Truffle Species Strongly Shapes its Surrounding Soil Mycobiota in a Pinus Armandii Forest

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Research Article

Keywords: hypogenous ectomycorrhizal fungi, truffles, soil nutrient, fungal community, mycobiota

Posted Date: March 11th, 2021

DOI: https://doi.org/10.21203/rs.3.rs-250458/v1

Abstract

Purpose: Truffles contribute to crucial dynamics in the soil systems, being involved in plentiful ecological functions important for ecosystems. Despite this, the interactions between truffles and surrounding mycobiota remain unknown. Here, we aimed to shed light on how much truffle species could affect their surrounding soil mycobiota.

Methods: Using traditional chemical analysis and ITS Illumina sequencing, we compared soil nutrients and the mycobiota in soil, gleba and peridium of the two truffle species, *Tuber indicum* (Ti) and *T. pseudohimalayense* (Tp), inhabiting the same *Pinus armandii* forest in southwestern China.

Result: Tp soil was more acidic and had higher nutrients (total C, N contents) than Ti soil. Fungal richness and diversity of ascomata tissues and surrounding soils were significantly higher in Tp than in Ti. Truffle species recruited unique soil mycobiota around its ascomata: in Ti soil, fungal taxa including *Suillus, Alternaria, Phacidium, Mycosphaerella, Halokirschsteiniothelia, Pseudogymnoascus* were abundant while in Tp soil the species of *Melanophyllum, Inocybe, Rhizopogon, Rhacidium,* and *Lecanicillium* showed higher abundances. Three dissimilarity tests including *adonis, anosim* and mrpp showed the differences of fungal community structure between the truffle and their surrounding soil was stronger in Tp than in Ti, and such differences extended to truffle tissues (peridium and gleba). Redundancy analysis further demonstrated that relationships between soil fungal taxa and soil properties had changed from negative (Tp) to positive (Ti) and shifted from a moisture-driving (Tp) to a total N-driving (Ti).

Conclusion: Overall, our results showed that the different interactions between truffle and soil system although the causative peculiarity of these associations needs to be further studied.

Background

Truffles (*Tuber* spp.) are hypogenous ectomycorrhizal fungi that produce fruiting bodies with economic value representing one of the most expensive foods worldwide (Zambonelli 2016). Due to this, the interest for truffles has increased during the last two centuries being extensively studied in many topics specially the ones related with mycelia, species diversity, associations with host trees and soil biological quality (Bonito et al. 2010; Liu et al. 2020; Innangi et al. 2020; Piñuela et al. 2021). During their life span, truffles inhabit in diverse and complex biotic environments, interacting as free mycelium, ascoma and/or mycorrhizal symbiont (Wenkart et al. 2001; Pacioni et al. 2014). The development of truffles starts with a pellet of mycelium that gradually grows into a globular ascoma which the outward cells differentiate into a protective layer (peridium) with pores as authentic entryways (Pacioni 1990; Zarivi et al. 2015). In most cases, during the process of truffle development, the inhibition in herbaceous growth and reduction in biodiversity of soil fungal communities occurred (De Miguel et al. 2014; Mello et al. 2015), due to phytotoxic metabolites emitted by some truffle species (*Tuber aestivum* Vittad., *T. melanosporum* Vittad., and *T. indicum* Cooke & Massee) (Pacioni et al. 1990) and allelopathy actions (Streiblová et al. 2012). Competition among mycelia from different species for soil nutrients and water might also be an

important ecological explanation for the phenomenon of biodiversity reduction (Sourzat 2004), and such competitive ability could vary among truffle *s*pecies. For instance, in greenhouse conditions, researches on the synthesis of different truffle ectomycorrhizae and the ecology of truffle-colonized seedlings have showed that i) the changes of soil nutrients varied among truffle species, for instance *T. melanosporum* inoculation on *Carya illinoinensis* seedlings had weak influence on soil N contents (Zhang et al. 2019), but *T. borchii* and *Tuber panzhihuanense* can strongly modify soil easily-available nutrients (i.e., N, P) and exchangeable cations (Li et al. 2019; Yang et al. 2019; Zhang et al. 2020); ii) truffle could shape ectomycorrhizosphere soil microbial community composition (Li et al. 2019; Yang et al. 2019; Zhang et al. 2019, 2020), and such influence was stronger for bacterial than for fungal community (Li et al. 2019; Yang et al. 2019; Liu et al. 2020). These studies have deepened our understanding of the complex "truffle-plant-soil-microbe" interactions; however, due to major experiments were truffle-cultivation oriented and mainly carried out in greenhouse conditions, changes of truffle-soil-microbe interaction in natural/field soil conditions could not well captured. On the other hand, although studies regarding the bacterial diversity associated with truffle are highly available (Mello et al. 2013; Barbieri et al. 2016; Zampieri et al. 2016), the knowledge of the fungal communities in truffles and/or surrounding soil is insufficient. In fact, the fungal communities have a close interaction with truffle peridium by colonizing through peridium pores to the gleba (Zambonelli 2016). At present, the tripartite interactions between truffle species, soil property and fungal community in truffle soil need to be further explored. For truffles that growth in natural conditions with same host trees, will the soil properties and the composition of fungal communities in soil around ascomata vary between truffle species? If so, what are the main relationships between truffle taxa and soil properties? To understand how truffle species could affect its surrounding soil physicochemical properties, soil mycobiota, and their interactive relationships, two commercially important truffle species (*Tuber indicum* and *T. pseudohimalayense*) were examined by routine soil chemical analyses, ITS Illumina sequencing and redundancy analysis. These two ectomycorrhizal fungal species display similar morphology (Manjón et al. 2009) and associate with the same *Pinus armandii* Franch. tree in southwest China (Juan et al. 2011). This study addressed the following hypotheses: H1: the two fungal species could differentially affect the surrounding soil properties; H2: the fungal diversity and community structure could be different in truffle producing soils and ascomata; and H3: the driving factor of the relationship between fungal taxa and soil properties would be different at each truffle's niche.

Results

Soil cation and nutrients changes

Soil moisture was similar among the three soil positions/treatments, no matter whether soil surrounded the ascomata or not (Table 1). Soil pH values were closed to neutral with the highest and lowest values in soils around and below the ascomata of *T. indicum* and the bulk soil, respectively (Table 1). The concentrations of Ca^{2+} and Mg^{2+} were significantly higher ($P < 0.05$) in the truffle soil of Ti, compared to Tp and the bulk soil (Table 1). In contrast, the concentrations of soil organic matter, total carbon and total

nitrogen were similar among the bulk soil, Ti soil and Tp soil (Table 1). Whereas, the C:N ratio and the alkaline hydrolysable nitrogen content were significantly higher ($P < 0.05$) in the truffle soil of Tp, compared to Ti soil (Table 1).

Table 1
Soil physicochemical properties in the soils around the ascomata of *Tuber indicum* and *Tuber pseudohimalyense.*

Treatment	Moisture (%)	pH	Ca^{2+} (mg kg^{-1})	Mg^{2+} (mg kg^{-1})	OM (g kg^{-1})	TC (g kg^{-1})	TN (g kg^{-1})	C:N ratio	AN (mg kg^{-1})
S_C	28 ± 3a	6.29 ± 0.10b	3935 ± 242b	627 ± 36b	80 ± 4a	46.3 ± 2.2a	3.64 ± 0.06a	12.7 ± 0.4b	319 ± 4a
S_{Ti}	29 ± 1a	6.62 ± 0.05a	6035 ± 221a	1116 ± 75a	78 ± 10a	45.1 ± 6.8a	3.68 ± 0.22a	12.2 ± 0.3b	283 ± 6b
S_{Tp}	30 ± 2a	6.42 ± 0.06ab	4421 ± 303b	786 ± 27b	88 ± 3a	51.2 ± 1.5a	3.78 ± 0.15a	13.6 ± 0.2a	316 ± 5a

Values (means ± SD, $n = 3$) followed by different letters are significantly different at $P < 0.05$ (ANOVA, *Tukey* HSD). Abbreviations: AN = alkaline hydrolysable nitrogen; OM = organic matter; S_C = bulk or control soils (ten meters away where had no any truffle); S_{Ti} = soils around and below the ascomata of *T. indicum;* S_{Tp} = soils around and below the ascomata of *T. pseudohimalayense;* TC = total carbon; TN = total nitrogen.

Fungal diversity changes

About 138,854 – 652,726 raw reads were obtained per sample and the reads length varied from 250 to 438 bp, with an average of 343 bp (Table S1). A range of 130,532–626,071 sequences from individual samples (mean = 317,012) were obtained (Table S1). A total of 6,657,258 high quality sequences from all 21 samples (soil, gleba and peridium of the two truffle species), were represented for 10 phyla, 21 classes, 96 orders and 179 families of fungi. The observed fungal species was around 200 and similar between Ti and Tp ascomata. The fungal community diversity was similar in the peridium and gleba as shown by the Shannon diversity index (considering both richness and evenness) (Fig. 1). The soil fungal richness and diversity from the soil around ascomata tissues were significantly higher ($P < 0.05$) in Tp than in Ti (Fig. 1). Similarly, the numbers of unique OTUs in the soil around truffle were also higher in Tp than in Ti (Fig. 2).

Distribution of fungal taxa and community structure

The two-way cluster analysis of the relative abundance of major fungal genera showed a clear separation of two clusters for truffle surrounding soils and ascomata tissues (Fig. 3).

In Ti soil, six fungal taxa including *Suillus, Alternaria, Phacidium, Mycosphaerella, Halokirschsteiniothelia, Pseudogymnoascus* were dominant (reddish color) corresponding to species of *Suillus, Alternaria* and *Phacidium* (dark red). Over twenty fungal taxa were in the Tp soil where species of *Melanophyllum, Inocybe, Rhizopogon, Rhacidium*, and *Lecanicillium* showed higher abundances. Compared with the truffle surrounding soils, fungal community inhabiting in the ascomata tissues showed less abundances, such as *Candida* in *T. indicum* and C*andida, Acremonium*, *Mortierella* in *T. pseudohimalayense* (Fig. 3). Principal coordinates analysis (PCoA) showed that in the overall fungal community structures (beta-diversity) of the control and truffle soils were separated from those of truffle peridium and gleba (Fig. 4). These results were statistically supported by the three dissimilarity tests including *adonis*, *anosim* and *mrpp* ($P < 0.05$). The differences of fungal community structure between the truffle and their surrounding soil was stronger in Tp (*anosim*, r = 0.98, $P = 0.001$; Fig. 4) than in Ti (*anosim*, r = 0.48, $P = 0.01$; Fig. 4). Such differences extended to truffle tissues (peridium and gleba). The truffle fungal community structure was also separated in Tp tissues (*anosim*, R = 0.90, $P = 0.001$) and in Ti tissues (*anosim*, R = 0.68, $P = 0.045$).

Effects of soil properties on fungal communities

Redundancy analysis (RDA) showed relationships of the most influential soil properties and fungal genera in both Ti and Tp soil. For Ti, the first and second axis of the RDA explained 92.7% and 7.3% variations in soil fungal taxa (Fig. 5A). The variation in soil fungal taxa was strongly driven by total N with a contribution (explained fitted variation) > 90%, and slightly driven by pH (7.5%). Among the 30 top fungal taxa, almost all of them (except for *Chalara*) exhibited positive correlations with soil pH and total N. Species of *Tuber*, *Rhizopogon, Inocybe*, and *Acremonium* showed pronounced correlations as shown by their closer coordinate positions (Fig. 5A) and was confirmed by significant positive correlations ($P < 0.001$; Figure S3). In Tp soil, the first and second axis of the RDA explained among the 79.0% and 20.1% variations for fungal taxa (Fig. 5B), mainly driven by soil moisture (78% variation explained) and partly by total N (21% explained; Fig. 5). Different from the relationships of soil properties and fungal genera in Ti, most fungal taxa in Tp surrounding soil exhibited negative correlations with the soil total N and moisture. There were only nine fungal taxa including *Tuber* which showed positive correlations, among them *Lecanicillium, Wilcoxina* and *Trichiophaea* were significant ($P < 0.001$; Figure S2).

Discussion

As we hyphothezied (H1), the soil properties were affected by the truffle species showing different Ca^{2+}, Mg^{2+}, C/N ratio in the truffles surrounding soils (Table 1). Ti seemed to be able to mobilize more Ca^{2+} and Mg^{2+} in the surrounding soils as indicated by the significantly higher ions concentrations in Ti soil as compared those of in Tp soil (Table 1). Similar results were reported by Hilszczańska et al. (2019) who mentioned that truffle abundance largely related to active carbonate content rather than soil pH. Most truffle species can tolerate a wide range of soil pH from slightly acidic, neutral (Li et al. 2017, 2018; Innangi et al. 2020) to alkaline (Mello et al. 2006). We found Tp has formed in slight acidic soil (pH = 6.4) in its surrounding as compared with Ti (pH = 6.6; Table 1). Noticeably, while the total C and N contents

were not significantly affected by truffle species, the C/N ratios were. The fungal species present in Tp soils (many members from Chytridiomycota) could be responsible for the reduction of C/N ratio, via mineralizing more organic N than organic C, with consequent variation in organic matter quality (Innangi et al. 2020). In addition, the significantly higher hydrolysable N found in Tp than in Ti surrounding soil (Table 1) supported this argument.

We hypothesized that the truffle species would form its unique soil fungal community around its ascomata and the difference in fungal taxa might also exist in truffle tissues (H2). As expected, Tp surrounding soil exhibited significantly higher fungal richness and diversity, compared to Ti (Fig. 1) and the change was in line with that in the soil C/N ratio and TC contents (Tp > Ti). The difference in the fungal diversity of the two truffle surrounding soils could relate to i) a closer association between soil fungi and nutrients such as TC, C/N ratio etc. (Liu et al. 2019; Yang et al. 2020); ii) differences in truffle mycelia might trigger soil fungal diversity variation in truffle surrounding area since the ascomata tissues appeared only when certain amounts of mycelia formed (Suz et al. 2008): the competitive ability of Ti mycelium could be stronger than that of Tp, which might occupy a wider ecological niche which in turn, leading to a significant decrease in the diversity of soil mycobiota. It was admitted that the mycelial abundance from the two truffle species was not recorded in the present study, however in the two truffle soils, the changes in the number of dominant/abundant fungal taxa (21 in Tp vs. 6 in Ti), as well as the unique fungal OTUs (Figs. 2 and 3) indirectly reflected various fungal competitive environments from surrounding soils.

When moving from control soil where truffles (T. *melanosporum*) were not presented, fungal community tend to shift from Basidiomycota- to Ascomycota-dominated (Mello et al. 2011). We found a relatively higher abundance of Ascomycota (65%) in Tp soil, compared to Ti soil (25%), which might reflect a stronger competition of Tp with other Basidiomycota (Napoli et al. 2010). Herein, we proposed a conceptual model to explain the effect of truffle species on its surrounding soil mycobiota and soil properties (Fig. 6).

In support of the H2, changes in soil fungal community influenced truffle inhabiting fungi composition and structure (Figs. 3 and 5). The changes in truffle surrounding soil properties and soil fungal diversity might induce further variations in the relationship between fungal taxa and soil properties (Fig. 6). As we hypothesized (H3), major relationships between truffle surrounding fungal taxa and soil properties had changed from a total N-driving (Ti) to a moisture-driving (Tp) (Figs. 5 and 6), accompanying by a shift from negative (Ti) to positive (Tp) (Figure S1). The tripartite interactions between truffle species, soil nutrients and fungal diversity finally thus shaped a varied relationship between soil and fungi. It should be noted that the conceptual model we proposed (Fig. 6) was based on the comparison between the two species within a similar habitat, which highlighted the important role from truffle itself. A systemic investigation in interactions between truffle and soil systems in further research is highly recommended, with consideration of multivariable properties such as soil texture, soil enzyme, and climatic factors. On the other hand, culture-based methods could be used in combination with Illumina sequencing to confirm the presence of certain fungal taxa associated with the ascomatas.

Conclusions

Our results showed a clear difference in soil properties and fungal diversity from soils around the two truffle species (*T. indicum* and *T. pseudohimalayense*) in the same *P. armandii* forest. The presence of *T. pseudohimalayense* appeared to have a stronger influence to modify the soil environment, leading to changes in its chemistry (lower pH and higher nutrient contents). Soil nutrients, truffle species, and other soil fungal taxa are interactive. Such influences could form a complex and competitive environment in truffle unique ecological niches, and the effect might further extend to truffle tissues. From the ecological point of view, whether the differences in the mycocenosis can affect the entire mycelial biomass of the two species of truffle needs to be explored to understand the interactions of hypogenous ectomycorrhizal fungi with soil systems.

Methods

Study site and sampling strategy

The sampling site is located in one of the Chinese truffle hotspots in Huidong county (26°22'48"N, 102°24'36"E, 2745 m a.s.l.) (Fu et al. 2016), Sichuan province, southwest China. The site is a pure *P. armandii* Franch forest with the following ecological traits: annual variation of air temperature ranged between 11 ~ 24°C; annual precipitation was 1099 mm (Fu et al. 2016), sandy loam (Haplic Luvisol, FAO Soil Classification System) soil (Fu et al. 2016). Sampling of soil and truffles was carried out at the truffle producing period on December 2018. In order to capture the variation present in the forest, we adopted a "3 × 6" sampling strategy, that is, 3 plots (100 m away from each other; size 200 × 200 m) were chosen as field ecological replicates, within each plot we randomly digged six truffles. Soil samples in each plot were respectively collected from soil around and below the ascomata of *T. indicum* (S_{Ti}), *T. pseudohimalayense* (S_{Tp}), and bulk or control soils (S_C, ten meters away where no ascomata were detected). Soils were immediately stored in a cooler and transported to the laboratory where they were sieved (2 mm) to remove stone, root, and microfauna under aseptic conditions. Half of each composite soil sample (six samples from each plot) was stored at − 20°C for microbial analysis and the rest soils were air-dried for chemical analyses.

Three composited fungal tissue samples (each having 18 cutting slices obtained with a sterilized scalpel from six fruiting bodies of *T. indicum* or *T. pseudohimalayense*) were also respectively collected from the gleba or peridium of *T. indicum* (G_{Ti} or P_{Ti}) and *T. pseudohimalayense* (G_{Tp} or P_{Tp}). After clean with sterilized milli-Q water, the peridium and gleba tissues of six selected ascomata from each plot were sampled using a sterilized scalpel, composited and then stored in sterilized self-sealing bags (60 mm × 85 mm) at − 20°C for subsequent DNA extraction.

Soil property analysis

Soil pH was determined in a soil and distilled water (1:2.5, W/V) mixture using a Delta 320 pH meter (Mettler-Toledo Instruments, Shanghai, China). Soil moisture was gravimetrically measured by oven drying at 105°C for 24 h. Soil organic matter was determined with the potassium dichromate external heating method (Guo 2009). Soil total carbon (TC) and total nitrogen (TN) were measured with an elemental analyzer (Vario MAX C/N, Hanau, Germany) (Parkinson and Allen 1975). Determination of alkaline hydrolyzable N, calcium (Ca^{2+}), and magnesium (Mg^{2+}) was based on the Chinese national standard method (Nu 1999).

DNA extraction and PCR amplification

DNA from soil and truffle samples were extracted using the MoBioPower Soil DNA kit (12888) and the DNeasy Plant Mini Kit (Qiagen SA, Germany), respectively. The ascomata of Ti and Tp were identified by both morphological and molecular techniques in the Kunming Institute of Botany, Chinese Academy of Sciences, Kunming, China. Polymerase chain reactions (PCR) were carried out following the previously described method (Xiong et al. 2016). Internal transcribed spacer 1 (ITS 1) was amplified for fungal community analyses, using universal primers ITS5-1737F and ITS2-2043R (Schultz 2005; Jeandroz et al. 2008). For PCR, all the samples were uniformly diluted to 20 ng/μL and PCR reactions were performed in triplicate in a 25 μL mixture (5 μL of 5× reaction buffer, 5 μL of 5×GC buffer, 2 μL of dNTP(2.5 mM), 1 μL of each primer, 2 μL of template DNA, 8.75 μL of DNase free water and 0.25 μL Q5 DNA polymerase). PCR thermal cycling conditions were 94°C for 5 min (initial denaturation), 30 cycles of 30 s at 94°C, 52°C 30 s, 72° C 30 s, with a final extension for 10 min at 72°C. Amplicons were extracted from 1 % agarose gels and purified with the EZNA Gel Extraction Kit (Omega, Bio-Tech, New York, USA) according to the manufacturer's guidelines and quantified with PicoGreen using a FLUOstar Optima microplate reader (BMG Labtech, Jena, Germany).

Illumina MiSeq sequencing and bioinformatics

Purified amplicons were pair-end sequenced 2 × 300 on the Illumina MiSeq platform (MAGIGE, Guangdong, China) using the MiSeq Reagent Kit v2 (600-cycles-PE, MS-102-3003). Sequences were processed and quality-filtered using the QIIME (V1.9.1) pipeline. Paired-end reads were truncated to 100 bp to remove low-quality sequence tails (average quality values < 20 over a 10-bp sliding window. The ≥ 10 bp that passed through quality screening overlapping sequences were assembled using the FLASH software (v1.2.11) (Magoč and Salzberg 2011). The remaining high-quality sequences were clustered into operational taxonomic units (OTUs) at a 97% similarity cutoff. For species identification, we compared our sequence with the one deposited the UNITE database (for ITS, http://unite.ut.ee/index.php) using a confidence threshold ≥ 0.5. The OTUs assigned to the same phylum, class, genus, and species level were grouped together based on their taxonomic affiliations.

Data processing and statistical analysis

Shannon index and the observed species were used to evaluate fungal diversity and richness in soils and ascomata, respectively. One-way analysis of variance (ANOVA) followed by Tukey HSD (at $P < 0.05$) was used to compare significant differences in soil properties of the control, *T. indicum* and *T.*

pseudohimalyense surrounding soils. Independent samples T-tests were applied to compare significant differences in diversity indices between *T. indicum* and *T. pseudohimalyense*. Beta-diversity from the overall microbial communities between paired samples were determined using the UniFrac metric (Lozupone et al. 2011) in the MOTHUR program (http://www.mothur. org). Principal Coordinate Analysis (PCoA) was performed by the vegan package of R software based on the weighted Unifrac distance matrix, and the obtained coordinate points were plotted using the ggplot 2 package in R software. Analysis of similarity (Anosim), non-parametric multivariate analysis of variance (Adonis) using distance matrices, and a multi-response permutation procedure (Mrpp) were used to examine fungal community differences (CLARKE 1993; Sickle 1997; Zapala and Schork 2006). Redundancy analysis (RDA) was used to analyze the relationship between fungal communities and soil properties. RDA is advantageous of assessing the explanatory power of each defined variable by parsing out other terms as constraints to calculate its proportion of total variance (O'Connor 1988).

Abbreviations

ANOVA: Analysis of variance; PCR: Polymerase chain reaction; QIIME: Quantitative Insights into Microbial Ecology; OTU: Operational taxonomic unit; rRNA: Ribosomal RNA

Declarations

Ethics approval and consent to participate

Not applicable.

Consent for publication

Not applicable.

Availability of data and materials

Raw sequence data were deposited in the NCBI under the accession number PRJNA649675.

Competing interests

The authors declare that they have no competing interests.

Funding

This research was funded by the CAS "Light of West China" Program (Y923217), Basic Research - General Program of Yunnan Province, China (202001AT070092), Science and Technology Service Network Initiative, Chinese Academy of Sciences (2017), and Guizhou Science and Technology Program (4002, 2018).

Authors' contributions

DL developed the concept. JPM and FQY designed all the experiments. DL and MH performed the lab experiments. DL performed the statistical analyses, constructed the figures and interpreted data. DL, JPM and XH wrote the manuscript. MH and XH critically reviewed the manuscript. All authors discussed the results, critically reviewed the manuscript and approved its publication.

Acknowledgments

The authors are grateful to Shanping Wan comments for the first draft, to assistant engineer Peng Zhang and Ran Wang for invaluable assistance during mushroom collecting. We thank technicians in the elemental content analysis center, and Mr. Zhonghua Li for technical assistance in laboratory and data analyses.

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Figures

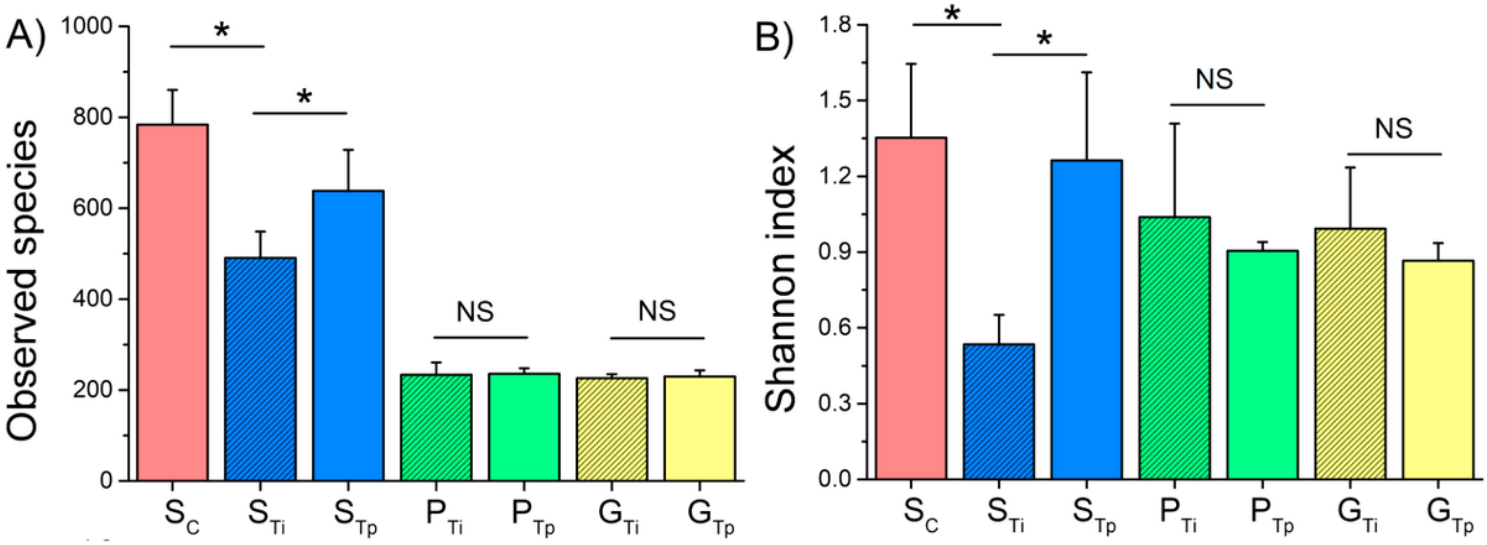

Figure 1

Fungal species richness (A) and shannon index (B) in the control and truffle surrounding soils, and fungal tissues of Tuber indicum (Ti) and T. pseudohimalyense (Tp) occurring in a P. armandii forest in Huidong, Sichuan, southwest China. For individual index boxes, differences between treatments were analyzed by independent samples T-tests; * P < 0.05. Abbreviations: GTi = gleba of T. indicum; GTp = gleba of T. pseudohimalyense; PTi = peridium of T. indicum; PTp = peridium of T. pseudohimalyense. SC = control soils; STi = soils around and below the ascomata of T. indicum; STp = soils around and below the ascomata of T. pseudohimalayense.

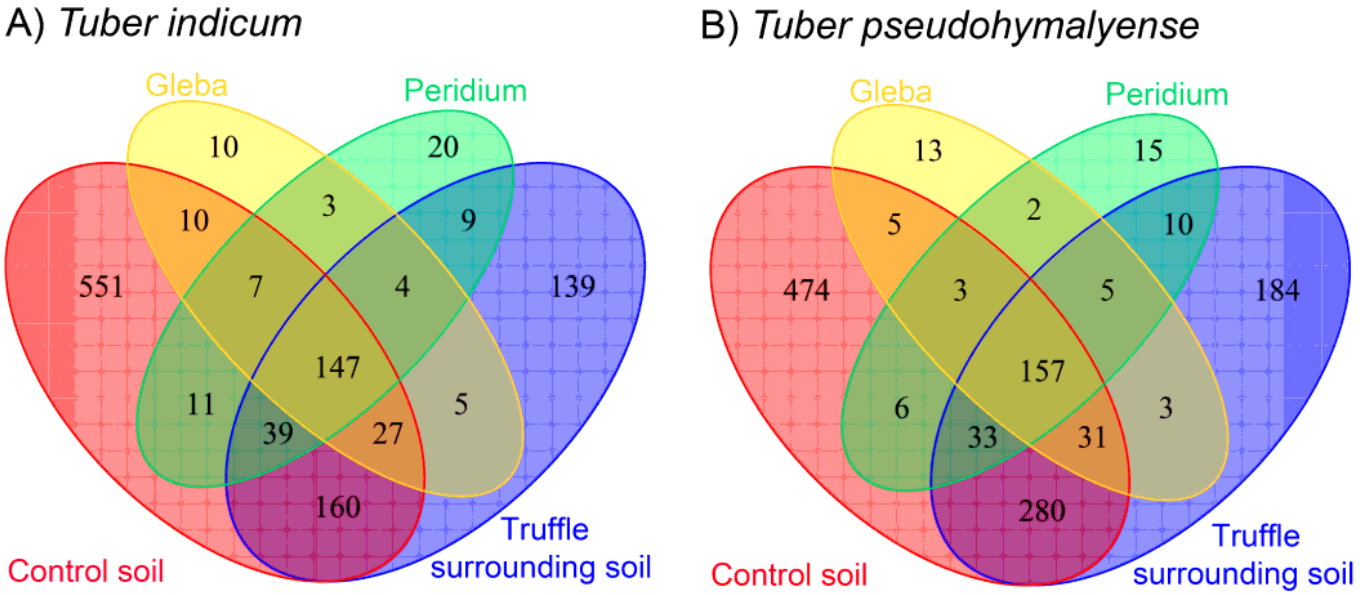

Figure 2

Shared and unique fungal OTUs in the control and truffle surrounding soils, and fungal tissues of A) Tuber indicum and B) T. pseudohimalyense occurring in a P. armandii forest in Huidong, Sichuan, southwest China. Abbreviations are shown in Figure 1.

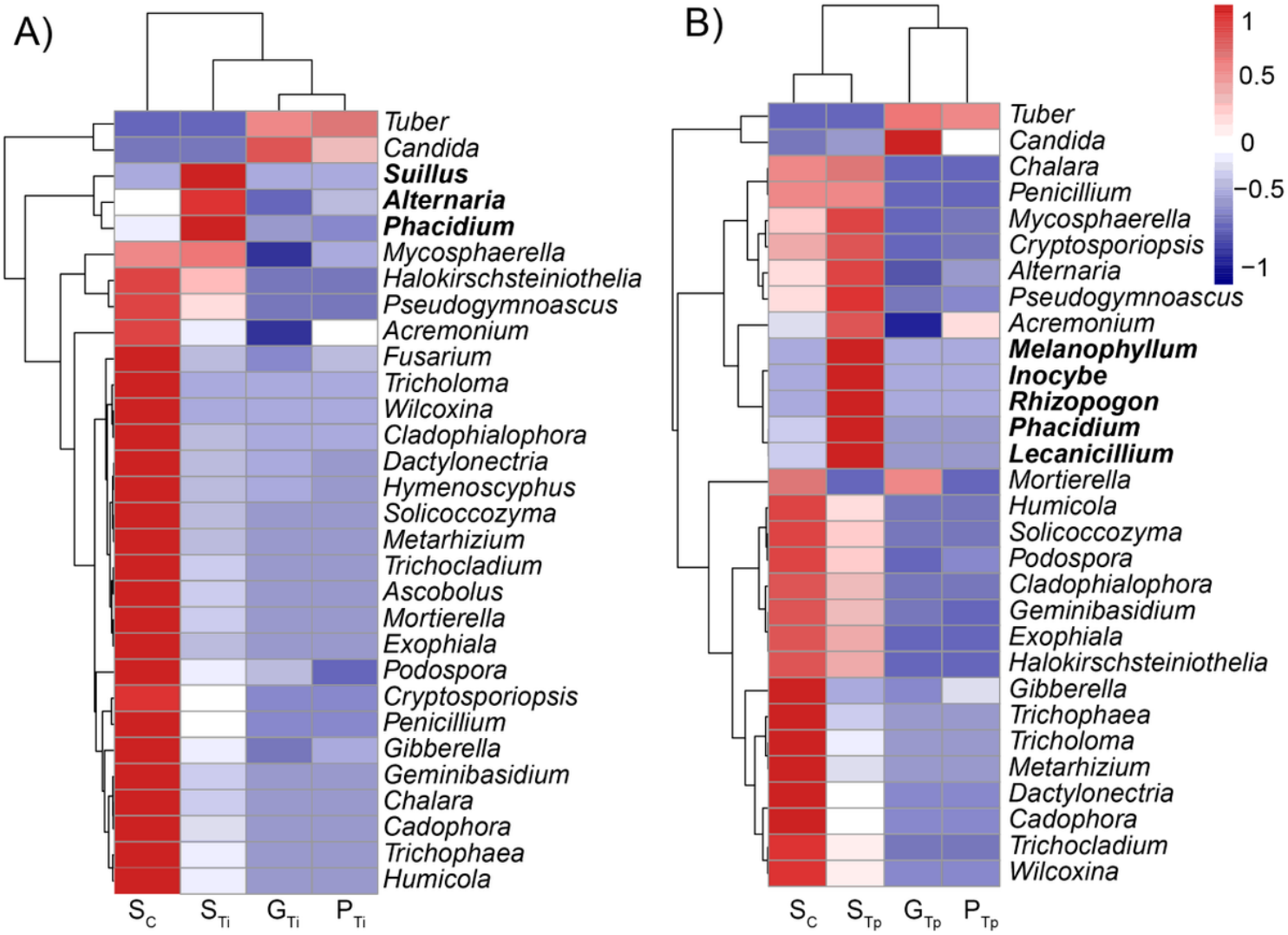

Figure 3

Cluster analysis based heatmaps of community composition (top 30 genera) in the control and truffle surrounding soils, and fungal tissues of A) Tuber indicum (Ti) and B) T. pseudohimalyense (Tp) occurring in a P. armandii forest in Huidong, Sichuan, southwest China. The horizontal and vertical cluster represents the bacterial genera and treatments clustering tree, respectively. In each square, the relative abundance of genus in each row has been standardized to obtain a “Z” value. The Z value of a genus is the value obtained by dividing the difference between the relative abundance of a sample and the average relative abundance of all samples by the standard deviation of all samples. The redder the square color, the higher the abundance of the genus among samples. Abbreviations are shown in Figure 1.

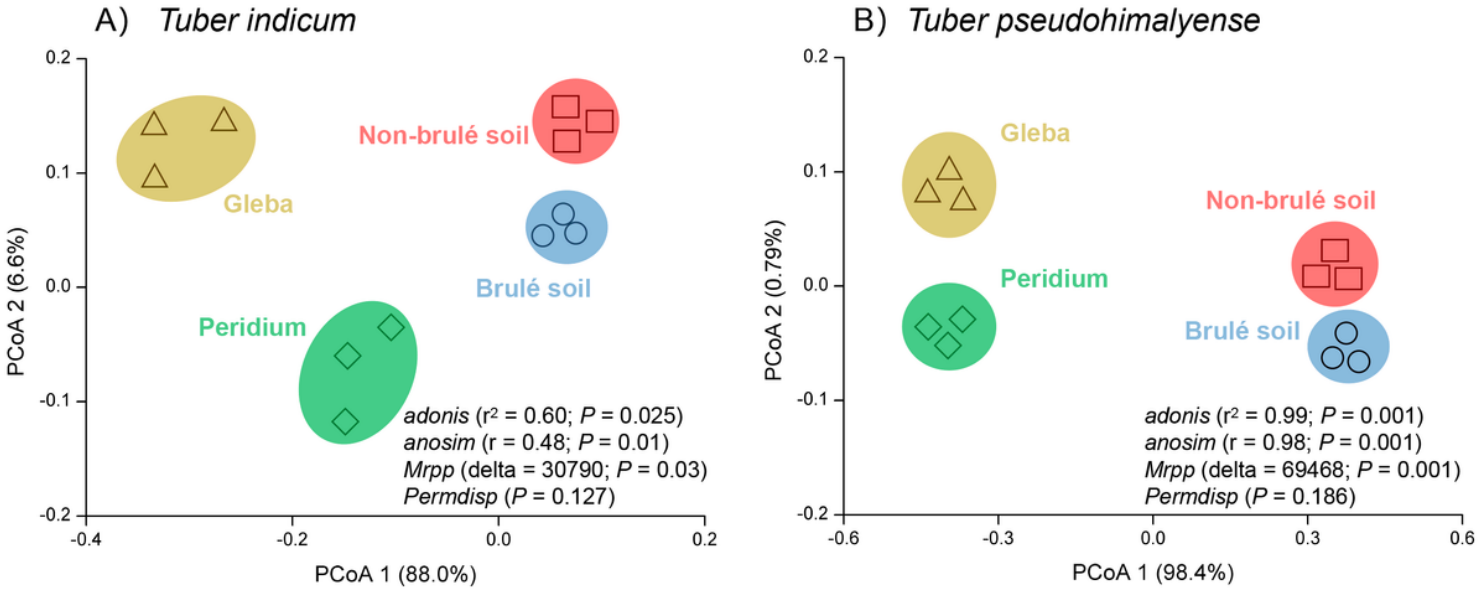

Figure 4

Fungal community compositions as indicated by principal coordinate analysis (PCoA) of pairwise Bray-Curtis distance in the truffle surrounding soils and fungal tissues of A) Tuber indicum and B) T. pseudohimalyense occurring in a P. armandii forest in Huidong, Sichuan, southwest China. Abbreviations are shown in Figure 1. Bray-Curtis distance-based results of three permutation dissimilarity tests were presented including analysis of similarity (anosim), non-parametric multivariate analysis of variance (adonis) using distance matrices, and a multiple response permutation procedure (Mrpp). Permutation test for hormogeneity of multivariate dispersion (Permdisp) was used to test heteroschedasticity between groups.

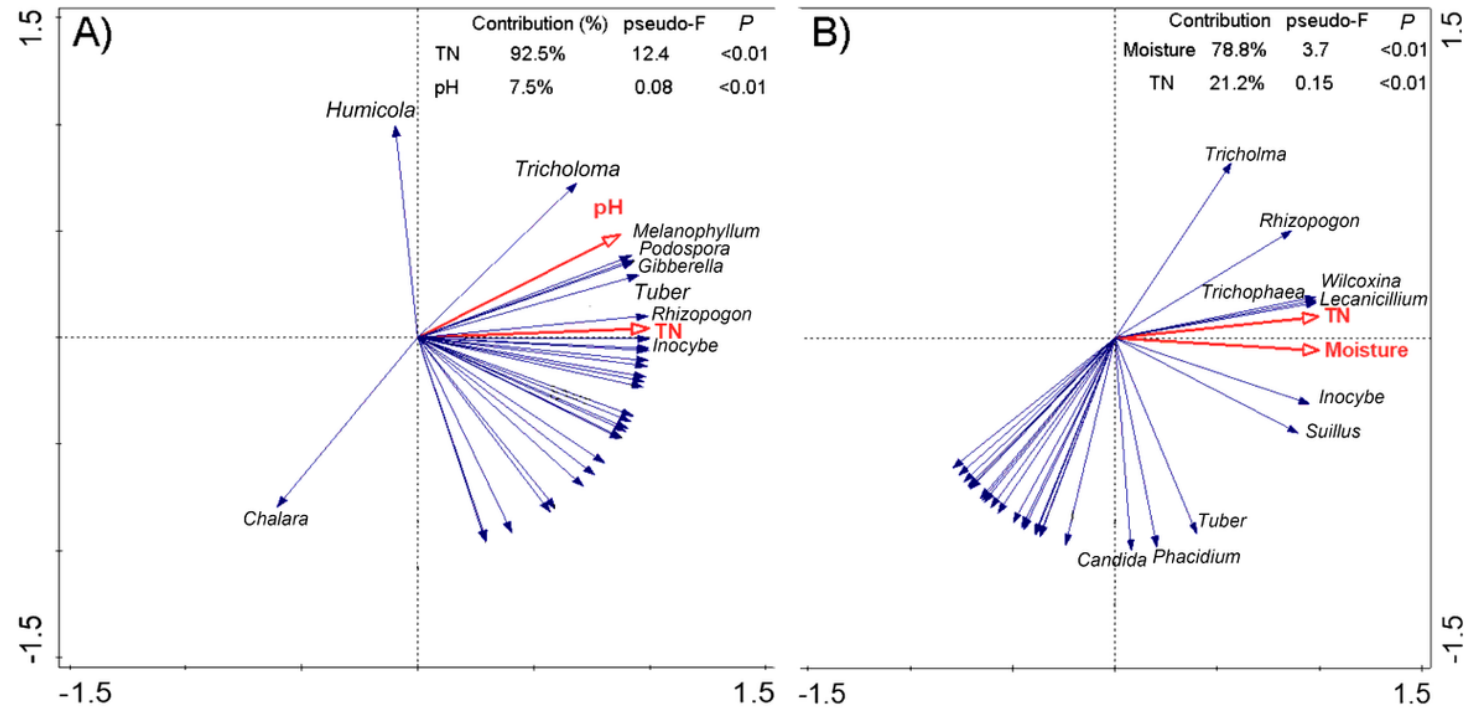

Figure 5

Redundancy analysis (RDA) showing the relationships of the ecological variables and fungal genera (Top 30) in the below and around soils of Tuber indicum (A) and T. pseudohimalyense (B) occurring in a P. armandii forest in Huidong, Sichuan, southwest China. The measured nine soil properties (see in Table 1) were used as constrains to select the most influential variables. The length of arrows represents the strength of the respective soil properties with the fungal genera. The angle between vectors indicates the degree of their associations (smaller angle means high correlation). To avoid the names of fungal genera overlapping, genera with loose assocations with soil major influencing factors were not showing alongside the blue arrow, can be found in Fig. 3.

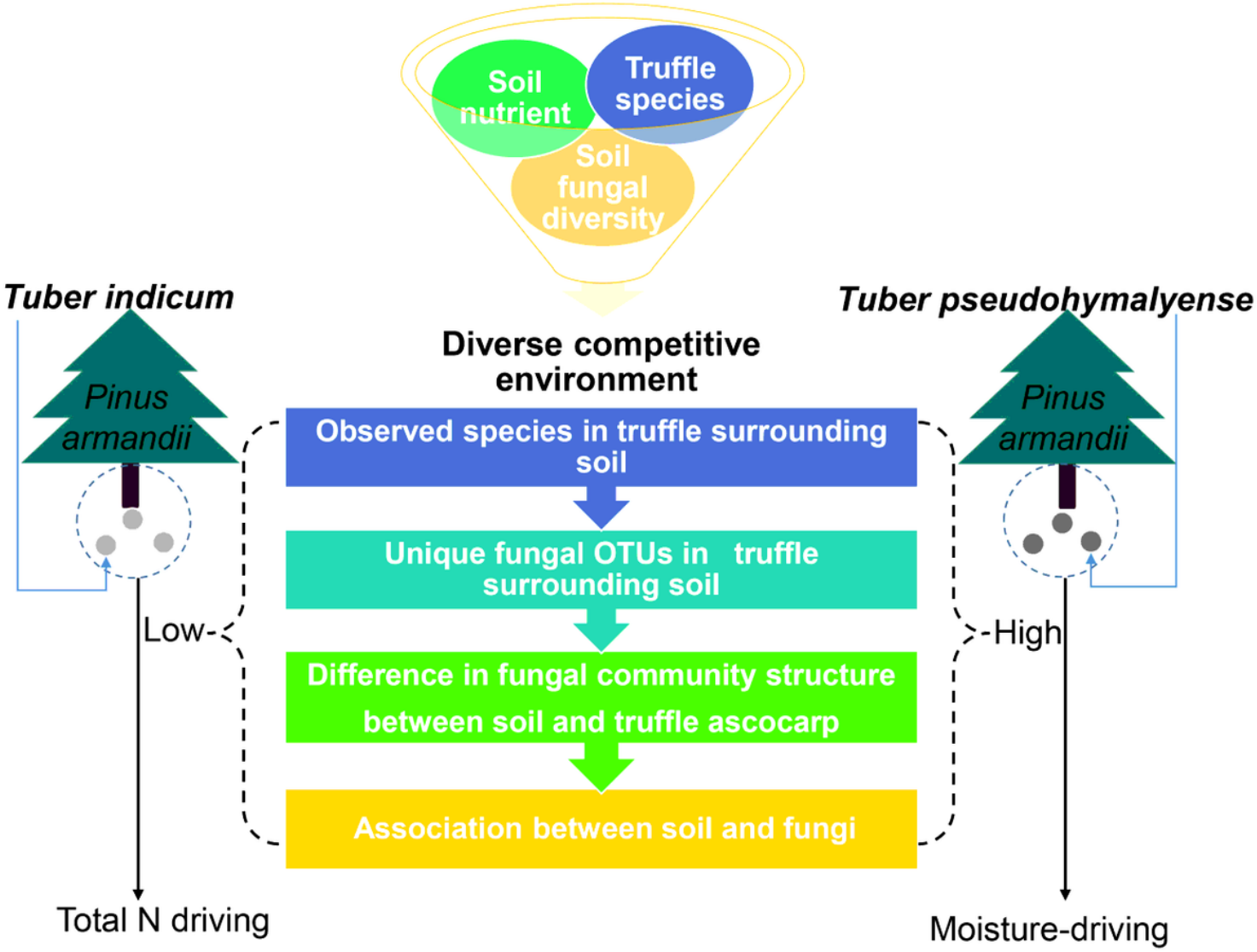

Figure 6

Diagram for the changes in the truffle surrounding soils and fungal tissues of Tuber indicum and T. pseudohimalyense occurring in a P. armandii forest.

Supplementary Files

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- SupplementaryTableandFigures.docx