dictyophora indusiata, Ganoderma lucidum* and *Agaricus bisporus* have all been shown to be susceptible to obstacles to continuous cultivation (Yuan et al., 2019; Fu et al., 2021; Yuan et al., 2021; Wu et al., 2022). For example, continuous cultivation of *A. bisporus* has been associated with an increase in soil-borne fungus such as *Penicillium* and *Mucor*. *Ganoderma lucidum,* a highly valued medicinal mushroom, faces similar challenges. Despite its market potential (Shen et al., 2024), continuous greenhouse cultivation of *G. lucidum* for 2-3 years often results in slow fruiting, smaller mushroom size, deformities and increased susceptibility to pests and diseases. Although improved

cultivation methods can alleviate some of these problems, yields often remain inconsistent. This decline is often attributed to the colonisation of the soil surface by competing fungal species that hinder the growth of *G. lucidum* (Ji et al., 2024). Similarly, *D. indusiata*, another nutritious and economically important fungus, suffers from yield fluctuations due to obstacles in continuous cultivation and increase in pests infestation and disease infection which hinders its sustainable production (Wu et al., 2022).

Black morels, like many other soil-cultivated fungi, face growing challenges related to continuous cropping practices (Chen et al., 2023). With the expansion of large-scale morel cultivation to meet global demand, these challenges are becoming increasingly evident and problematic. Continuous cultivation in morel production often leads to a decline in yields, sometimes even the complete absence of fruiting bodies (Liu et al., 2022). Despite these challenges, several black morel species have been successfully domesticated for large-scale production in recent years, a lucrative endeavour driven by the high market value of these valuable mushrooms (Liu et al., 2023). This has led to rapid growth of the industry both in China and internationally (Xu et al., 2022; Liu et al., 2023). However, the obstacle of continuous cultivation, characterised by declining yields, is a significant barrier to fully exploiting the potential of the morel industry (Liu et al., 2022; Chen et al., 2023).

2.2.1(a) Changes in soil physicochemical characteristics

Soil is a naturally occurring resource consisting of a mixture of minerals, organic matter, water and air (Philippot et al., 2024; Rillig et al., 2024). Soil is the basis for plant