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Fungal genome size and composition reflect ecological strategies along soil fertility gradients

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Author Contributions

HZ and JP conceived the study and analysed the data. HZ compiled the fungal traits dataset. AB led the AMI project and JP performed taxonomic assignments for zOTUs. HZ performed the statistical analyses with help from JP. HZ, HL and JP wrote the manuscript, and all co-authors provided input on subsequent drafts.

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Abstract

Genomic traits reflect the evolutionary processes that have led to ecological variation among extant organisms, including variation in how they acquire and use resources. Soil fungi have diverse nutritional strategies and exhibit extensive variation in fitness along resource gradients. We tested for trade-offs in genomic traits with mycelial nutritional traits and hypothesize that such trade-offs differ among fungal guilds as they reflect contrasting resource exploitation and habitat preferences. We found species with large genomes exhibited nutrient-poor mycelium and low GC content. These patterns were observed across fungal guilds but with varying explanatory power. We then matched trait data to fungal species observed in 463 Australian grassland, woodland and forest soil samples. Fungi with large genomes and lower GC content dominated in nutrient-poor soils, associated with shifts in guild composition and with species turnover within guilds. These findings highlight fundamental mechanisms that underpin successful ecological strategies for soil fungi.

Introduction

A pervasive challenge in ecology is to understand and predict how organisms adapt to their local environments and respond to environmental changes, with recent efforts attempting to address this challenge with microbial organisms (Widder *et al.* 2016; Fierer 2017). There has been growing interest in using trait-based approaches to study microbial ecological strategies and their responses to local and global environmental factors due to the direct ecological implications of functional traits on organism's fitness and tolerance to biotic and abiotic factors (Kraft *et al.* 2008; Martiny *et al.* 2015). This is particularly the case for fungi (Aguilar-Trigueros *et al.* 2014; Põlme *et al.* 2020; Zanne *et al.* 2020), which are ubiquitous organisms that control critical aspects of ecosystem functioning including plant nutrition, host health and fitness and nutrient cycling in terrestrial ecosystems (Tedersoo *et al.* 2014; Naranjo-Ortiz & Gabaldón 2019). Most trait-based studies of fungi have targeted phenotypic traits related to mycelium construction (e.g., hyphal extension and branching rates, hyphal chemistry; Camenzind *et al.* 2020; Camenzind *et al.* 2021), reproduction (e.g., spore size and sporulation behaviour; Aguilar-Trigueros *et al.* 2019; Chan *et al.* 2019, 2020) and resource uptake (gene expression of enzymatic pathways; Talbot *et al.* 2015). However, these traits have been studied largely (if not entirely) under highly controlled conditions, which limits our understanding of how fungal traits can shape species distributions in their natural environment.

All living organisms require nutrients to grow and reproduce. Genomic traits such as genome size (DNA content of the entire genome) and genomic DNA base composition of guanine-cytosine (GC) content in organisms are associated with their adaptation strategies to changes in nutrient availability in the environment (Giovannoni *et al.* 2014; Shenhav & Zeevi 2020). Nucleic acids are among the most cellular nutrient-rich molecules, with carbon (C) : nitrogen (N) : phosphorus (P) stoichiometry being 12 : 4 : 1 (Sterner & Elser 2002). Thus, species with large genomes, which are more demanding and costly to build and maintain than small genomes, are expected to be less competitive when N and P (and in some cases other nutrients) are limited (Leitch & Leitch 2013). Evidence from plant studies suggests that species with large genomes are more likely to successfully compete and dominate in natural plant communities when levels of resources such as N and P are high in soil (Šmarda *et al.* 2013; Guignard *et al.* 2016). The same patterns exist in bacteria: species with small genomes possess growth advantages in nutrient-depleted environments due to their reduced nutrient requirements than species with large genomes (Giovannoni *et al.* 2014). These studies highlight that mapping microbial species and their genomic traits along resource gradients

can provide insight into their eco-evolutionary adaptations to resource limitations (Gudelj *et al.* 2010; Barberán *et al.* 2014).

Trait-trait correlations reflect fundamental constraints related to organisms' ecological strategies (Westoby *et al.* 2002; Reich *et al.* 2003) because one must optimize their performance by strategically allocating limited resources to different features (Bazzaz & Grace 1997). These trait trade-offs can exist at the genome level. For example, nutrient limitation can select for genomes with low GC content due to the adenine-thymine (AT) base pair having a lower nitrogen-to-carbon ratio (N: C= 7: 10, =0.7) than the GC pair (N: C= 8: 9, ~0.9). Therefore, organisms with large genomes may adapt to higher N requirements by reducing the use of the relatively N-rich GC base pair (Kelly 2018). In addition, tissue nutrient concentration reflects an organism's nutrient demand and utilization (Aerts & Chapin III 1999) and determines organism's growth efficiency (Sinsabaugh *et al.* 2013). A link between genomic traits and tissue nutrient concentrations can be expected if species with different genome sizes have different nutrient requirements, exploit nutrients with varying efficiency, or store or convert nutrients to biomass at different rates (Faizullah *et al.* 2021). Besides the potential linkage between GC content and N economy, others have found a direct connection of GC content with recombination of homologous chromosomes in organisms, along with other factors such as mutation rates, natural selection, and genetic drift can influence GC content of genomes. But how nutrient availability is involved in the GC content of soil fungi is less investigated.

A striking property of fungi is their diversity of lifestyles reflected by different guilds (e.g. mycorrhizal, pathogenic, saprotrophic guilds). Each guild exhibits specific trophic behaviours, i.e., resource exploitation and habitat or host preferences (Johnson *et al.* 2013; Naranjo-Ortiz & Gabaldón 2019), which might affect their sensitivity to changes in resource availability (Maaroufi *et al.* 2019; Lekberg *et al.* 2021). The fitness of free-living (e.g., saprotrophic) fungi is expected to be directly linked to abiotic resource conditions, while the fitness of symbiotic (e.g., mycorrhizal and pathogenic) fungi is linked to nutrients and carbon source-sink dynamics during interactions with the host (and, for mycorrhizal guilds, competition of soil resources with saprotrophs). In addition, recent studies suggested that genome size varies among fungi with different lifestyles (Spanu 2012; Miyauchi *et al.* 2020). Spanu (2012) suggested that the acquisition of biotrophy in plant pathogens was associated with an expanded genome. Genome size variation during plant-host interactions or specific sources in nutrient acquisition among different fungal guilds might determine the strength of the correlation between genome size and nutrient demand for growth. Thus, considering

fungal guilds help to understand how trophic behaviour shapes an organism's genome traits and nutrient adaptation (Bahram & Netherway 2021).

Here we compiled species-level data on two genomic traits (genome size from 2,437 and GC content from 1,276 fungal species) and estimated their inter-correlations and relationships with fungal nutrient concentrations to assess their ecological trade-offs and adaptative strategies among and within fungal guilds. We also investigated the distribution of these genomic traits in relation to nutrient availability in 463 soil samples taken from grassland, woodland and forest ecosystems over natural soil nutrient gradients across Australia. We hypothesized that nutrient-depleted environments favour fungi with smaller genomes and lower GC content. We also hypothesised that such environmental selection would be stronger on genomic traits for plant symbiotic fungi than saprotrophs given that plant symbiotic fungi will respond to changes in resource availability as well as to how their plant hosts respond to those changes (eg., greater resource-sharing or stronger competition for limited resources).

Material and Methods

Fungal genomic and nutritional traits

We retrieved fungal genome size (Mbp, 1C-values) and GC content (%) data from the National Center for Biotechnology Information (NCBI) genome database (https://www.ncbi.nlm.nih.gov/genome/), Mycocosm (Grigoriev *et al.* 2014) (https://mycocosm.jgi.doe.gov/mycocosm/home) and Fungal Genome Size Database (http://www.zbi.ee/fungal-genomesize) (Kullman *et al.* 2005). We assigned the most recently accepted taxonomic names from Catalogue of Life (https://www.catalogueoflife.org/) to our database by comparing accepted and synonym names using the *cp_nu_suggest* function from the *rcol* R package (Chamberlain 2021). After checking synonymic names, the genomic traits were averaged for the same species from the three resources. We kept the genomic trait data for species that could be aligned with fungal guild information derived from the FUNGuild database with a confidence level of "probable" or "highly probable" (Nguyen *et al.* 2016). This resulted in our dataset containing 2,437 and 1,276 guild-annotated species with genome size and GC content data, respectively; we obtained genome size data for all 1,276 species with GC content data. For both genomic traits, the guilds included were 7 Arbuscular mycorrhizal (AM), 78 ectomycorrhizal (EcM), 317 plant pathogenic and 874 saprotrophic species.

We retrieved fungal trait data related to growth and nutrition from the Fungal Functional Trait database (Fun[Fun]) (Zanne *et al.* 2020), including N and P concentrations in fungal mycelial tissues obtained from the database but originally derived from Zhang and Elser (2017). There were 534 observations for 280 species in mycelium N and 654 observations for 275 species in mycelium P. For species with multiple observations, we calculated a mean trait value. After cross-referencing each database, we found matching information for genome size with 70 and 85 species for mycelium N and P, respectively. For matches with GC content, we found 32 and 47 species with estimates of mycelium N and P, respectively. The numbers of observations at the guild-level for the overlap between genomic traits and fungal nutrients were supplied in Table 1. The majority of the observations with overlapping data were from ECM and saprotrophic fungi, followed by plant pathogenic fungi.

Study sites and soil sampling across Australia

In this study, we used data generated from soil samples collected across Australia during the period from 2005 to 2018 as part of the Australian Microbiome Initiative (AMI; formerly the Biomes of Soil Environments [BASE] project; Bissett *et al.* 2016). We obtained the sequencing data from https://www.australianmicrobiome.com/ on 27 August 2020. A total of 463 soil samples from 409 sites were included in this study, selected based on the criteria described below (see Appendix S1 for a list of sample numbers used here). The soil samples were collected according to the methods described in Bissett *et al.* (2016). Briefly, composite soil samples were generated from 9-25 soil cores collected to a depth of 10 cm within 25 × 25 m plots (details see Fig. S1). Each soil sample was separated into two subsamples. The first subsample was frozen and transported to the Adelaide node of the Australian Genome Research Facility (AGRF) laboratories for DNA extraction and sequencing. The second subsample was air-dried for soil available N and P measurements (Bissett *et al.* 2016). Note that we used soil available nutrients (inorganic form) as the proxy for indicating fungal nutrient limitation although some fungi, particularly saprotrophs but also EcM fungi, can also access organically bound soil nutrients in addition to those in inorganic forms. In addition, biotrophic pathogens can obtain essential nutrients from host tissues, which will vary to a certain extent but not entirely with soil nutrient availability. Thus, estimates of relationships may lack precision for these guilds, and further studies could

also benefit from estimating soil total N and P as well as autecological studies of how free-living and symbiotic fungi acquire and allocate N and P from different source pools.

Fungal DNA extraction, sequencing and bioinformatics

Genomic DNA extraction and bioinformatic analysis were conducted according to methods described in Bissett *et al.* (2016). Briefly, soil gDNA was extracted in triplicate using MoBio PowerSoil extraction kits (MO BIO Laboratories Inc., USA) following the manufacturer's instructions. DNA amplicons targeting the fungal ITS1-5.8S-ITS2 region were prepared and sequenced at the Australian Genome Research Facility (Melbourne, Australia) and Ramaciotti Centre for Genomics (Sydney, Australia). ITS amplicons were sequenced on the Illumina MiSEQ platform with MiSeq Reagent Kit v3 600 cycle chemistry, to produce 300 bp paired-end reads. The sequenced region (ITS1-5.8S-ITS2) is 550bp on average in fungi but can be much larger (Nilsson *et al.* 2015), thus for many reads there was not sufficient (or any) overlap between the forward and reverse pair of each template sequence to merge them into a single sequence read. To ensure that those long fungal ITS1-5.8S-ITS2 sequences were not excluded from our analysis, the ITS1 and ITS2 regions were separately extracted from forward and reverse reads, respectively, using ITSx (Bengtsson-Palme *et al.* 2013) and both regions were processed. Zero-radius operational taxonomic units (zOTUs) were generated from identified ITS regions and frequencies of zOTUs within each sample were determined. Taxonomic and guild identities of zOTUs were assigned and matched with fungal genomic traits (Appendix S2). Eventually, there were 225 fungal species with genome size data occurring in 463 soil samples and 145 species with GC content data in 460 soil samples across Australia (Fig. S1-S2).

Statistical analyses

To assess correlations among traits, we used the phylogenetic generalized least squares (PGLS) method to account for the shared evolutionary histories among fungal species. We constructed the fungal phylogenetic tree using the fungal mega-phylogeny published by Li *et al.* (2021) (time-calibrated tree) as the backbone. For those genera and species that were absent from the meta-phylogeny, we used V.PhyloMaker (https://github.com/jinyizju/) to add them to their respective families (in the case of genera) and genera (in the case of species) in the mega-phylogeny under Scenario 3 (Jin & Qian 2019). The polytomies were resolved by *multi2di* function in the R package ape (Paradis & Schliep 2019). We estimated differences in genome size and GC content among fungal guilds, as a fixed effect, by fitting PGLS-based models using the *phylolm* R package (Tung

Ho & Ané 2014). In addition, we examined the relationships between (i) fungal genome size and GC content; and (ii) each of genome size and GC content versus each of mycelial N concentration and mycelial P concentration. To determine differences in resource acquisition strategies among guilds, we tested these relationships separately across four guilds: AM, EcM, pathogens and saprotrophic fungi. These PGLS-regressions were performed using the *phylolm* function. For the correlations in (ii), we also calculated the independent variation explained by each predictor (R^2) using the R package *rr2* (Ives & Li 2018).

We evaluated distributions of fungal genomic traits in soils and their correlations with soil available nutrients. We calculated the community-weighted mean genome size (CWM-GS) and GC content (CWM-GC) using the fungal species-sample table generated after summing read counts associated with zOTUs assigned to the same species. To generate CWM, weighted averages of genomic traits were calculated for each soil sample using the relative abundance of each fungal species as weights. CWM was calculated across the entire fungal community and individually for subsets of species assigned to each of three major fungal guilds (AM fungi were represented by too few species for a robust analysis to be performed, thus were not included) and correlated with soil available nutrients. Note that when calculating CWM, values can be potentially affected by coverage in each sample, i.e., the proportion of zOTU counts that could be assigned to a species for which trait data were available and, therefore, were used to calculate CWM. To assess whether samples with very low species coverage may cause spurious correlations, we analysed the data twice using the following strategies: (i) using all soil samples for which trait data could be assigned to at least one zOTU, and (ii) excluding those soil samples for which the percentage of zOTU read counts that could be assigned trait data was lower than the median proportion across all samples.

Linear mixed-effects models were used to evaluate the effects of soil available nutrients within each vegetation category on (i) CWM-GS and CWM-GC at the community level, (ii) relative abundance of each fungal guild, and (iii) CWM-GS and CWM-GC at the fungal guild level (e.g., CWM was calculated for each of the four fungal guilds, separately). For all mixed-models, we included one of each soil nutrient (NH_4^+, NO_3^-, PO_4^-) as fixed effects and the ITS fragment used to generate zOTUs (ITS1 and ITS2) as random effects, to account for potential biases between the two regions in their ability to detect fungal species and, thus, result in different members of the fungal community being detected (Bazzicalupo *et al.* 2013). Before the modelling using functions in the *lme4* package (Bates *et al.* 2015), soil nutrients and CWM-GS values were log-transformed (natural logarithms). Dispersion

was checked using the *DHARMa* package (Hartig 2022). While determining statistical significance, Kenward-Roger degrees of freedom were calculated using the Anova function from the *car* package (Fox *et al.* 2012). Marginal R^2 values (variance explained only by fixed effects) were calculated using the r.squaredGLMM function from the *MuMIn* package (Barton 2015). All the analyses were performed using R version 4.0 (R Core Team 2020).

Results

Genomic, growth and nutritional traits correlations across fungal guilds

Fungal genome size was negatively correlated with GC content across all four guilds (Fig. 1), with AM and ECM fungi having significantly larger genomes and lower GC contents on average than pathogenic and saprotrophic fungi (Table S1). Furthermore, this pattern was still observed even when we restricted the analysis to only the subset of species observed within the Australian Microbiome database (Fig. S3). When using all species for which we collected trait data, we observed relatively strong negative correlations for EcM (r^2 = 0.23, $P < 0.001$; slope 95% confidence interval (CI) was [-0.061, -0.025]; Fig. 1b) and plant pathogens (r^2 = 0.32, $P < 0.001$; 95% CI [-0.021, -0.015]; Fig. 1c) and a much weaker negative correlation for saprotrophs (r^2 = 0.03, $P < 0.001$; 95% CI [-0.007, -0.003]; Fig. 1d).

Fungal genome size negatively correlated with tissue N (r^2 = 0.17, P = 0.002; slope 95% CI was [-4.143, -1.175]; Fig. 2a) and P concentrations (r^2 = 0.14, P = 0.01; slope 95% CI [-0.244, -0.043]; Fig. 2b). Furthermore, fungal GC content was negatively correlated with N (r^2 = 0.14, P = 0.009; slope 95% CI [-1.278, -0.200]; Fig. 2c) and P concentrations (r^2 = 0.10, P = 0.028; slope 95% CI [-0.054, -0.004]; Fig. 2d) in fungal tissues. When comparing the relative importance of the genomic traits to explain variation in growth and nutritional traits, genome size explained greater variation than GC content in predicting concentrations of N and P (Table S2).

However, the correlational patterns between genomic traits and nutritional traits were also dependent on specific fungal guilds: the negative correlation between genome size and fungal N was only observed for EcM and saprotrophic fungi; while the negative correlation of genome size and P was only observed among saprotrophs (Fig. 2ab). In contrast, the negative correlations between GC content and fungal nutrients were not significant within most guilds (Fig. 2cd), except for that with fungal P for saprotrophic fungi.

Biogeography of fungal traits at the community level

On average, 3% of sequence reads per sample could be assigned trait data (range: 0.002% to 72%). This is equivalent to a range from 18 to 464220 sequence reads (mean: 16970) being assigned trait data in each sample (Appendix S3). Contrary to our initial hypothesis, we found that nutrient-depleted soils favoured fungi with larger genomes. This pattern was explained, in part by variation in soil N and P availability, with the response being most consistent and with the largest effect sizes in forest samples. In general, CWM-GS was negatively correlated with soil available NH_4^+ and NO_3^-, with correlations being strongest for forests (NH_4^+: $r^2 = 0.22$, $P < 0.001$; NO_3^-: $r^2 = 0.22$, $P < 0.001$), weak but significant for woodlands (NH_4^+: $r^2 = 0.02$, $P < 0.001$; NO_3^-: $r^2 = 0.07$, $P < 0.001$), and not observed for grasslands (Fig. 3abc). Similarly, CWM-GS was negatively correlated with soil available PO_4^-, although this correlation was only significant and relatively weak for forests ($r^2 = 0.05$, $P < 0.001$; Fig. 3d). In contrast, CWM-GC was positively correlated with soil available nutrients as we expected in our initial hypothesis, with most of these correlations being significant (Fig. 3e-g), except for correlations with soil available NH_4^+ and NO_3^- in grasslands (Fig. 3ef; Table S3). We also observed that these relationships between trait CWMs and soil available nutrients were generally consistent when we limited our analyses to samples in which trait data could be assigned to a *relatively* high proportion of community members (Table S3, which compares the analysis using all samples to one using only those for which a proportion of reads that could be assigned trait data was higher than the median value for all samples (1.2%)).

Guild-specific biogeographical patterns of fungal traits

The response patterns of genomic traits along the soil nutrient gradient at the community level can be explained by both shifts in the relative abundance among different fungal guilds and by species turnover within specific fungal guilds. For guild composition, EcM fungi exhibited generally negative responses (Fig. 4a), and plant-pathogenic (Fig. 4d) and saprotrophic fungi (Fig. 4g; Fig. S4; Table S4) both exhibited generally positive responses.

For CWM-GS responses to increased soil nutrients, most effect sizes were neutral for EcM, except for two negative responses (NO_3^- in the woodland and PO_4^- in the forest; Fig. 4b). In contrast, associations with CWM-GS were positive for plant pathogens (Fig. 4e), while negative for saprotrophic fungi (Fig. 4h; Fig. S5; Table S5). For CWM-GC responses to increased soil nutrients, most effect sizes were neutral for ECM fungi, except for two positive responses (response to NO_3^- in the woodland and PO_4^- in the forest; Fig. 4c). Most

associations with CWM-GC were positive for plant pathogens (Fig. 4f), while both positive and negative responses existed for saprotrophic fungi (Fig. 4i; Fig S6; Table S6).

Discussion

Our study demonstrates that genomic traits can be potentially used to explain variations in fungal functional traits and biogeographical patterns of fungi along large environmental gradients. First, fungi with large genomes produced nutrient-poor tissue and adjusted their nucleotide composition by reducing the frequency of the expensive GC base-pair. Second, soils across Australia with low levels of nutrient availability favoured fungi with large genomes and low GC content. Such genomic patterns at the community level along nutrient gradients were generally consistent across different forms/types of nutrients and in different vegetations. Finally, partially supporting our hypothesis, the environmental selection on genomic traits along the nutrient gradient would be stronger in symbiotic fungi than saprotrophic fungi, but this was only true for pathogenic but not for mycorrhizal fungi. Together, our results reveal fundamental mechanisms that underpin genome size and nucleotide selection in soil fungal communities along soil nutrient gradients, although in some cases the explanatory power was weak. This is not surprising given that tissue nutrient concentration and soil mineral nutrient stocks only partially reflect the range of biological and environmental controls on fungal distributions in soils (Tedersoo *et al.* 2014).

Trade-offs among fungal genomic, growth and nutritional traits

The negative correlation between genome size and GC content in fungal genomes, especially for pathogenic fungi, supports the existence of a close relationship between the size and composition of fungal genomes. Previous studies found that community-averaged GC content and genome size in bacteria were negatively correlated in soil but, in the same study, these two genomic traits were positively correlated under marine conditions (Chuckran *et al.* 2021; Chuckran *et al.* 2022). Thus, their results support our findings and suggest similar selection pressures on genome size and GC content for soil bacteria and soil fungi. In bacteria and archaea, several environmental factors in addition to nutrient availability are known to drive the selection on genome size and genomic GC-content, mainly including environmental selection (such as growth temperature and the availability of oxygen; see Foerstner *et al.* (2005); Sabath *et al.* (2013)) or GC-biased gene conversion (which favours G/C nucleotides during DNA recombination; see Webster and Hurst (2012) and Lassalle *et al.* (2015)). In our study, we mainly focused on nutrients due to their importance for genome construction

during the growth of fungi, but acknowledge that further variation in these genomic traits could be predicted with additional environmental drivers. In fungi, we found fungal genome sizes were negatively correlated with fungal N and P concentrations, suggesting that species with small genomes produced nutrient-rich tissue. We propose that this fungal behaviour has a similar function as in plants, for which the plant leaf economic spectrum suggests that nutrient-rich tissue is associated with high nutrient demand during rapid growth, and that small genomes facilitate a faster cell division and therefore a higher growth rate (Rayburn *et al.* 1994; Knight *et al.* 2005).

For bacterial and archaeal communities, inconsistent patterns have been described when linking genome size to copiotrophy/oligotrophy in previous studies, with both supporting (Liu *et al.* 2023) and non-supporting (Westoby *et al.* 2021) evidence being revealed. We previously found that bacterial genome sizes weakly but significantly correlated with soil carbon and P concentrations, in that bacteria with small genomes exhibited fewer negative responses to increasing soil carbon or more positive responses to increasing soil P, suggesting that higher soil fertility favours bacteria with smaller genomes (Liu *et al.* 2023). Fungi are generally considered to be more oligotrophic than bacteria, but it may be possible to assess these relationships in targeted studies of fungi exhibiting generally more copiotrophic strategies, such as those in the Zygomycota (Ho *et al.* 2017).

Nutrient-depleted soil environments favour fungi with large genomes and low GC content

CWM-GS significantly increased, and CWM-GC content decreased, in response to decreasing soil available nutrients, indicating that fungi with large genomes and low GC content dominated in nutrient-depleted sites. These patterns were generally found in each vegetation type (grassland, woodland and forest) or soil nutrient type (NH_4^+, NO_3- and PO_4^-), but were more pronounced in forest ecosystems, possibly due to the high prevalence of EcM fungi in forests and presumably a tighter relationship with available N (Table S4). Additional analyses showed weak but significant correlations between the relative abundance of EcM fungi and their genomic traits at the community level, where CWM-GS was increased (r^2 = 0.02, $P < 0.0001$) and CWM-GC content decreased ($r^2 = 0.03$, $P < 0.0001$) when the relative abundance of EcM fungi increased.

The opposite responses between CWM-GS and CWM-GC content along these nutrient gradients may be explained by the trade-off between the two genomic traits: fungi with large genomes tended likely to reduce the cost of genome construction by lowering the GC content. Genome size increases in low-nutrient environments can reflect several potential

mechanisms to cope with these conditions. For example, undergoing gene duplication can facilitate production of the enzymes and proteins required to cope with nutrient stress (Konstantinidis & Tiedje 2004; Giovannoni *et al.* 2005), suggesting a mechanism for EcM and saprotrophic fungi that produce extracellular hydrolytic enzymes for nutrient mineralization. Such mechanism aligns with an active and fitness-enhancing response of an organism capable of enhanced resource acquisition. As at a certain point it may no longer be adaptive to have a large genome under nutrient-limiting conditions (given that it is accompanied by a high genome construction cost), reducing GC content to build large genomes can offset the cost because a reduction in GC content decreases the amount of nitrogen required for DNA synthesis. These findings suggest that shifts in size and composition of organisms' genomes can be an important evolutionary and ecological strategy to adapt to their local nutritional environments.

To our knowledge, our study provided the first link between fungal genomic traits and fungal distribution patterns across large nutrient gradients. Previous investigations on this topic have focused on genome size patterns in plants (Pellicer *et al.* 2018) and bacteria (Chuckran *et al.* 2021). For plants, positive associations between CWM-GS and soil nutrients were reported (Šmarda *et al.* 2013; Guignard *et al.* 2016). Plants with large genomes are more likely to dominate in communities where nutrient availability is high in soil, probably because plants with large genomes require more nutrients to build genomes (Pellicer *et al.* 2018). For bacteria, inconsistent patterns have been described previously as both positive and negative correlations between genome size and nutrients were revealed. For example, bacteria with smaller genomes were associated with more harsh or nutrient-limited environments (Chuckran *et al.* 2022). In contrast, whole-genome shotgun sequencing data of *Lactobacillus* (phylum Firmicutes) suggested that deep, nutrient-depleted marine environments were dominated by bacteria with large genomes (Makarova *et al.* 2006).

Guild-level responses along soil nutrient gradients

Correlations between genomic traits and soil nutrient availabilities at the whole fungal community level could be derived from two major processes: (i) shifts in fungal guild structure and (ii) species turnover within fungal guilds. For the first process, the relative abundance for fungi with relatively large genomes (i.e., EcM; Fig. 1) significantly decreased while the relative abundance of fungi with relatively small genomes (i.e., plant pathogenic and saprotrophic fungi) significantly increased when soil nutrients increased to high levels; such shifts in fungal guild structure resulted in a lower CWM-GS under nutrient-enriched

sites. For the second process, the strength of such negative correlations varied depending on vegetation type, nutrient form and fungal guild. For example, when observed to be statistically significant, genomic traits of saprotrophs tended to exhibit relatively weak correlations across many combinations of vegetation types and nutrient forms compared with EcM and pathogenic fungi. The latter two groups also exhibited noticeable variations, with EcM fungi showing significant correlations ranging from weak to strong in woodlands and forests, and pathogenic fungi generally showing stronger correlations in grasslands. The distinct responses among guilds highlight the importance of considering different fungal groups independently when investigating their genomic trait patterns along environmental gradients.

Pathogens stood out because their CWM-GS and CWM-GC content was the only guild that mainly showed positive responses to soil fertility, i.e., supporting our original hypothesis that nutrient-depleted environments favour fungal species with smaller genomes and lower GC content. The nitrogen disease hypothesis states that plant growth at high N availability may result in increased plant susceptibility to pathogens because of increased foliar nitrogen concentrations (Mitchell *et al.* 2003). Our results expand this theory by showing that pathogens with large genomes and higher GC content could be particularly favoured in nutrient-enriched environments. Spanu and Kämper (2010) proposed that acquisition of biotrophy in plant fungal pathogens is associated with an expanded genome, with obligate pathogenic fungi such as powdery mildews and rust fungi having greater genome size than necrotrophic pathogens. However, in our study, the proportion of DNA reads that could be assigned to biotrophic fungi, including rust and mildew fungi, was low (0.2% on average) among all pathogens. Thus, the changes in genomic size along soil nutrients should be also driven by other pathogen types (e.g. necrotrophic pathogens). Variation in genome size can also be related to polyploidy, the amount of transposable elements and the potential function of predicted genes encoding secreted proteins and other effectors in pathogens (Lo Presti *et al.* 2015; Lorrain *et al.* 2019). We found that P availability had stronger explanatory power than N availability in explaining genome GC content for pathogens in grassland and woodland soils. This suggests that other, non-C/N-related, mechanisms may have been involved. In addition, P availability does influence plant and fungal growth and ecophysiologies, and acquisition of limited P can indirectly affect organisms' growing environments, modifying pH, redox state or availability of other nutrients availability, all of which could lead to environmental selection on GC content

(Foerstner et al. 2005). Determining which of these drivers is responsible for shaping genome GC content in pathogenic (and other) fungi warrants future research.

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Competing interests:

The authors declare no competing interests.

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Table 1 Guild-level sample sizes (number of species) for the overlap between genome size (GS) or genome GC content and other fungal traits, including fungal N and P concentration. Specific fungal guilds are arbuscular mycorrhizal (AM), ectomycorrhizal (EcM), plant pathogenic and saprotrophic fungi.

Model	AM	EcM	Pathogen	Saprotroph
GS ~ fungal N	0	34	11	25
GS ~ fungal P	1	39	12	33
GC ~ fungal N	0	10	9	13
GC ~ fungal P	0	14	10	23
Total numbers	1	97	42	94

Fig. 1

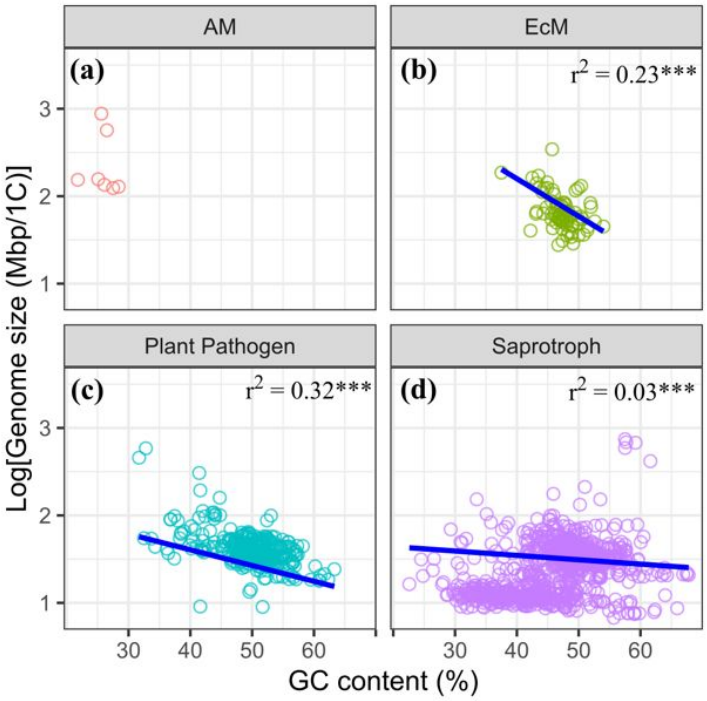

Fig. 1 Correlations between genome size (log-transformed) and GC content (%) for species within different fungal guilds. All trait estimates were averaged at the species level. Blue lines indicate correlations being significant (*** $P < 0.001$) and were fitted considering phylogenetic correlations among fungal species. Points with different colours indicate different fungal guilds: arbuscular mycorrhizal (AM, red, n = 7), ectomycorrhizal (EcM, green, n = 78), plant pathogen (blue, n = 317), and saprotrophic (purple, n = 874) fungi. The data in panel (d) represent both yeasts and filamentous fungi, which differ in both genome size and GC content, on average (Fig. S7).

Fig. 2

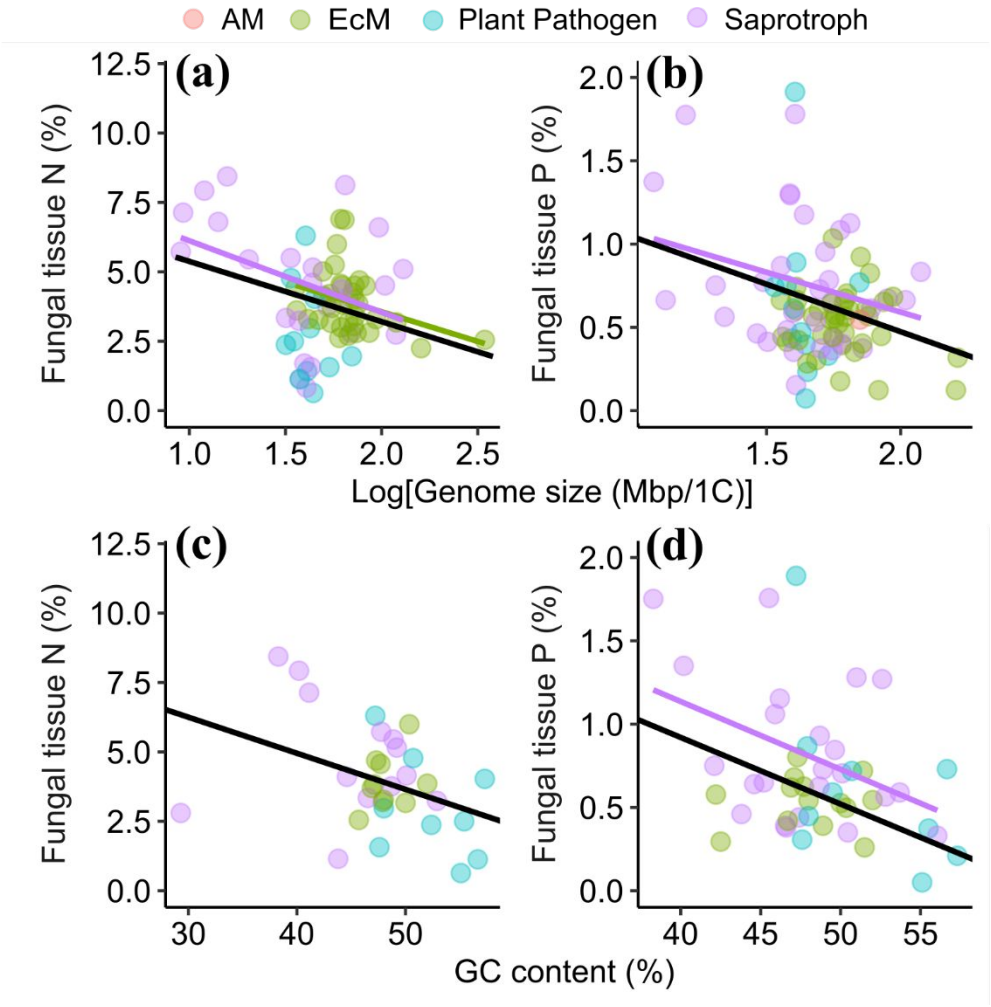

Fig. 2 Correlations of genome size (log-transformed) or genome GC content (%) with fungal nutrient concentrations, including N (%, a, c) or P (%, b, d) concentration in fungal tissues. All trait estimates were averaged at the species level. Lines were added when correlations were significant across all species (black lines) and within specific fungal guilds (coloured lines). Points with different colours indicate different fungal guilds: arbuscular mycorrhizal (AM, red), ectomycorrhizal (EcM, green), plant pathogen (blue), and saprotrophic (purple) fungi. Points are darker with more overlapping. Models were fitted considering the phylogenetic correlations among fungi species, with both genome size and GC content as predictors in explaining the variation of fungal tissue N and P. Statistical results and explained variation for each predictor were provided in Table S3.

Fig. 3

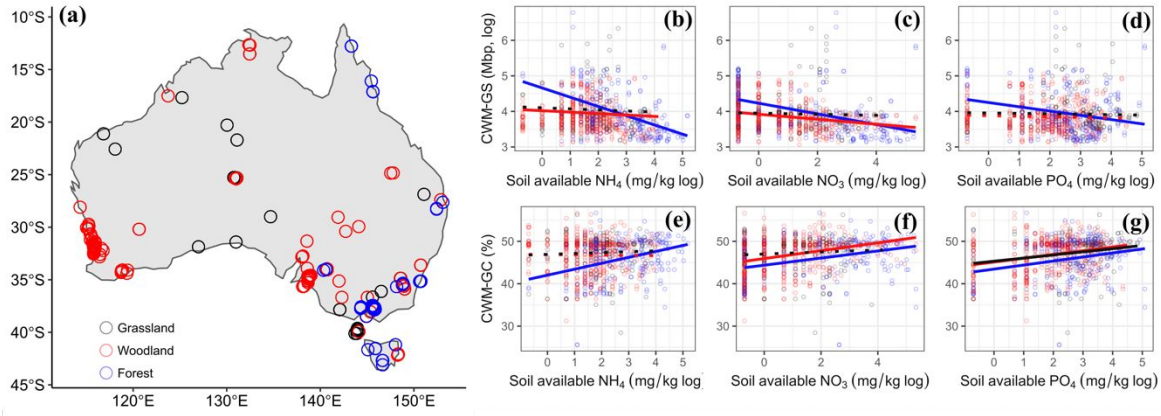

Fig. 3 Correlations between community weighted mean genome size (CWM-GS, Mbp/1C, log-transformed) or GC content (CWM-GC, %) and soil available nutrients (NH_4^+, NO_3^-, PO_4^-) for soils from grassland (black), woodland (red), and forest (blue) samples across Australia; 463 soil samples were collected from 409 locations. Correlations were fitted with a linear mixed model that included soil available nutrients as fixed effects and with DNA region sequenced (ITS1 or ITS2) as random effects. Points are darker with more overlapping. Solid lines indicate the correlations were significant ($P < 0.05$) while dashed lines indicate non-significant (model fitting results were supplied in Table S4).

Fig. 4

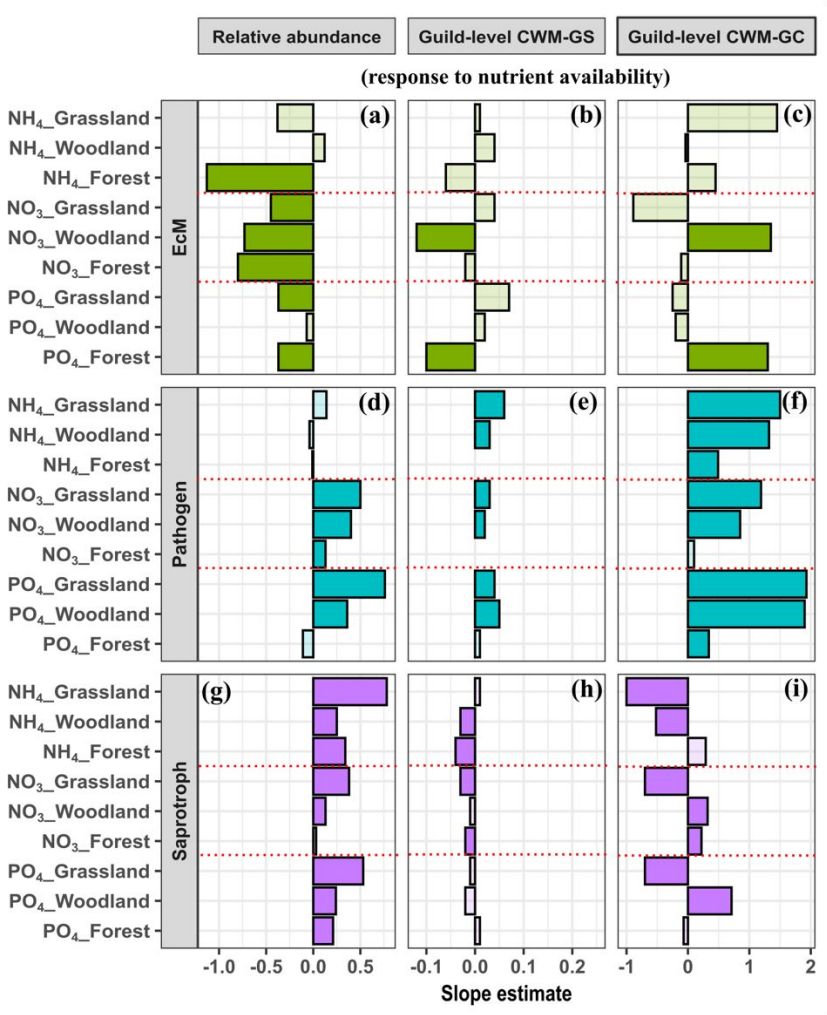

Fig. 4 Relative abundance of fungal guilds, guild-level community weighted mean genome size (CWM-GS) and GC (CWM-GC) content in relation to increasing soil nutrient availability for ectomycorrhizal (EcM, green), plant pathogen (blue), and saprotrophic (purple) fungi across Australia. Bars display the effect size of these responses, i.e., the estimated slopes from models that were fitted with one of the soil nutrients (NH_4^+, NO_3^-, PO_4^-) as the predictor for grassland, woodland and forest samples, respectively. Parameters were estimated with a linear mixed model that included the DNA region sequenced (ITS1 or ITS2) as random effects (detailed results were supplied in Fig. S4-6 and Table S4-6). In each panel, positive slopes indicate response variables increased with increasing soil nutrient availability while negative slopes indicate the opposite pattern. Solid bars indicate the responses were significant ($P < 0.05$) while faded bars indicate responses were not significant.