Long-term no-till: A major driver of fungal communities in dryland wheat cropping systems

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Abstract

In the dryland Pacific Northwest wheat cropping systems, no-till is becoming more prevalent as a way to reduce soil erosion and fuel inputs. Tillage can have a profound effect on microbial communities and soilborne fungal pathogens, such as Rhizoctonia. We compared the fungal communities in long-term no-till (NT) plots adjacent to conventionally tilled (CT) plots, over three years at two locations in Washington state and one location in Idaho, US. We used pyrosequencing of the fungal ITS gene and identified 422 OTUs after rarefaction. Fungal richness was higher in NT compared to CT, in two of the locations. Humicola nigrescens, Cryptococcus terreus, Cadophora spp., Hydnodontaceae spp., and Exophiala spp. were more abundant in NT, while species of Glarea, Coniochaetales, Mycosphaerella tassiana, Cryptococcus bhutanensis, Chaetomium perlicum, and Ulocladium chartarum were more abundant in CT in most locations. Other abundant groups that did not show any trends were Fusarium, Mortierella, Penicillium, Aspergillus, and Macroventuria. Plant pathogens such as Rhizoctonia (Ceratobasidiaceae) were not abundant enough to see tillage differences, but Microdochium bolleyi, a weak root pathogen, was more abundant in NT. Our results suggest that NT fungi are better adapted at utilizing intact, decaying roots as a food source and may exist as root endophytes. CT fungi can utilize mature plant residues that are turned into the soil with tillage as pioneer colonizers, and then produce large numbers of conidia. But a larger proportion of the fungal community is not affected by tillage and may be niche generalists.

Introduction

Since the dawn of agriculture, tillage has played an important role by maintaining a disturbance that favors domesticated plants [1]. Disturbance by mechanical implements is used to prepare and cultivate the seed-bed, control weeds, break up residue, and incorporate manures and fertilizer. However, tillage can have a detrimental effect on soil sustainability by reducing organic matter through exposure to oxygen and microbial decomposition, breaking up soil aggregates and fungal networks [2, 3], but most importantly by increasing soil erosion from...
water and wind. The rate of soil loss from agriculture far exceeds the rate of soil formation [4].

In 1997, an estimated 2.0 billion tons of soil were lost in the US [5]. One way to reduce soil loss is to stop tillage by using no-till methods. This practice has increased significantly in the US over the last 30 years, especially in soybean, corn, and wheat cropping systems. Overall in the US in 2012, 96 million acres were planted with no-till out of 278 million total acres under cultivation [6]. In reduced tillage or no-till (NT), also called direct-seeding, seeds are planted directly into the residue of the previous crop with minimal disturbance of the residue [7]. Besides reducing soil erosion, no-till can conserve soil moisture, increase water infiltration, increase soil organic matter, and result in increased earthworm populations [8].

Tillage can also have a profound effect on soil microbial communities, by breaking up soil aggregates, exposing microsites to oxygen, and increasing microbial activity. Studies of the bacterial component of soil communities have demonstrated significant impacts of tillage on bacterial community composition and microbial activity [9, 10], though others have found little or no significant impact of tillage [11–14]. The literature on effects of tillage on the arbuscular mycorrhizal fungi (AMF) is more consistent. Numerous studies show either detrimental effects or shifts in the communities of AMF, where diversity, richness, and abundance of AMF increased in no-till or reduced tillage systems [11, 15–18]. However, most of the literature on AMF or other fungal groups is based on phospholipid fatty acid (PLFA) or fatty acid methyl esterase (FAME), which quantifies broad groups of microbes based on molecules in their membranes. These methods do not have the resolution to delineate fungal community composition in detail. In the early 2000s, more researchers used PCR amplification, cloning and sequencing of ribosomal genes that have taxonomic applications in fungi—internal transcribed spacer (ITS) and large ribosomal subunit (LSU). However, these methods are still limited by the size of the library of clones that can be made, usually just a few hundred [19]. The use of next-generation sequencing (NGS) offers the ability to examine fungal communities in far greater detail in agricultural ecosystems, especially the effect of tillage.

Fungi play a major role in no-till systems in terms of carbon cycling, soil health, and disease or disease suppression. In the dryland wheat cropping systems of the Pacific Northwest (PNW), Rhizoctonia bare patch and root rot can increase significantly when tillage is stopped, during the conversion from conventional to no-till or direct seeding [20, 21]. It often takes two years for the disease to increase, but once it does, yield is significantly reduced. However, in long-term no-till systems, Rhizoctonia is not a problem, based on a comparison of no-till fields to adjacent conventionally-tilled fields [22]. Could shifts in the microbial community be responsible for suppression of Rhizoctonia? Bacterial communities can be affected by tillage in the PNW [23], but tillage had a much weaker effect on bacterial communities compared to proximity to root or location. The bacterial communities in the rhizosphere which would have the most profound effect on root rotting fungi, were more buffered from the effects of tillage than those in the bulk soil. Very few bacterial taxa were consistently shown to be affected by tillage. What about fungal communities? In Australia, fungi were implicated in the suppression of Rhizoctonia in a long-term no-till system [24]. Fungi are the primary decomposers of plant residue and play an important role in the carbon cycle. They also produce hyphal networks in the soil that may be sensitive to disruption by conventional tillage practices. Because of the profound effect of tillage on residue decomposition, we hypothesize that fungal communities will be significantly affected in terms of diversity, richness and composition. More specifically, we hypothesize that certain taxa will be favored by lack of tillage (NT) and others will be more predominant in conventional tillage (CT). To address these hypotheses, we sampled plots that had been in long-term no-till (12–32 years), and adjacent plots that were in long-term conventional tillage. This was done at two dryland wheat locations in eastern Washington and one location in northern Idaho, with samples taken over three years.
**Materials and methods**

**Survey sites and tillage treatments**

Three experimental sites included in the study were in the Palouse region of Idaho and Washington. The Palouse region has Palouse silt loam soils with an average pH of 5.6, 1.78% organic C, and 0.12% total N within the top 10 cm [25]. One location was in Idaho (Kambitsch Farm) and two locations were in Washington (USDA-ARS Palouse Conservation Farm, PCFS, and R. J. Cook Agronomy Farm, or the Cook Farm). The Kambitsch Farm, Cook Farm, and PCFS have been managed and maintained by University of Idaho, Moscow, ID; Washington State University, Pullman, WA; and USDA-ARS, Pullman, WA, respectively.

The Kambitsch Farm is located at north of Genesee, ID (46˚35’17.0”N 116˚56’49.8”W). This farm had five replicated NT and CT treatment plots. The crop rotation was winter wheat-spring wheat or spring barley-grain legume (pea, lentil or chickpea). The Cook Farm, Pullman, WA (46˚47’00.6”N 117˚04’40.6”W) had long-term direct seed cropping systems research program. This experimental site had no-till treatment for 13 years. Adjacent to the NT block, had a conventional tillage block. Conventional tillage was done with chisel plows. Since this site did not have replicated treatment plots, four directional quadrants (NE, NW, SE, and SW) were considered as replications for each treatment (CT vs NT). PCFS site (46˚45’30.0”N 117˚11’35.4”W) had two treatments, conventional tillage and no-till management for 35 years. There were 4 replications. The crop rotation was winter wheat/spring wheat in the no-till plot. The conventional tillage plot with a winter wheat/fallow rotation was adjacent to each no-till plot. Soil was fall chiseled, spring-disked, and rod weeded 3 to 5 times during the fallow years. In all plots, the winter wheat part of the rotation was sampled.

**Soil sampling and DNA extraction**

Soil samples were collected in 2012, 2013, and 2014 at Kambitsch Farm and PCFS sites whereas samples were collected from Cook Farm in 2013 and 2014. Bulk soil consisted of three sub-samples collected randomly from each plot. Crop residue on the soil surface was removed and soil was collected from the top 20 cm at the tillering stage of wheat and from other plots simultaneously. Each sub-sample was approximately 1 kg. Each sample was sieved through a 2-mm mesh to eliminate crop residues and debris. Three sub-samples from each plot were composited and homogenized. Soil samples were stored in 50 ml plastic tubes (Thermo Fisher Scientific, MA), stored at -20˚C and thawed just before DNA extraction. Total DNA was extracted from 0.5 g of each soil sample using UltraClean Soil DNA Kit (MO BIO Laboratories, CA) as described previously [26] and stored at -20˚C for subsequent procedures.

**Sequencing of the Fungal ITS region**

A total of 83 DNA samples (23 samples collected in 2012, 30 in 2013, and 30 in 2014) was used for NGS (454 pyrosequencing). The ITS 1–4 region was amplified using fungi specific primers (ITS1-F: 5’-CTTGGTCATTTAGAGGAAG TAA-3’; ITS4: 5’-TCCTCCGCTTATTGATATGC−3’ [27] as per MrDNA (Shallowater, TX) protocols. Amplicons were sequenced using a Roche 454 FLX titanium instruments and with reagents following the manufacturer’s guidelines. Raw sequence data has been submitted to the NCBI Sequence Read Archive under the study accession #SRP114697.

**DNA sequence processing**

Raw 454 flowgrams were trimmed to 450 flows and denoised in MOTHUR v1.36.1 [28] using shhh.seqs. Barcodes and primers were trimmed and any sequence with a homopolymer >8bp
or <200bp in length were discarded. Denoised sequences from both runs were combined and chimeric sequences were identified with the usearch61 algorithm implemented in identify-
chimeric_seqs.py in QIIME v1.9.1 [29]. Open-reference OTU picking was performed with
pick_open_reference_otus.py using the UNITEv7 dynamic (31.01.16) reference set using a
97% similarity threshold. Taxonomy was assigned to OTUs by blasting representative
sequences against the UNITE general release (31.01.16). To ensure that only high-quality
OTUs were included, those with low e-values (<10e-40), low match length to representative
sequences (query length/subject length<0.75), or <10 total sequences were discarded. After
processing, sequencing yielded an average of 7,562 sequences/sample (+/- 2023), with a mini-
mum of 2157 and a maximum of 15,524. To ensure equal sampling depth, samples were rare-
fied to 3,679 sequences/sample prior to analyses and samples (n = 1) with less than this
number of sequences were discarded.

Statistical analyses
The composition of fungal communities was evaluated using non-metric multidimensional
scaling (NMDS) plots of Bray-Curtis distances using the metaMDS function of the vegan pack-
age in R [30, 31]. The significance of different factors to fungal community structure was
assessed using PERMANOVA implemented in the adonis function in vegan with 1000 permu-
tations to determine significance. Fungal richness, the inverse Simpson’s diversity index (1/D),
and the Shannon diversity index (S’) were calculated using vegan and compared using a 3-way
ANOVA with year, location, and tillage as factors. The Simpson’s index is more weighted
toward community evenness, while the Shannon index is weighted towards community rich-
ness. Proportions of fungal phyla were compared across tillage treatments, locations, and years
using Kruskal-Wallis tests. Relative abundances of abundant fungal general and OTUs (genera
<0.5% total relative abundance or OTUs with >200 total sequence counts) were compared
with ANOVA after log10(1+x) transformation of sequence counts using a Benjamini-Hoch-
berg correction for false discovery rates.

Results
Fungal community composition
After sequence processing and quality filtering, 626,182 sequences belonging to 987 fungal OTUs
remained (297,999 sequences among 422 OTUs after rarefaction). Fungal communities were domi-
nated by members of the Ascomycota (~82.5% ± 9.6% of all sequences; S1 Fig) followed by the Bas-
diomycota (~12.5% ± 8.7% of all sequences; S1 Fig). There was a significantly greater proportion of
Basidiomycetes in no-till fields versus conventionally-tilled (NT: 15.4 ± 4.6%; CT: 7.7 ± 10.5%;
Kruskal-Wallis test p = 0.001) sites, and significantly small proportion of Ascomycetes (79.2% ±
10.9% in NT, 86 ± 0.06% in CT; Kruskal-Wallis test p = 0.002). The relative abundances of these
phyla did not differ significantly among locations (Kruskal-Wallis test: Ascomycota χ² = 2.69,
p = 0.26; Basidiomycota χ² = 2.92, p = 0.23) or years (Kruskal-Wallis test: Ascomycota χ² = 4.78,
p = 0.091; Basidiomycota χ² = 1.73, p = 0.42). Zygomycota, Chytridiomycota, and unidentified
phyla composed smaller proportions of communities that did not differ significantly among tillage
treatments (S1 Fig; Kruskal-Wallis test p>0.62), though relative abundances of these minor phyla
differed significantly among locations and years (data not shown).

Fungal communities varied significantly among locations, tillage treatments, and years (Fig
1, Table 1). Overall, location explained the largest amount of variation in fungal community
structure (r² = 0.17), followed by sampling year (r² = 0.09) and tillage treatment (r² = 0.08).
However, within each location, fungal communities from NT fields were consistently distinct
from those CT fields, regardless of the year in which they were sampled (Fig 2; Table 2).
Within each location there was also significant year-to-year variation in fungal community structure in both CT and NT fields (Table 2). Interestingly, the relative importance of tillage treatment and year in determining fungal community structure varied among locations (Table 2). Notably, tillage had a much weaker influence on fungal communities at the Kambitsch Farm than the other two locations. The Kambitsch Farm had a shorter history of no-till than the other sites. There were also significant tillage × location and tillage × year interactions (Table 2).

Fungal community diversity metrics

Despite the large effect of tillage on fungal community structure within each location, fungal richness and diversity was most strongly related to the year of sampling (Table 3, Fig 3). In

Table 1. Factors determining fungal community structure as assessed by PERMANOVA.

| Factor                     | F-value | \( \mathcal{R}^2 \) | p-value |
|----------------------------|---------|----------------------|---------|
| Tillage                    | 9.55    | 0.08                 | 0.001   |
| Location                   | 10.99   | 0.17                 | 0.001   |
| Year                       | 5.76    | 0.09                 | 0.001   |
| Tillage × Location         | 5.05    | 0.08                 | 0.001   |
| Tillage × Year             | 1.91    | 0.03                 | 0.003   |
| Location × Year            | 2.86    | 0.07                 | 0.001   |
| Tillage × Location × Year  | 2.14    | 0.05                 | 0.001   |

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Fig 2. NMDS plots of fungal communities within locations colored by tillage treatment (red = conventional tillage, CT; blue = no-till, NT). Symbols indicate different sampling years.

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general, communities sampled in 2014 had greater fungal richness and diversity than those from 2013 or 2012, suggesting that yearly (or seasonal) environmental variation has a significant impact on fungal diversity. Tillage treatment was a significant factor determining fungal richness ($p = 0.02$), where fungal communities from NT fields tended to have a greater number of OTUs than those from CT fields for the PCFS and Cook Farm. However, this was not the case for the Kambitsch Farm and individual contrasts between tillage treatments were not statistically significant (data not shown). In contrast to the PCFS or Cook farms, the Kambitsch location tended to have greater diversity when assessed by the inverse Simpson’s index, suggesting greater evenness of fungal communities under CT at this location.

**Variation in fungal genera among tillage practices**

The relative abundances of fungal genera often varied significantly among tillage treatments, locations, and years (Fig 4; S1 Table). For example, fungal communities from fields under long-term no-till management consistently had higher proportions of *Exophiala* and *Humicola* than communities from conventionally tilled fields. For some genera (eg. *Cadophora*), relative abundances were greater in communities from no-till systems in some locations (Kambitsch and PCFS), but not others (Cook), suggesting that location may influence the response of some fungal genera to long-term no-till practices. In contrast to no-till, some genera had greater relative abundance in communities from conventionally tilled fields. These included *Chalara*, *Glarea*, *Mycosphaerella*, and *Ulocladium*. However, patterns in *Glarea* and *Ulocladium* were not present in all locations, again suggesting some location-specific impacts of tillage practices, as indicated by significant location × tillage interactions (S1 Table). Other abundant genera, such as *Cryptococcus* and *Macroventuria*, were equally abundant across all tillage types.

**Table 3. ANOVA for fungal richness, inverse Simpson’s diversity, and Shannon diversity indices among tillage treatments, farm locations, and sampling years.**

| Factor                | Richness (no. OTUs) | Inverse Simpson’s (1/D) | Shannon Index (S’) |
|-----------------------|---------------------|-------------------------|--------------------|
|                       | F-value             | p-value                 | F-value           | p-value    | F-value | p-value |
| Tillage               | 5.73                | 0.02                    |                   |            |         |        |
| Location              | 2.23                | 0.11                    | 8.31              | 0.0007     | 2.6     | 0.083   |
| Year                  | 45.95               | <0.0001                 | 20.78             | <0.0001    | 19.15   | <0.0001 |
| Tillage × Location    | 2.25                | 0.11                    | 3.2               | 0.048      | 3.1     | 0.053   |
| Tillage × Year        | 0.49                | 0.45                    | 0.57              | 0.45       | 0.29    | 0.59    |
| Location × Year       | 1.05                | 0.36                    | 0.62              | 0.54       | 0.2     | 0.82    |
| Tillage × Location × Year | 1.94           | 0.15                    | 1.64              | 0.2        | 3.62    | 0.033   |

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At the taxonomic rank of family, fungal groups also frequently differed among tillage treatments, locations, and years (data not shown).

Variation in individual fungal OTUs

Many fungal OTUs were identified that were significantly more or less abundant between tillage treatments (Table 4, S2 Table; n = 41 OTUs with FDR p-value < 0.05), locations (n = 24 OTUs), and years (n = 10 OTUs). There were also significant tillage x location interactions with n = 15 OTUs. OTUs identified as *Humicola nigrescens*, *Cryptococcus terreus*, *Hydnodontaceae* spp., and *Exophiala* were among the most abundant that had greater relative abundances in fungal communities from no-till fields (Table 4, S2 Table), though other less abundant groups, such as *Microdochium bolleyi*, may also play important roles in these communities. Abundant OTUs that were more frequent in conventionally tilled soil communities included...
representatives of Chalara, Mortierella, and Coniochaetales spp., as well as those identified as Cryptococcus bhutanensis, Chaetomium perlucidum, and Ulocladium chartarum (Table 4, S2 Table). In general, patterns in the relative abundances of OTUs between tillage treatments were consistent among locations, though in some cases OTUs only differed in two of the three locations. For example, Hydnodontaceae sp (SH175275.07FU_GU055572) was of higher relative abundance in no-till soils at the Cook and PCFS locations, but almost absent from Kambitsch soils. Similarly, the OTU identified as Cryptococcus bhutanensis had a greater relative abundance in conventionally tilled soils at the Cook and PCFS locations, but not at PCFS. Conversely, Cryptococcus terreus was more abundant in no-till in the Cook and PCFS, but not Kambitsch. Thus, although most fungal OTUs respond consistently to tillage practices, populations of others may be more strongly controlled by location-specific factors, such as soil characteristics or local species interactions.
One of the most common genera of wheat pathogens is *Fusarium* (*Fusarium pseudograminearum* and *F. culmorum*), the cause of Fusarium crown rot. We identified 8 OTUs classified

| Taxonomy          | OTU identifier | Till | Yr | Loc | Till × Yr | Yr × Loc | Till × Loc × Yr |
|-------------------|----------------|------|----|-----|------------|-----------|-----------------|
| Humicola nigrescens SH374010.07FU_AY706334_ref | 0+ | 0.079 | 1 | 1 | 0+ | 1 | 1 |
| Glarea lozoyensis SH198390.07FU_FJ005111_rep | 0+ | 0.001 | 1 | 1 | 0.020 | 1 | 0.109 |
| Mycosphaerella tassiana SH216250.07FU_EF679363_ref | 0.007 | 0+ | 1 | 1 | 1 | 0.155 | 1 |
| Cryptococcus terreus SH357827.07FU_AF444351_ref | 0+ | 0.642 | 0.426 | 1 | 0.009 | 1 | 1 |
| Ulocladium chartarum SH216785.07FU_AF294988_ref | 0.028 | 1 | 1 | 1 | 1 | 1 | 1 |
| Heliotiales sp. SH204310.07FU_JX974734_rep | 0.001 | 0+ | 0+ | 1 | 1 | 1 | 0.137 |
| Tremellomyces sp. SH190741.07FU_HG532069_rep | 0+ | 1 | 0.008 | 1 | 0+ | 1 | 1 |
| Heliotiales sp. New.ReferenceOTU20 | 0.016 | 0+ | 0.016 | 1 | 1 | 0.779 | 1 |
| Hypocreales sp. SH175275.07FU_GU055572_rep | 0.003 | 0.642 | 1 | 1 | 0.218 | 1 | 1 |
| Mortierella sp. SH180194.07FU_KF428242_rep | 0+ | 0.135 | 0+ | 1 | 1 | 0.105 | 1 |
| Hydnodontaceae sp. SH186054.07FU_HQ2212160_rep | 0+ | 1 | 0.021 | 1 | 0.031 | 1 | 1 |
| Coniochaetales sp. SH011282.07FU_KC965268_rep | 0.019 | 1 | 0.397 | 1 | 1 | 1 | 1 |
| Mortierella rishikeshensis SH180109.07FU_HQ630308_ref | 0.013 | 0+ | 0.835 | 1 | 0.442 | 0.416 | 1 |
| Incertae sedis sp. SH408326.07FU_FJ427063_rep | 0+ | 1 | 0+ | 1 | 1 | 1 | 1 |
| Cryptococcus bhutanensis SH278429.07FU_AF145317_ref | 0+ | 1 | 0+ | 1 | 0.003 | 1 | 1 |
| Chalara sp. SH204486.07FU_AY969323_rep | 0+ | 0.058 | 0.924 | 1 | 1 | 1 | 1 |
| Chaetomium perlucidum SH195314.07FU_HQ607856_rep | 0+ | 1 | 0.001 | 0.204 | 0+ | 1 | 1 |
| Microdochium bolleyi SH213512.07FU_KF646098_rep | 0.031 | 0.013 | 1 | 1 | 1 | 1 | 1 |
| Tetracadium sp. SH020300.07FU_KC966090_rep | 0+ | 1 | 0+ | 1 | 0.218 | 1 | 0.928 |
| Coniochaetales sp. New.ReferenceOTU4 | 0.001 | 1 | 1 | 1 | 1 | 1 | 1 |
| Hymenula cerealis SH186776.07FU_HQ322364_ref | 0.004 | 1 | 0+ | 1 | 0+ | 1 | 0.946 |
| Phialocephala curvata New.CleanUp.ReferenceOTU2 | 0.002 | 0.303 | 0+ | 1 | 1 | 1 | 1 |
| Chaetomium gallicicum SH195324.07FU_JN573175_rep | 0+ | 0.076 | 0+ | 1 | 0+ | 1 | 0.037 |
| Fungi sp. SH008253.07FU_FR871193_rep | 0+ | 0.047 | 0+ | 1 | 0.642 | 1 | 1 |
| Ascomycota sp. SH185508.07FU_KC007266_rep | 0+ | 0.023 | 1 | 1 | 0.030 | 1 | 1 |
| Lasiosphaeriaceae sp. New.ReferenceOTU5 | 0+ | 0.817 | 0+ | 1 | 0+ | 0.005 | 0.008 |
| Cryptococcus sp. New.ReferenceOTU12 | 0+ | 1 | 0+ | 1 | 0+ | 1 | 1 |
| Penicillium novae-zeelandiae SH407703.07FU_JN617688_ref | 0+ | 1 | 1 | 1 | 1 | 1 | 1 |
| Trechisporales sp. SH218947.07FU_JF691365_rep | 0+ | 1 | 0+ | 1 | 0+ | 1 | 1 |

0+, value < 0.001. OTU identifiers correspond to representative sequences in the UNITE v.7 dynamic (31.01.16) reference set.

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### Plant pathogenic genera and OTUs

One of the most common genera of wheat pathogens is *Fusarium* (*Fusarium pseudograminearum* and *F. culmorum*), the cause of Fusarium crown rot. We identified 8 OTUs classified...
as *Fusarium* and 8 in the family Nectriaceae, with very abundant sequences, but because of the conserved nature of ITS sequences in this genus, they could not be identified to species. Another group of pathogens is *Rhizoctonia solani* AG-8 and other *Ceratobasidium* species, which cause root rots and would be classified in the family Ceratobasidiaceae. We identified 2 OTUs in this family, which were not very abundant. We identified one OTU of *Hymenula cerealis* (*Cephalosporium graminis*), causal agent of Cephalosporium stripe, a wilt disease. This was only found at PCFS CT, a field which was previously inoculated with the pathogen as part of a variety screening site. We identified one OTU of *Microdochium nivale*, cause of a snow mold, but this taxa was also rare. An OTU of *Microdochium bolleyi* was also identified, which was most abundant in no-till. This is a common root parasite, but is considered weakly virulent or non-pathogenic.

**Discussion**

This work demonstrates that tillage practices have a profound impact on soil fungal communities in agricultural systems. Though a few recent studies have used next-generation sequencing to look at how tillage affects the soil microbiome, most of these have investigated other components of the soil community, such as bacteria or arbuscular mycorrhizal fungi. For example, Yin et al. [26] described bacterial communities at the same locations used in this work, and showed that tillage had rather minor effects on overall community structure, compared to location and proximity to the root (bulk soil vs rhizosphere). We hypothesized that fungal communities would be more strongly influenced, because of the crucial role that fungi play in residue decomposition, compared to bacteria.

Degrune et al. [32] found tillage significantly affected fungal communities, but that tillage was less important than soil depth. Unlike most previous work with high-throughput sequencing, they found that fungal diversity and richness declined with reduced tillage compared to conventional tillage, and that several taxa present in conventional tillage were lost in the reduced tillage treatment. Our findings were contrary to this, and fit most previous work in finding higher abundance, richness and diversity with no-till [15, 17, 33]. Although we did not specifically look at AMF, most of the literature, including recent work with high-throughput sequences, has confirmed the detrimental effects of tillage on AMF. Detheridge et al. [34] found that tillage had no major effect on fungal communities, except that AMF were more abundant in no-till and pathogenic fungi were more abundant in plowed soils.

One of the most obvious interactions in wheat cropping systems between fungi and tillage concerns plant pathogenic fungi that rot roots and crowns of wheat. There is abundant literature on how no-till or reduced tillage may increase fungal diseases of wheat, especially those that survive in crop residue. For example, Fusarium head blight caused by *Fusarium graminearum* (*Gibberella zeae*) is increased when residue is left on the surface, because the pathogen can overwinter and produce fruiting bodies and ascospores on the straw. Although it is typically too dry for Fusarium head blight in the dryland PNW, Fusarium crown rot caused by *F. pseudograminearum* and *F. culmorum* are widespread and major yield reducers [35, 36]. Some studies have shown increased crown rot under no-till [37], but we find it also in conventional systems. Unfortunately, the sequencing of the fungal ITS region does not provide sufficient resolution to identify *Fusarium* species. Sequencing with the translation elongation factor 1 alpha is the standard for identifying *Fusarium*. However, we identified 8 OTUs of *Fusarium* and 8 OTUs belonging to Nectriaceae, the family that contains *Fusarium*. Interestingly, *Fusarium* were one of most abundant genera overall, but showed no trends with tillage treatments. From other surveys in the world [38], at least a dozen species can colonize wheat straw, including *F. equisiti*, *F. acuminatum*, and *F. avenaceum*. Additional sequencing using alternative
targets specific to *Fusarium* (eg. RPB2; [39]) may reveal interesting patterns in this genus with tillage. Another group that is affected by tillage is *Rhizoctonia solani* AG-8, which increases when tillage is stopped [37]. We detected two OTUs in the Ceratobasidiaceae, but the abundances were low and no trends were observed. However, we were successful in identifying two other potential pathogen groups at the species level. *Microdochium nivale*, cause of a snow mold, was in low abundance and showed no trends. However, *Microdochium bolleyi* was of relatively high abundance, and increased in the no-till treatments across all locations and years. This taxon is considered to be a weak parasite of wheat roots [40], a potential biocontrol agent against other root pathogens [41] and has been shown in another study to be more common in no-till, based on isolation from wheat roots [42]. We also detected *Hymenula cerealis*, also known as *Cephalosporium gramineum*. This causes a wilt disease, and was only found in high levels in the PCFS CT site. This was the site of a previous *Cephalosporium* stripe nursery that had been inoculated with the pathogen in previous years.

There were a few groups of saprophytic fungi that were more predominant in no-till. One was *Humicola nigrescens*, (Family Chaetomiaceae), a dematiaceous fungus. *Humicola* contains 20 species, mostly isolated from soil and plant tissue [43]. Many species are thermophilic [44]. It is closely related to *Trichocladium*. *T. aspergum* was found to be the dominant late colonizer of rye straw [45] and a dominant member of the soil cellulolytic community [46]. *Exophiala* was also more predominant in no-till. Like *Humicola*, this is a dark mycelial, dark-spored genus is found in soil, leaf litter, and wood. Some species are also pathogen of mammals, amphibians and fish [43]. *Cadophora* spp. are dark-septate root endophytes. Some are pathogens of soybean [47], grape [48] and tree species such as willow [49]. Some are also considered potential biocontrol agents [50] and may benefit tree health [51].

More groups of fungi were more abundant in the conventionally tilled treatment. One of the most abundant was *Mycosphaerella tassiana*, the perfect stage of *Cladosporium herbarum*. It is also classified as *Davidiella* (Family Mycosphaerellaceae). *Cladosporium* are extremely common in soil, plant surfaces and are excellent colonizers of necrotic plant tissue. They produce abundant spores and are often found in air samples, both indoor and outdoor. *Ulocladium* (Family Pleosporaceae) occupies a similar niche, with abundant spores, and were also more abundant in conventionally tilled soils. Two OTUs in the order Coniochaetales were higher in conventional tillage. This group in the class Sordariomycetates forms perithecia, and are found on wood, bark and the soil [52].

One unusual finding was the genus *Glarea*. First described in the late 1990s, the most widely studied species is *G. lozoyensis*, which produces a novel antifungal compound pneumocandin, with applications in medicine [53]. The most abundant *Glarea* OTU showed 99% similarity with *G. lozoyensis*. It was more abundant in conventional tillage, and was found at all three locations. Another genus found more abundant in conventional tillage was *Chalara* (Family Helotiaceae). This is related to *Thielaviopsis basicola*, which is a pathogen of rotation crops such as pea and causes black root rot.

But a larger number of genera did not show any general trend with tillage, although they were very abundant. This included *Fusarium* and *Mortierella*. However, individual OTUs within a genus may show a trend, for example a *Mortierella* sp. OTU which was more common in conventional tillage. *Mortierella* is in the Division Zygomycota and is a very common soil fungus and root colonizer. Also common were *Penicillium* and *Aspergillus* (Family Trichocomaceae), common cellulolytic colonizers of soil and plant residue. The most unusual finding was *Macroventuria* (Family Didymellaceae). The taxonomic status of this was recently resolved [54] and one OTU showed 97.5% similarity to *M. anomochaeta*, originally isolated from decaying canvas in South Africa and sequenced [54]. It probably has cellulolytic capability, but may be part of the *Didymella-Phoma* complex which also contains pathogens of rotation crops such
as peas and chickpeas. As with all identifications based on a limited sequence, a caveat needs to be made that only by isolating and characterizing the isolate, can one be completely sure of species identification. In addition, there can be PCR primer bias that can favor certain groups. But nevertheless, *Macroventuria* was a very abundant taxon.

Although Ascomycota was the predominant phylum, there were a few groups of basidiomycetes that were common and more predominant in no-till. We detected 2 OTUs in the family Hydnodontaceae and one in the order Trechisporales. The order Trechisporales contains one family, Hydnodontaceae, with 15 genera [52]. They produce a macroscopic thallus, a corticioid, recupinate flat structure, with a hymenium covered with basidia and basidiospores. There is not much literature on this group, but like most basidiomycetes in the Agaricales, they are prolific producers of enzymes that break down lignins and wood. The genus *Trechispora* has a wide spread distribution in forest ecosystem, but little is known about corticioid fungi in agricultural soils. Lynch and Thorn [55] examined a clone library from DNA extracted from an agricultural soil in Michigan. They sequenced the large and small subunit RNA and identified 215 homobasidiomycetes species, including the order Ceratobasidiales (*Rhizoctonia, Waitea*) and Tremellales (*Cryptococcus*), but none in the order Trechosporiales.

The most abundant Basidiomycete group was the genus *Cryptococcus* (Family Tremellaceae), a very common soil inhabitant worldwide, with the vegetative form of a single-celled yeast [56]. It is found in forest and grassland soils based on DNA sequences [57–59]. *Cryptococcus* is a colonizer of wheat roots [60] and strawberry roots [61]. It also contains the human pathogens *C. neoformans* and *C. gatti* [62]. These are able to survive dry soil conditions by the formation of a polysaccharide capsule and melanin [62]. We identified 4 species that were highly abundant, *C. terreus* (more abundant in no-till), and *C. victoriae, C. aerius* and *C. bhutanensis* (more abundant in conventional tillage). This group of fungi is probably not capable of degrading complex plant structural components, like the homobasidiomycetes, but is perhaps using simpler components of breakdown products [63]. A number of them have been shown to be effective biocontrol agents [63] and may play a role in the suppression of root diseases.

After an initial increase in wheat root diseases, especially *Rhizoctonia*, disease often declines in long-term no-till systems [20, 22]. The buildup of populations of pathogen-antagonistic fungi has been implicated as the responsible agents for disease suppression [24]. As such, the taxa found as more abundant in no-till soils may be good candidates for exploration as biocontrol agents. However, targeted culturing approaches will be needed to isolate and investigate the pathogen-antagonistic potential and other functional characteristics of these taxa. Similarly, because amplicon sequencing provides only relative abundance data, complementary quantitative approaches, such as qPCR, will offer additional insight into the absolute abundances of these groups and their importance in soil communities in different cropping systems.

In conclusion, the fungal community in wheat soils is highly influenced by the tillage system. This effect was evident, despite the fact that location and year of sampling also had major effects. Even though we sampled the winter wheat part of the rotation in all treatments, the previous rotations were different, which could explain some of the variation. We identified a consortia of taxa in high abundance that were more dominant in no-till and one that was more dominant in conventional tillage, consistent across locations and years. Our results suggest that taxa more dominant in no-till are better adapted at utilizing intact, decaying roots as a food source and may exist as root endophytes. This would give them a competitive advantage in colonizing the dying root. However, another possibility is that these populations are negatively impacted by tillage, which can break up hyphal networks or cause modify other components of the soil microbiome, leading to a secondary effects on fungi. Our results suggest that taxa more common in conventionally tilled systems can utilize fresh, mature plant residues that are turned into the soil with tillage as pioneer colonizers, and then produce large numbers of conidia that...
are not as affected by tillage as the mycelial life stage. However, a larger portion of the community also showed no significant difference in abundance in both systems. Taxa unaffected by tillage treatments may be niche generalists, survive in deeper soil layers so as to avoid negative effects, or may be less affected by the bacterial microflora which can be stimulated by tillage.

Supporting information
S1 Fig. Phylum-level composition of no-till (NT) and conventionally tilled (CT) fields in different locations and years.
(TIF)

S1 Table. FDR corrected p-values for ANOVAs on Log10 transformed sequence counts comparing tillage, location, and year effects on abundant fungal genera. Mean sequence counts and standard deviations are presented for each location/tillage combination.
(DOCX)

S2 Table. OTUs significantly influenced by tillage (ANOVA FDR adjusted p-value < 0.05). Mean sequence counts (± standard deviation) for each tillage-location combination are presented.
(DOCX)

S3 Table. Rarefied OTU table.
(XLSX)

S4 Table. OTU representative sequences.
(FNA)

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References
1. Lal R, Reicosky DC, Hanson JD. Evolution of the plow over 10,000 years and the rationale for no-till farming. Soil Tillage Res. 2007; 93: 1–12.
2. Ritz K, Young IM. Interactions between soil structure and fungi. Mycologist. 2004; 18: 52–59.
3. Young IM, Ritz K. Tillage, habitat space and function of soil microbes. Soil Till Res. 2000; 53: 201–213.
4. Montgomery DR. Soil erosion and agricultural sustainability. P Natl Acad Sci 2007; 104: 13268–13272.
5. Uri ND, Lewis JA. Agriculture and the dynamics of soil erosion in the United States. J Sustain Agric. 1999; 14: 63–82.
6. USDA-NASS. 2012 Census of Agriculture Highlights. 2012;ACH12-6/July 2014.
7. Derpsch R, Friedrich T, Kasssam A, Li HW. Current status of adoption of no-till farming in the world and some of its main benefits. Int J Agric Biol Eng. 2010; 3:1–25.
8. Huggins DR, Reganold JP. No-till: How farmers are saving the soil by parking their plows. Scientific American. 2008. Available from: https://www.scientificamerican.com/article/no-till/.
9. Govaerts B, Mezzalama M, Sayre KD, Crossa J, Lichter K, Troch V, et al. Long-term consequences of tillage, residue management, and crop rotation on selected soil micro-flora groups in the subtropical highlands. Appl Soil Ecol. 2008; 38: 197–210.
10. Gonzalez-Chavez MDA, Aitkenhead-Peterson JA, Gentry TJ, Zuberer D, Hons F, Loeppert R. Soil microbial community, C, N, and P responses to long-term tillage and crop rotation. Soil Till Res. 2010; 106: 285–293.
11. Helgason BL, Walley FL, Germida JJ. Long-term no-till management affects microbial biomass but not community composition in Canadian prairie agroecosystems. Soil Biol Biochem. 2010; 42: 2192–2202.
12. Wortmann CS, Quincke JA, Drijber RA, Mamo M, Franti T. Soil microbial community change and recovery after one-time tillage of continuous no-till. Agron J. 2008; 100: 1681.
13. Jangid K, Williams MA, Franzluebbers AJ, Schmidt TM, Coleman DC, Whitman WB. Land-use history has a stronger impact on soil microbial community composition than aboveground vegetation and soil properties. Soil Biol Biochem. 2011; 43: 2184–2193.
14. Jiang X, Wright A, Wang X, Liang F. Tillage-induced changes in fungal and bacterial biomass associated with soil aggregates: a long-term field study in a subtropical rice soil in China. Appl Soil Ecol. 2011; 48: 168–173.
15. Mmouthia LW, Acosta-Martinez V, DeBruyn J, Schaeffer S, Tyler D, Odoi E, et al. Long term tillage, cover crop and fertiliser effect on microbial community structure, activity: Implication for soil quality. Soil Biol Biochem. 2015; 89: 24–34.
16. Murugan R, Koch HJ, Joergensen RG. Long-term influence of different tillage intensities on soil microbial biomass, residues and community structure at different depths. Biol Fertil Soils. 2014; 50: 487–498.
17. Sale V, Aguileria P, Laczko E, Madov P, Berner A, Zihlmann U, et al. Impact of conservation tillage and organic farming on the diversity of arbuscular mycorrhizal fungi. Soil Biol Biochem. 2015; 84: 38–52.
18. Zhang S, Li Q, Lu Y, Sun X, Jia S, Zhang X, et al. Conservation tillage positively influences the micro-flora and microfauna in the black soil of Northeast China. Soil Till Res 2015; 148: 46–52.
19. Turrini A, Sbrana C, Avio L, Njeru EM, Bocci G, Barberi P, and Giovannetti M. Changes in the composition of native root arbuscular mycorrhizal fungal communities during a short-term cover crop-maize succession. Biol Fertil Soils 2016; 52: 643–653.
20. Schroeder KL, Paulitz TC. Root diseases of wheat and barley during the transition from conventional tillage to direct seeding. Plant Dis. 2006; 90: 1247–1253.
21. Schillingwer WF, Paulitz TC. Natural suppression of Rhizoctonia bare patch in a long-term no-till cropping systems experiment. Plant Dis. 2004; 98: 389–394.
22. Schroeder KL. The dynamics of root diseases of wheat and barley in transition from conventional tillage to direct seeding. PhD dissertation, Washington State University, Pullman. 2004.
23. Yin C, Schroeder K, Mueth N, Schlatter D, Dhingra A, Hulbert S, et al. Bacterial communities on wheat grown under long-term conventional tillage and no-till in the Pacific Northwest of the US. Phytobiome. 2017; 1: 83–90.
24. Penton CR, Gupta VVSR, Tiedje JM, Neate SM, Ophel-Keller K, Gillings M, Harvey P, et al. Fungal community structure in disease suppressive soils assessed by 28S LSU gene sequencing. PLoS ONE. 2014; 9: 1–12.
25. Johnson-Maynard JL, Umiker KJ, Guy SO. Earthworm dynamics and soil physical properties in the first three years of no-till management. Soil Till Res. 2007; 94: 338–345.
26. Yin C, Hulbert SH, Schroeder KL, Mavrodi O, Mavrodi D, Dhingra A, et al. Role of bacterial communities in the natural suppression of Rhizoctonia solani/bare patch disease of wheat (Triticum aestivum). Appl Environ Microbiol. 2013; 79: 7428–7438. https://doi.org/10.1128/AEM.01610-13 PMID: 24056471
27. White TJ, Bruns T, Lee S, Taylor JW. Amplification and direct sequencing of fungal ribosomal RNA genes for phylogenetics. PCR Protocols: a guide to methods and applications. 1990; 18: 315–322.
28. Schloss PD, Westcott SL, Ryabin T, Hall JR, Hartmann M, Hollister EB, et al. Introducing mothur: open-source, platform-independent, community-supported software for describing and comparing microbial communities. Appl Environ Microbiol. 2009; 75: 7537–41. https://doi.org/10.1128/AEM.01541-09 PMID: 19801464

29. Caporaso JG, Kuczynski J, Stombaugh J, Bittinger K, Bushman FD, Costello EK, et al. QIIME allows analysis of high-throughput community sequencing data. Nature Methods. 2010; 7: 335–336. https://doi.org/10.1038/nmeth.f.303 PMID: 20383131

30. R Core Team. R: A language and environment for statistical computing (R Foundation for Statistical Computing, Vienna, Austria). 2016; Available from: https://www.CRAN.R-project.org.

31. Oksanen J, Blanchet F, Kindt R, Legendre P, Minchin P, O’Hara R, et al. Vegan: Community Ecology Package. Oulu: University of Oulu. 2016; Available from: http://CRAN.R-project.org/package=vegan

32. Degrune F, Theodorakopoulos N, Dufrene M, Colinet G, Bodson B, Hiel MP, et al. No favorable effect of reduced tillage on microbial community diversity in a silty loam soil (Belgium). Agr Ecosyst Environ. 2016; 224: 2–21.

33. Wang Z, Chen Q, Liu L, Wen X, Liao Y. Responses of soil fungi to 5-year conservation tillage treatments in the drylands of northern China. Appl Soil Ecol 2016; 101:132–140

34. Detheridge A, Brand G, Fychan R, Crotty FV, Sanderson R, Griffith GW, et al. The legacy effect of cover crops on soil fungal populations in a cereal rotation. Agr Ecosyst Environ 2016; 228: 49–61.

35. Smiley RW, Gourlie JA, Easley SA, Patterson LM, Whittaker RG. Crop damage estimates for crown rot pathogens under direct seeding in the Pacific Northwest, USA. Can J Plant Pathol. 2002; 24: 416–428.

36. Poole G, Smiley RW, Walker C, Huggins D, Rupp R, Abatzoglou J, et al. Effect of climate on the distribution of Fusarium spp. causing crown rot of wheat in the Pacific Northwest of the United States. Phytopathology. 2013; 103: 1130–1140. https://doi.org/10.1094/PHYTO-07-12-0181-R PMID: 24102211

37. Paulitz TC, Smiley RW, Cook RJ. Insights into the prevalence and management of soilborne cereal pathogens under direct seeding in the Pacific Northwest, USA. Can J Plant Pathol. 2002; 24: 416–428.

38. Zhang XX, Sunm HY, Shen CM, Li W, Yu HS, Chen HG. Survey of Fusarium culmorum and Bipolaris sorokiniana: effects of selected fungal antagonists on growth and yield components. Plant Path. 1995; 44: 467–77.

39. Fernandez MR, Basnyat P, Zentner RP. Response of common root rot in wheat to crop management in eastern Saskatchewan. Can J Plant Sci. 2007; 87: 953–963.

40. Fernandez MR, Holzgang G. Fungal populations in roots and crowns of oat crops in Saskatchewan. Can J Plant Sci. 2009; 89: 549–557.

41. Knudsen IM, Hockenhull J, Jensen DF. Biocontrol of seedling diseases of barley and wheat caused by Fusarium culmorum and Bipolaris sorokiniana. Plant Path. 1995; 44: 467–77.

42. Fernandez MR, Basnyat P, Zentner RP. Response of common root rot in wheat to crop management in eastern Saskatchewan. Can J Plant Sci. 2007; 87: 953–963.

43. Seifert K, Morgan-Jones G, Gams W, Kendrick B. The Genera of Hyphomycetes. CBS-KNAW Fungal Biodiversity Centre, Utrecht, The Netherlands. 2011.

44. Tiscornia S, Sequi C, Bettucci L. Composition and characterization of fungal communities from different composted materials. Cryptogamie Mycol. 2009; 30: 363–376.

45. Poll C, Brune T, Begerow D, Kandel E. Small-scale diversity and succession of fungi in the detritusphere of rye residues. Microb Ecol. 2010; 59: 130–140. https://doi.org/10.1007/s00248-009-9541-9 PMID: 19495854

46. Eichorst SA, Kuste CR. Identification of cellulose-responsive bacterial and fungal communities in geographically and edaphically different soils by using stable isotope probing. Appl Environ Microbiol. 2012; 78: 2316–2327.

47. Cummings JA, Bergstrom GC. First report of brown stem rot caused by Cadophora gregata in soybean in New York. Plant Dis. 2015; 99: 1284.

48. Agusti-Brisach C, Gramaje D, Garcia-Jimenez J, Armengol J. Detection of black-foot and Petri disease pathogens in soils of grapevine nurseries and vineyards using bat plants. Plant Soil. 2013; 364: 5–13.

49. Hosseini-Nasabnia Z, Van Rees K, Vujanovic V. Preventing unwanted spread of invasive fungal species in willow (Salix spp.) plantations. Can J Plant Pathol. 2016; 38: 325–337.

50. Khastini RO, Ogawara T, Sato Y, Narisawa K. Control of Fusarium wilt in melon by the fungal endophyte, Cadophora sp. Eur J Plant Path. 2014; 139: 333–342.

51. Alberton O, Kuyper TW, Summerbell RC. Dark septate endophytic fungi increase growth of Scots pine seedlings under elevated CO2 through enhanced nitrogen use efficiency. Plant Soil. 2010; 328: 459–470.
52. Cannon PF, Kirk PM. Fungal Families of the World. CABI, UK; 2007.
53. Bills GF, Platas G, Pelaez F, Masurekar P. Reclassification of a pneumocandin-producing anamorph, 
Glarea lozoyensis gen. et sp. nov., previously identified as Zalerion arboricola. Mycol Res. 1999; 103: 
179–192.
54. Aveskamp MM, de Gruyter J, Woudenberg JHC, Verkley GJM, Crous PW. Highlights of the 
Didymellaceae: A polyphasic approach to characterise Phoma and related pleosporalean genera. Stud Mycol. 
2010; 5: 1–60.
55. Lynch MKJ, Thorn RG. Diversity of basidiomycetes in Michigan agricultural soils. Appl Environ Microb. 
2006; 72: 7050–7056.
56. Vishniac HS. A multivariate analysis of soil yeasts isolated from a latitudinal gradient. Microb Ecol. 
2006; 52: 90–103. https://doi.org/10.1007/s00248-006-9066-4 PMID: 16708262
57. Bruée M, Reich M, Mural C, Morin E, Nilsson R H, Uroz S, et al. 454 Pyrosequencing analyses of forest 
soils reveal an unexpectedly high fungal diversity. New Phytol. 2009; 184: 449–456. https://doi.org/10. 
1111/j.1469-8137.2009.03003.x PMID: 19703112
58. Yarwood SA, Bottomley PJ, Myrold D. Soil microbial communities associated with Douglas-fir and red 
alder stands at high- and low-productivity forest sites in Oregon, USA. Microb Ecol. 2010; 60: 606–617. 
https://doi.org/10.1007/s00248-010-9675-9 PMID: 20449582
59. Yurkov AM, Kemler M, Begerow D. Assessment of yeast diversity in soils under different management 
regimes. Fungal Ecol. 2012; 5: 24–35.
60. Taheri AE, Hamel C, Gan Y. Pyrosequencing reveals the impact of foliar fungicide application to chick- 
pea on root fungal communities of durum wheat in subsequent year. Fungal Ecol. 2015; 15: 73–81.
61. Nallanchakravartula S, Mahmood S, Alstrom S, Finlay RD. Influence of soil type, cultivar and 
Verticillium dahliae on the structure of the root and rhizosphere soil fungal microbiome of strawberry. PLoS 
ONE 9(10): 2014;e111455. https://doi.org/10.1371/journal.pone.0111455 PMID: 25347069
62. Kwon-Chung KJ, Fraser JA, Doering TL, Wang ZA, Jumaa G, Idnurm A, et al. Cryptococcus neoform- 
mans and Cryptococcus gattii, the etiologic agents of Cryptococcosis. Cold Spring Harbor Perspectives 
in Medicine. 2014; 4: https://doi.org/10.1101/cshperspect.a019760 PMID: 24985132
63. Botha A. The importance and ecology of yeasts in soil. Soil Biol Biochem. 2011; 43: 1–8.