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OPINION

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The emerging threat of human-use antifungals in sustainable and circular agriculture schemes

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Societal Impact Statement

Rapidly growing global populations mandate greater crop productivity despite increasingly scarce natural resources, including freshwater. The adoption of sustainable agricultural practices seek to address such issues, but an unintended consequence is the exposure of agricultural soils and associated biota to emerging contaminants including azole pharmaceutical antifungals. We show that environmentally relevant exposure to three commonly prescribed azole antifungals can reduce mycorrhizal ^{33}P transfer from the soil into the host plant. This suggests that exposure to azoles may have a significant impact on mycorrhizal-mediated transfer of nutrients in soil-plant systems. Understanding the unintended consequences of sustainable agricultural practices is needed to ensure the security and safety of future food production systems.

Summary

- Sustainable farming practices are increasingly necessary to meet the demands of a growing population under constraints imposed by climate change. These practices, in particular the reuse of wastewater and amending soil with wastewater derived biosolids, provide a pathway for man-made chemicals to enter the agricultural environment.
- Among the chemicals commonly detected in wastewater and biosolids are pharmaceutical azole antifungals. Fungi, in particular mycorrhiza-forming fungal symbionts of plant roots, are key drivers of nutrient cycling in the soil–plant system. As such, greater understanding of the impacts of azole antifungal exposure in agricultural systems is urgently needed.
- We exposed wheat (*Triticum aestivum* L. cv. 'Skyfall') and arbuscular mycorrhizal fungi to environmentally relevant concentrations of three azole antifungals (clotrimazole, miconazole nitrate and fluconazole). We traced the mycorrhizal-acquired ^{33}P from the soil into the host plant in contaminated versus non-contaminated soils and found ^{33}P transfer from mycorrhizal fungi to host plants was reduced in soils containing antifungals. This represents a potentially major disruption to soil nutrient flows as a result of soil contamination.

- Our work raises the major issue of exposure of soil biota to pharmaceuticals such as azole antifungals, introduced via sustainable agricultural practices, as a potentially globally important disruptive influence on soil nutrient cycles. The impacts of these compounds on non-target organisms, beneficial mycorrhizal fungi in particular, could have major implications on security and sustainability of future food systems.

KEYWORDS
antifungal, arbuscular mycorrhizal fungi, azole, emerging contaminants, nutrient cycling, organic fertiliser, pharmaceutical pollution, wastewater reuse

1 | BACKGROUND

By 2050, food production needs to meet the demands of a global population exceeding nine billion people. This represents a necessary increase in agricultural outputs of up to 70% (FAO, 2009), including an additional one billion tonnes of cereal (FAO, 2012). These agricultural demands need to be met under climate stressed conditions, for example, doubling of the incidence of drought over the last four decades (FAO, 2012) whilst accounting for increasing energy costs and a dwindling supply of finite raw resources such as rock phosphorus (Thirkell et al., 2020) and freshwater for irrigation (Elliott et al., 2014). These challenges are forcing modern agriculture to adopt more sustainable practices to meet growing food demands whilst mitigating the impacts of climate change.

Reusing wastewaters, such as domestic and municipal wastewater or surface runoff, provides an alternative source of irrigation for agricultural land and has been adopted in many arid countries for decades (Valipour & Singh, 2016). This practice not only supplements freshwater supplies but also has the added benefit of being rich in plant essential nutrients, potentially providing a route away from the costly and environmentally damaging chemical-based fertilisers routinely used in modern intensive agriculture (Thirkell et al., 2017). Application of biosolids of municipal or animal agriculture origin is also increasingly widespread in sustainable agriculture systems (Smith, 2009). By eliminating waste and supporting the continual reuse of resources, recycling of wastewater and use of organic fertilisers in agriculture follows circular economy principles (Toop et al., 2017) potentially supporting many national plans for adopting sustainable agricultural practices. Circular economy principles have also gained prominence through the links to the UN's Sustainable Development Goals (SDGs) in the Post-2015 Development Agenda (Schroeder et al., 2019). By creating agricultural systems that require minimal inputs and in which waste does not exist, this contributes to achieving SDG 2 End hunger (via sustainable food production), SDG 12 Responsible consumption and production, SDG 13 Climate action and SDG 15 Sustainable use of terrestrial ecosystems. The circular economy and SDG agenda are closely linked and mutually reinforcing.

The products of wastewater treatment, wastewater effluent and biosolids that are frequently used in farming, are known reservoirs of potentially hundreds of chemical contaminants originating from products used in everyday life. Chief among these chemicals are pharmaceuticals. Significant portions of human and veterinary medicines consumed are excreted unchanged in urine and faeces where they enter the sewage system (Bound & Voulvoulis, 2005). Wastewater treatment plants comprise a variety of different treatment technologies ranging from trickling filter beds to activated carbon adsorption and chemical disinfection which are designed to remove conventional contaminants including nutrients, metals and microbial pathogens (Verlicchi et al., 2012). Emerging contaminants, such as pharmaceuticals, are inefficiently removed in the treatment process (Burns et al., 2018; Jelic et al., 2011; Verlicchi et al., 2012), meaning that, depending on their physicochemical properties, a portion remains in finished wastewater effluents or is partitioned into the sewage sludge

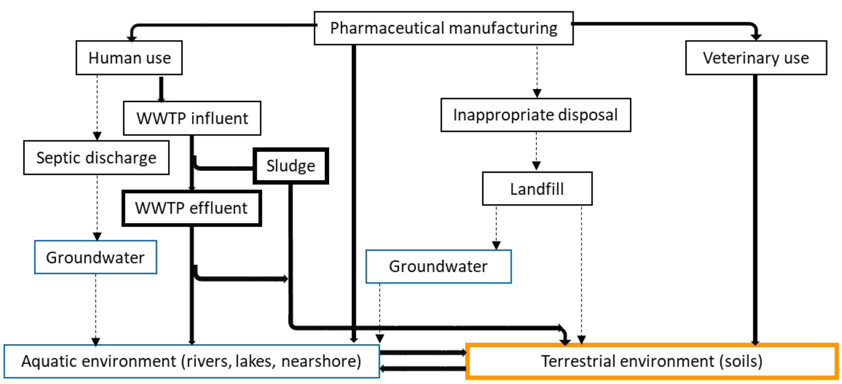

FIGURE 1 Emission sources and pathways linked to pharmaceutical exposure in the environment

that ultimately becomes biosolids. As such, human medicines have been quantified in effluents and biosolids which are then released into the environment as a means of disposal (Patel et al., 2019) (Figure 1). This has led to the widespread detection of pharmaceuticals in environmental matrices such as rivers, sediments and soils (aus der Beek et al., 2016).

Although use of wastewater as a source of irrigation is increasing globally, farmers in semi-arid regions have been using wastewater in agriculture as a solution to the problem of water scarcity for many years. One of the most widely documented cases of this is Mexico's Mezquital Valley which comprises over 90,000 hectares and has a long history of wastewater use in agriculture (for over 100 years). Through repeated irrigation events, concentrations of these chemicals can build up over time, with persistent pharmaceuticals such as carbamazepine detected in soils up to low μg/kg concentrations (typically <10 μg/kg) (Dalkmann et al., 2012; Siemens et al., 2008). It should also be noted that in countries dealing with limited wastewater treatment infrastructure and sewage connectivity, wastewater can often be released directly into the environment with little or no treatment and this can result in elevated levels of exposure (Ashfaq et al., 2017). Despite the widespread and increasing application of wastewater and biosolids to agricultural soils across the world, little is known about the upstream effects on soil biota and plants resulting from the inadvertent release of pharmaceuticals. Of particular concern are the impacts of bioactive pharmaceuticals on soil microbial and fungal process and the potential for plant—and therefore crop—assimilation of these compounds and their subsequent entry into the human food chain.

2 | ANTIFUNGALS

Agricultural yields are highly sensitive to reduction by pests and disease pressures and have been ever since large-scale, intensive farming systems began. Today, fungal pathogens are responsible for around 20% of crop losses, with a further 10% of yields subject to post-harvest losses (Fisher et al., 2018). It has long been common practice to use a wide range of synthetic antifungal agents to prevent such losses. The pesticides used are mainly triazoles, one of the most commonly used classes of fungicides for the treatment of fungal phytopathogens.

Antifungal azoles also play an important role in the management of human fungal diseases and are routinely administered as therapeutic agents in human health care formulations, including topical treatments or oral medicines (Snelders et al., 2012). Azole fungicides are also frequently present in personal care products such as hair shampoos, soaps, toothpastes and shower gels (Chen & Ying, 2015). Based on an analysis of prescription data in the United Kingdom, nearly 1.5 tonnes of azole antifungals, including commonly used imidazoles (e.g., climbazole, clotrimazole, ketoconazole and miconazole) and triazoles (e.g., fluconazole, itraconazole and metconazole) were prescribed for use in 2018 alone (Figure 3).

Through their use as prescription drugs or in human health care products, azole antifungals are either washed off into wastewater following topical application or excreted after ingestion. This results in a proportion of the unchanged parent compound entering the sewage system (Chen et al., 2014). The precise amount of antifungal eliminated into waste streams following ingestion depends on the pharmacokinetics of the specific drug, including factors controlling human metabolism such as age and gender (Brüggemann et al., 2009). Fluconazole and posconazole are primarily renally excreted with approximately 80% of the drug eliminated unchanged (Brüggemann et al., 2009). Comparatively, itraconazole is extensively metabolised by the liver, with the major metabolite, droxy-itraconazole excreted in urine (Templeton et al., 2008). Nevertheless, a fraction of azole antifungal, with retained biological potency is released into sewage system and undergoes wastewater treatment. It should be noted that not only are the parent compounds bioactive but also their degradation products can be toxic and the technologies employed during wastewater treatment are not designed to remove these chemicals, meaning they frequently pass through the treatment process in a bioactive state (Liu et al., 2017).

Azole antifungals used in human medicinal products are particularly resistant to biodegradation and can readily adsorb to sludge (Cai et al., 2021). Assessment of the removal of azole substances following mechanical, biological and chemical treatment in China found the pharmaceutical fluconazole, clotrimazole, econazole, ketoconazole and miconazole were constantly detected in the final wastewater effluent at concentrations <100 ng/L. The latter four were also routinely detected in the sludge, whereas fluconazole largely remained in the aqueous phase. Ketoconazole is more readily bio-transformed, whereas clotrimazole, econazole and miconazole are more likely to be adsorbed onto and persist in sewage sludge in concentrations ranging between 5 and 268 ng/g (dry weight) (Peng et al., 2012). Once in the environment, azole fungicides become ubiquitous contaminants, being distributed across different environmental compartments (e.g., soils, rivers and sediments; reviewed by Chen & Ying, 2015; Chen et al., 2014; Huang et al., 2013). In bio-solid amended soils, concentrations of azole antifungals are reported to range between 0.53 and 64.5 ng/g in China (Chen et al., 2013a, 2013b), 30 and 90 ng/g in the United States (Walters et al., 2010) and 150 and 340 ng/g in Canada (Gottschall et al., 2012). The presence and concentrations of these fungicides in soil following use of wastewater as a source of irrigation remain unclear, potentially due to azole compounds often not being included as target compounds in screening (Chen & Ying, 2015). Nevertheless, as these chemicals are persistent and widely reported in wastewater effluents, there is a very high likelihood that azole fungicides are also introduced into agricultural systems via the use of wastewater irrigation.

3 | DRUG-PLANT INTERACTIONS

The conserved biological potency of bioactive chemicals presents a significant risk to non-target organisms. It has been widely reported that the pharmaceutical compounds introduced into soils are assimilated by and accumulate in plants with the physio-chemical properties of the chemical and surrounding soil properties playing critical roles in controlling the degree of pharmaceutical phytoaccumulation (Carter et al., 2014; Li et al., 2018). For azole compounds specifically, it has been shown that ketoconazole is taken up and accumulates in grasses (*Lolium perenne*, *Poa pratensis* and *Poa trivialis*) and watercress (*Nasturtium officinale*) (Chitescu et al., 2013). However, beyond these specific examples, data on azole antifungal accumulation in soil–plant systems and the mechanisms by which uptake occurs are lacking. Under hydroponic conditions, translocation of selected azoles from the nutrient solution to the aerial part of lambs lettuce (*Valerianella locusta* L.) was found to be highly dependent on the hydrophobicity of the azole (García-Valcárcel et al., 2016). It remains unclear as to the role that soil will play in influencing the availability of azoles for plant uptake. Furthermore, the indirect impacts on plant health, growth and accumulation of pharmaceuticals via effects on soil microbial and fungal community structure and function are unknown. Pharmaceuticals are designed to interact with specific molecular targets in humans, and it has been identified that these targets can often have orthologs in other species, including plants (Verbruggen et al., 2018). Following uptake and accumulation of bioactive chemicals, there is potential for mode of action related effects in plants.

To date, it has been established that pharmaceuticals can interact with key plant functions which may affect plant growth and development. Impacts such as lethality largely manifest at high concentrations, not typical of the concentrations expected to accumulate in the environment (Knight et al., 2018; Timmerer et al., 2020). However, recent evidence shows that pharmaceuticals at low environmental concentrations can manifest in sub-lethal toxic responses in the plant (Fu et al., 2019). Specific sub-lethal responses to pharmaceutical exposure include changes in phytohormone homeostasis (Carter et al., 2015), activation of detoxification pathways (Bartha et al., 2010; Sun et al., 2018) and changes in stress response protein markers (Gorovits et al., 2020). Research demonstrating sub-lethal impacts such as those described here is still in its infancy with a limited number of chemicals and end-points considered. Ultimately, these findings suggest the potential for widespread effects on crop yield and thus agricultural productivity.

Although the levels of exposure for azole compounds reported in environmental compartments are significantly less than therapeutic levels (often 100–500 mg per daily dose), the impacts of continuous trace level exposure to non-target organisms is a major concern. A significant knowledge gap remains around the conserved biological potency of antifungals in the environment and, in particular, the mechanisms by which these compounds are assimilated by plants from the soil. Key to understanding these mechanisms will be determining the impact of antifungal contamination on the community composition, structure and function of the microbes and fungi that play vital roles in soil nutrient cycling and plant nutrition. Of particular interest is the potential interaction between pharmaceutical antifungals with the groups of soil fungi that form mycorrhizal associations with plant roots. Arbuscular mycorrhizal (AM) fungi form intimate associations with >80% land plants including most major crops (Smith & Reed, 2008). They often play a major role in plant nutrition through their engagement in bidirectional exchange of soil nutrients, such as nitrogen and phosphorus, in return for plant-fixed carbon compounds such as sugars and fatty acids (Luginbuehl et al., 2017; Figure 2). In human medicine, antifungal chemicals are drugs that are designed to selectively eliminate fungal pathogens from a host. Specifically, azole antifungal drugs such as fluconazole, itraconazole and

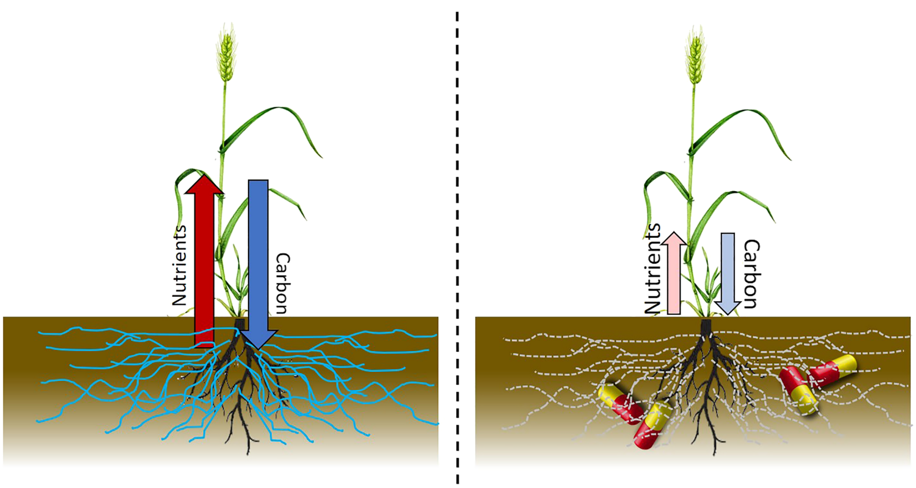

FIGURE 2 Extraradical mycorrhizal fungal hyphae extend beyond the host plant root system and facilitate plant nutrient uptake from the soil (red arrows) in exchange for plant-fixed carbon which moves into the soil via fungal mycelium (blue arrows) as demonstrated on the left. The impact of inadvertent pharmaceutical contamination of soil on mycorrhizal-acquired nutrient assimilation in host plants (red arrow), and return of plant-fixed carbon to mycorrhizal symbionts (blue arrow) represents an important knowledge gap in the use of organic soil amendments (i.e., wastewater and biosolids) and application of mycorrhizal fungi to cropping systems

ketoconazole inhibit cytochrome P_{450}-dependent enzymes (particularly C14-demethylase) involved in the biosynthesis of ergosterol, which is required for fungal cell membrane structure and function (Dixon & Walsh, 1996). Azole antifungals which have been inadvertently introduced into the soil environment could therefore interact with mycorrhizal hyphae and impact on plant nutrient acquisition and ultimately plant health (Figure 2).

4 | IMPACTS OF AZOLE ANTIFUNGALS ON SOIL–PLANT PROCESSES

To test the potential impacts of soil contamination by pharmaceutical azole antifungals on the function of associations between wheat (*Triticum aestivum* L. cv. 'Skyfall') and arbuscular mycorrhizal fungi, we exposed wheat and mycorrhizal associates to environmentally relevant levels of the azole antifungals clotrimazole, miconazole nitrate and fluconazole. These compounds were selected on the basis of high consumption and previous detection in biosolids (Figure 3; Peng et al., 2012). Soil was spiked with a mixture of the three antifungals (ratio of 1:1:1) which resulted in a nominal concentration of 100 ng/g (see the supporting information for experimental details). Concentrations were within the range of previously measured concentrations for azole compounds in biosolid-amended soils (0.1–340 ng/g; Chen & Ying, 2015). This is not dissimilar to recommended application rate (250 ng/g) of the fungicide, propiconazole (Kling & Jakobsen, 1997). Using ^{33}P-orthophosphate, we traced the movement of mycorrhizal-acquired ^{33}P from the soil into the host plant in contaminated soils versus non-contaminated soils. Full experimental detail is available in the supporting information (Methods S1).

Despite there being uniformly typically low colonisation by mycorrhizal fungi in wheat roots across treatments (Table S1), particularly when compared to experiments using the same wheat cultivar and mycorrhizal inoculum (Elliott et al., 2021), we found transfer of ^{33}P from fungus-to-plant was reduced in plants grown in azole-contaminated soils compared to those grown in non-contaminated soils (Figure 4). Similar significant differences between treatments were found in terms of total ^{33}P (Figure S1). This is consistent with Schweiger and Jakobsen (1998), who demonstrated reduced hyphal ^{32}P uptake by pea (*Pisum sativum* L. cv. Finale) exposed to the pesticide propiconazole at concentrations of 1 μg/g, or 10× higher than the concentrations of our study. Although the mechanisms underpinning this effect are currently unknown and require further exploration, our results suggest the contamination of soils with azole antifungals could have a disruptive effect on mycorrhizal function, potentially indicative of negative impacts of pharmaceuticals on soil fungal and microbial soil nutrient cycling processes. Future work should seek to understand the impacts of azole antifungals on plant-to-fungal carbon dynamics and on the AM fungal community structure, both within and outwith host plant root systems.

In addition to facilitating the transfer of phosphorus to the plant, as observed in this study (Figure 4), mycorrhizal associations are also integral in terms of mobilising other key nutrients such as nitrogen,

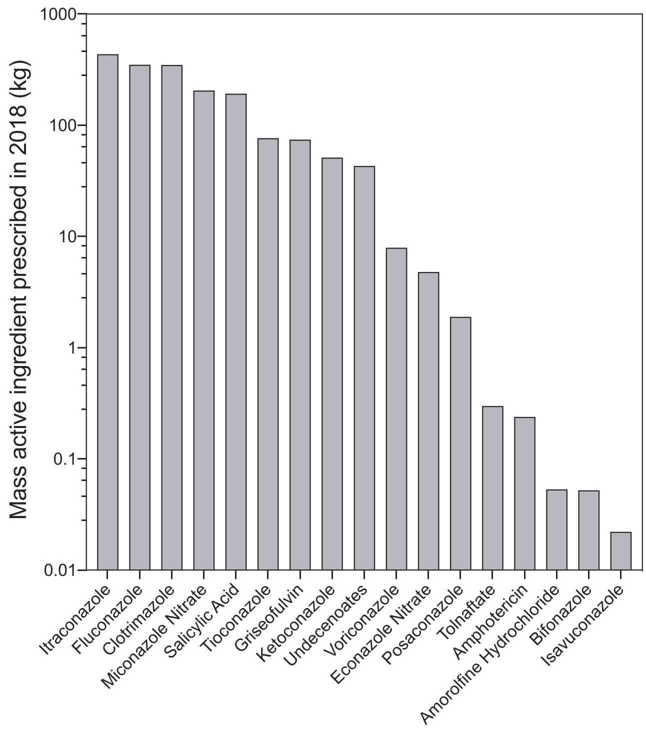

FIGURE 3 Mass of azole antifungal compounds prescribed for use in the United Kingdom in 2018. Data from the National Health System (NHS) Prescription Cost Analysis (NHS, 2017)

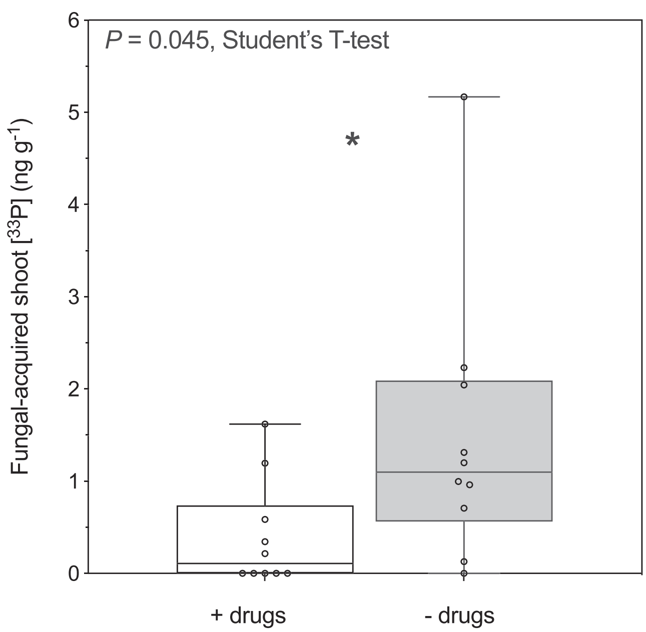

FIGURE 4 Mycorrhizal fungal-acquired ^{33}P detected in wheat shoots in plants grown in soils contaminated with environmentally relevant concentrations of azole antifungals (white boxes, $n = 10$) and control treated plants grown in uncontaminated soil (grey bars, $n = 10$) in terms of concentration of ^{33}P per g plant material. * indicates significant difference where $P < 0.05$ (Student's T test)

potassium and iron to crops whilst offering benefits such as enhanced soil structure by improving its aggregation and stability (Rillig & Mummey, 2006). Additional work is therefore needed to explore a wider suite of mycorrhizal associated benefits to understand the broader ecological consequences resulting from the inadvertent release of pharmaceuticals following sludge application and wastewater reuse. We evaluated the impacts of azole exposure in only a single cultivar of wheat using a commercial AMF inoculant. The degree to which plants benefit from the mycorrhizal associations and the degree of dependency on the association is known to differ among plant and fungal species (Field et al., 2012; Smith & Reed, 2008), and even genotype (Johnson et al., 2012), including in crops (Elliott et al., 2021; Thirkell et al., 2019). As such, further work is now needed to elucidate pharmaceutical-induced differences in mycorrhizal colonisation and subsequent nutrient transfer across a variety of crop species, cultivars and mycorrhizal fungi. This would allow us to understand if species and genotypes with a greater mycorrhizal dependency in terms of deriving plant nutritional benefit from the fungal associations are more affected by pharmaceutical-driven changes in mycorrhizal colonisation and function.

Interestingly, the observed effects on ^{33}P transfer from fungus-to-plant occurred without any significant impacts ($P = 0.12$, Student's T test) on the degree of mycorrhizal colonisation in response to azole exposure (Table S1), although these were very low across both treatments. There was no correlation apparent between root colonisation by AMF and plant shoot ^{33}P assimilation. Comparatively, impacts on mycorrhizal colonisation have been previously observed in wetland plant species (*Eclipta prostrata*, *Hibiscus laevis* and *Sesbania herbacea*) following exposure to the personal care product, triclosan, an antimicrobial agent commonly found in toothpaste and other products. In an aqueous exposure, triclosan caused significant reductions in hyphal and arbuscular colonisation ($P < 0.05$) at concentrations >0.4 μg/L (Twanabasu et al., 2013). It remains unknown whether this inhibition resulted in a subsequent effect on fungus–plant nutrient transfer. Based on our findings which suggest plant-drug interactions could affect mycorrhizal fungi in terms of nutrient acquisition from the soil environment or development of external hyphal mycelium, it would be interesting to explore this further to understand if these observed impacts on hyphal and arbuscular colonisation can affect plant health and ultimately plant performance.

Our study was based on antifungal exposure representative of the concentrations found in wastewater derived biosolids, almost certainly resulting from pharmaceutical use. Although the field application of biosolids represents an unintentional exposure pathway for azole compounds, the intentional use of azole antifungals as antimycotic treatments in agriculture represents much higher chemical exposure that can include multi-year applications at a rate of 100 g/ha (Hof, 2001) with soil half-lives greater than 2 years (Bromilow et al., 1999). There has been no research reporting the impacts of pharmaceutical azole compounds on beneficial fungi including mycorrhizal symbionts. Studies on pesticide–mycorrhizal interactions are also limited. The few investigating azole compounds have reported conflicting effects on the impacts of hyphae activity (Hage-Ahmed et al., 2019). If we intend to move towards a future less dependent on inorganic fertilisers, this will require alternative sources for organic nutrient additions (i.e., municipal biosolids). In tandem, future strategies for crop breeding should consider plant traits relevant to mycorrhizal fungal responsiveness and function to maximise the potential benefits in terms of soil nutrient capture of crop-mycorrhizal associations. Understanding the exposure and effects of antifungals on non-target soil fungi is essential to realising this more sustainable future.

5 | WIDER IMPLICATIONS AND FUTURE RESEARCH DIRECTION

There are currently >1,500 active pharmaceuticals in use in the UK alone (Guo et al., 2016), and we know very little about how these chemicals might interact in soil–plant systems. More research is urgently needed to explore the potential for drug–soil–plant interactions using a wider range of crops, accounting for the variability in plant genotype, environmental conditions, soil biota and soil properties known to influence bioactive pharmaceutical fate in soil–plant systems.

In particular, data on the impacts of land-applied emerging contaminants on mycorrhizal colonisation and subsequent mycorrhizal-derived benefits are limited. A recent study highlighted the fact that legacy pesticide residues in fields impacted arbuscular mycorrhizal fungi abundance years after switching to organic management (Riedo et al., 2021). Our preliminary findings suggest that the inadvertent release of pharmaceuticals in agricultural systems has the potential to impact on mycorrhizal nutrient transfer to shoots with unknown implications for wider nutrient cycling processes. Given the significant role that arbuscular mycorrhizal fungal communities play in terms of influencing plant growth, plant community structure and ultimately ecosystem services, together with their potential for application in sustainable agricultural systems (Bender et al., 2016), it is essential that we explore this further.

Given the widespread and necessary implementation of sustainable agricultural practices and support for adopting circular economy principles, it is imperative that we are able adequately quantify the risks associated with the introduction of bioactive chemicals in the agricultural environment. Understanding the risks will enable scientists to explore potential mitigation options to reduce the impacts associated with these chemicals soil–plant systems, such as the addition of chemical sorbents or pre-treatment of waste products prior to land application.

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CONFLICT OF INTEREST

The authors declare no conflicts of interest.

AUTHOR CONTRIBUTIONS

JBS, KJF and LJC planned and designed the research. TJT carried out the experiments. KJF and TJT conducted data analysis. JBS, KJF and LJC interpreted data and wrote the manuscript.

DATA AVAILABILITY STATEMENT

Data generated in this project is available on request from the authors.

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REFERENCES

Ashfaq, M., Nawaz Khan, N., Saif Ur Rehman, M., Mustafa, G., Faizan Nazar, M., Sun, Q., Iqbal, J., Mulla, S. I., & Yu, C.-P. (2017). Ecological risk assessment of pharmaceuticals in the receiving environment of pharmaceutical wastewater in Pakistan. *Ecotoxicology and Environmental Safety*, *136*, 31–39.

aus der Beek, T., Weber, F. A., Bergmann, A., Hickmann, S., Ebert, I., Hein, A., & Küster, A. (2016). Pharmaceuticals in the environment—Global occurrences and perspectives. *Environmental Toxicology and Chemistry*, *35*(4), 823–835. https://doi.org/10.1002/etc.3339

Bartha, B., Huber, C., Harpaintner, R., & Schröder, P. (2010). Effects of acetaminophen in *Brassica juncea* L. Czern.: Investigation of uptake, translocation, detoxification, and the induced defense pathways. *Environmental Science and Pollution Research International*, *17*(9), 1553–1562. https://doi.org/10.1007/s11356-010-0342-y

Bender, S. F., Wagg, C., & van der Heijden, M. G. A. (2016). An underground revolution: Biodiversity and soil ecological engineering for agricultural sustainability. *Trends in Ecology & Evolution*, *31*(6), 440–452. https://doi.org/10.1016/j.tree.2016.02.016

Bound, J. P., & Voulvoulis, N. (2005). Household disposal of pharmaceuticals as a pathway for aquatic contamination in the United Kingdom. *Environmental Health Perspectives*, *113*(12), 1705–1711. https://doi.org/10.1289/ehp.8315

Bromilow, R. H., Evans, A. A., & Nicholls, P. H. (1999). Factors affecting degradation rates of five triazole fungicides in two soil types: 1. Laboratory incubations. *Pesticide Science*, *55*, 1129–1134.

Brüggemann, R. J., Alffenaar, J. W., Blijlevens, N. M., Billaud, E. M., Kosterink, J. G., Verweij, P. E., & Burger, D. M. (2009). Clinical relevance of the pharmacokinetic interactions of azole antifungal drugs with other coadministered agents. *Clinical Infectious Diseases: An Official Publication of the Infectious Diseases Society of America*, *48*(10), 1441–1458. https://doi.org/10.1086/598327

Burns, E. E., Carter, L. J., Kolpin, D. W., Thomas-Oates, J., & Boxall, A. B. A. (2018). Temporal and spatial variation in pharmaceutical concentrations in an urban river system. *Water Research*, *15*(137), 72–85. https://doi.org/10.1016/j.watres.2018.02.066

Cai, W., Ye, P., Yang, B., Shi, Z., Xiong, Q., Gao, F., Liu, Y., Zhao, J., & Ying, G. (2021). Biodegradation of typical azole fungicides in activated sludge under aerobic conditions. *Journal of Environmental Sciences (China)*, *103*, 288–297. https://doi.org/10.1016/j.jes.2020.11.007

Carter, L. J., Harris, E., Williams, M., Ryan, J. J., Kookana, R. S., & Boxall, A. B. (2014). Fate and uptake of pharmaceuticals in soil-plant systems. *Journal of Agricultural and Food Chemistry*, *62*(4), 816–825. https://doi.org/10.1021/jf404282y

Carter, L. J., Williams, M., Böttcher, C., & Kookana, R. S. (2015). Uptake of pharmaceuticals influences plant development and affects nutrient and hormone homeostases. *Environmental Science & Technology*, *49*(20), 12509–12518. https://doi.org/10.1021/acs.est.5b03468

Chen, Z. F., & Ying, G. G. (2015). Occurrence, fate and ecological risk of five typical azole fungicides as therapeutic and personal care products in the environment: A review. *Environment International*, *84*, 142–153. https://doi.org/10.1016/j.envint.2015.07.022

Chen, Z. F., Ying, G. G., Liu, Y. S., Zhang, Q. Q., Zhao, J. L., Liu, S. S., Chen, J., Peng, F. J., Lai, H. J., & Pan, C. G. (2014). Triclosan as a surrogate for household biocides: An investigation into biocides in aquatic environments of a highly urbanized region. *Water Research*, *58*, 269–279. https://doi.org/10.1016/j.watres.2014.03.072

Chen, Z. F., Ying, G. G., Ma, Y. B., Lai, H. J., Chen, F., & Pan, C. G. (2013a). Occurrence and dissipation of three azole biocides climbazole, clotrimazole and miconazole in biosolid-amended soils. *Science of the Total Environment*, *1*(452–453), 377–383. https://doi.org/10.1016/j.scitotenv.2013.03.004

Chen, Z. F., Ying, G. G., Ma, Y. B., Lai, H. J., Chen, F., & Pan, C. G. (2013b). Typical azole biocides in biosolid-amended soils and plants following biosolid applications. *Journal of Agricultural and Food Chemistry*, *61*(26), 6198–6206. https://doi.org/10.1021/jf4013949

Chitescu, C. L., Nicolau, A. I., & Stolker, A. A. (2013). Uptake of oxytetracycline, sulfamethoxazole and ketoconazole from fertilised soils by plants. *Food Additives & Contaminants. Part a, Chemistry, Analysis, Control, Exposure & Risk Assessment*, *30*(6), 1138–1146. https://doi.org/10.1080/19440049.2012.725479

Dalkmann, P., Broszat, M., Siebe, C., Willaschek, E., Sakinc, T., Huebner, J., Amelung, W., Grohmann, E., & Siemens, J. (2012). Accumulation of pharmaceuticals, enterococcus, and resistance genes in soils irrigated with wastewater for zero to 100 years in central Mexico. *PLoS ONE*, *7*(9), e45397. https://doi.org/10.1371/journal.pone.0045397

Dixon, D. M., & Walsh, T. J. (1996). Antifungal agents. In S. Baron (Ed.), *Medical Microbiology* (4th ed.). Galveston (TX): University of Texas Medical Branch at Galveston. Chapter 76. Available from: https://www.ncbi.nlm.nih.gov/books/NBK8263/

Elliott, A. E., Daniell, T. J., Cameron, D. D., & Field, K. J. (2021). Commercial arbuscular mycorrhizal inoculum increases root colonisation in wheat but does not increase assimilation of mycorrhizal-acquired nutrients. *Plants, People, Planet*. https://doi.org/10.1002/ppp3.10094

Elliott, J., Deryng, D., Müller, C., Frieler, K., Konzmann, M., Gerten, D., Glotter, M., Flörke, M., Wada, Y., Best, N., Eisner, S., Fekete, B. M., Folberth, C., Foster, I., Gosling, S. N., Haddeland, I., Khabarov, N., Ludwig, F., Masaki, Y., … Wisser, D. (2014). Constraints and potentials of future irrigation water availability on agricultural production under climate change. *Proceedings of the National Academy of Sciences of the United States of America*, *111*(9), 3239–3244. https://doi.org/10.1073/pnas.1222474110

FAO. (2009). FAO—News Article: 2050: A third more mouths to feed. Food and Agriculture Organization of The United Nations. http://www.fao.org/news/story/en/item/35571/icode/

FAO. (2012). Coping with water scarcity: An action framework for agriculture and food security. FAO Water Reports 38. Food and Agriculture Organization of The United Nations. http://www.fao.org/

Field, K. J., Cameron, D. D., Leake, J. R., Tille, S., Bidartondo, M. I., & Beerling, D. J. (2012). Contrasting arbuscular mycorrhizal responses of vascular and non-vascular plants to a simulated Palaeozoic CO_2 decline. *Nature Communications*, *3*, 835. https://doi.org/10.1038/ncomms1831

Fisher, M. C., Hawkins, N. J., Sanglard, D., & Gurr, S. J. (2018). Worldwide emergence of resistance to antifungal drugs challenges human health and food security. *Science*, *360*(6390), 739–742.

Fu, Q., Malchi, T., Carter, L. J., Li, H., Gan, J., & Chefetz, B. (2019). Pharmaceutical and personal care products: From wastewater treatment into agro-food systems. *Environmental Science & Technology*, *53*(24), 14083–14090. https://doi.org/10.1021/acs.est.9b06206

García-Valcárcel, A. I., Loureiro, I., Escorial, C., Molero, E., & Tadeo, J. L. (2016). Uptake of azoles by lamb's lettuce (Valerianella locusta L.) grown in hydroponic conditions. *Ecotoxicology and Environmental Safety*, *124*, 138–146. https://doi.org/10.1016/j.ecoenv.2015.10.021

Gorovits, R., Sobol, I., Akama, K., Chefetz, B., & Czosnek, H. (2020). Pharmaceuticals in treated wastewater induce a stress response in tomato plants. *Scientific Reports*, *10*(1), 1856. https://doi.org/10.1038/s41598-020-58776-z

Gottschall, N., Topp, E., Metcalfe, C., Edwards, M., Payne, M., Kleywegt, S., Russell, P., & Lapen, D. R. (2012). Pharmaceutical and personal care products in groundwater, subsurface drainage, soil, and wheat grain, following a high single application of municipal biosolids to a field. *Chemosphere*, *87*(2), 194–203. https://doi.org/10.1016/j.chemosphere.2011.12.018

Guo, J., Sinclair, C. J., Selby, K., & Boxall, A. B. (2016). Toxicological and ecotoxicological risk-based prioritization of pharmaceuticals in the natural environment. *Environmental Toxicology and Chemistry*, *35*(6), 1550–1559. https://doi.org/10.1002/etc.3319

Hage-Ahmed, K., Rosner, K., & Steinkellner, S. (2019). Arbuscular mycorrhizal fungi and their response to pesticides. *Pest Management Science*, *75*(3), 583–590. https://doi.org/10.1002/ps.5220

Hof, H. (2001). Critical annotations to the use of azole antifungals for plant protection. *Antimicrobial Agents and Chemotherapy*, *45*(11), 2987–2990. https://doi.org/10.1128/AAC.45.11.2987-2990.2001

Huang, Q., Wang, Z., Wang, C., & Peng, X. (2013). Chiral profiling of azole antifungals in municipal wastewater and recipient rivers of the Pearl River Delta, China. *Environmental Science and Pollution Research International*, *20*(12), 8890–8899. https://doi.org/10.1007/s11356-013-1862-z

Jelic, A., Gros, M., Ginebreda, A., Cespedes-Sánchez, R., Ventura, F., Petrovic, M., & Barcelo, D. (2011). Occurrence, partition and removal of pharmaceuticals in sewage water and sludge during wastewater treatment. *Water Research*, *45*(3), 1165–1176. https://doi.org/10.1016/j.watres.2010.11.010

Johnson, D., Martin, F., Cairney, J. W., & Anderson, I. C. (2012). The importance of individuals: Intraspecific diversity of mycorrhizal plants and fungi in ecosystems. *New Phytologist*, *194*(3), 614–628.

Kling, M., & Jakobsen, I. (1997). Direct application of carbendazim and propiconazole at field rates to the external mycelium of three arbuscular mycorrhizal fungi species: Effect on ^{32}P transport and succinate dehydrogenase activity. *Mycorrhiza*, *7*, 33–37.

Knight, E. R., Carter, L. J., & McLaughlin, M. J. (2018). Bioaccumulation, uptake, and toxicity of carbamazepine in soil-plant systems. *Environmental Toxicology and Chemistry*, *37*(4), 1122–1130. https://doi.org/10.1002/etc.4053

Li, Y., Sallach, J. B., Zhang, W., Boyd, S. A., & Li, H. (2018). Insight into the distribution of pharmaceuticals in soil-water-plant systems. *Water Research*, *152*, 38–46. https://doi.org/10.1016/j.watres.2018.12.039

Liu, W. R., Yang, Y. Y., Liu, Y. S., Zhang, L. J., Zhao, J. L., Zhang, Q. Q., Zhang, M., Zhang, J. N., Jiang, Y. X., & Ying, G. G. (2017). Biocides in wastewater treatment plants: Mass balance analysis and pollution load estimation. *Journal of Hazardous Materials*, *329*, 310–320. https://doi.org/10.1016/j.jhazmat.2017.01.057

Luginbuehl, L. H., Menard, G. N., Kurup, S., Van Erp, H., Radhakrishnan, G. V., Breakspear, A., Oldroyd, G. E., & Eastmond, P. J. (2017). Fatty acids in arbuscular mycorrhizal fungi are synthesized by the host plant. *Science*, *356*(6343), 1175–1178. https://doi.org/10.1126/science.aan0081

National Health Service. (2017). Prescription Cost Analysis - England, 2017 [PAS] - NHS Digital. NHS Digit. Available: https://digital.nhs.uk/data-and-information/publications/statistical/prescription-cost-analysis/prescription-cost-analysis-england-2018

Patel, M., Kumar, R., Kishor, K., Mlsna, T., Pittman, C. U. Jr., & Mohan, D. (2019). Pharmaceuticals of emerging concern in aquatic systems: Chemistry, occurrence, effects, and removal methods. *Chemical Reviews*, *119*(6), 3510–3673. https://doi.org/10.1021/acs.chemrev.8b00299

Peng, X., Huang, Q., Zhang, K., Yu, Y., Wang, Z., & Wang, C. (2012). Distribution, behavior and fate of azole antifungals during mechanical, biological, and chemical treatments in sewage treatment plants in China. *The Science of the Total Environment*, *426*, 311–317. https://doi.org/10.1016/j.scitotenv.2012.03.067

Riedo, J., Wettstein, F. E., Rösch, A., Herzog, C., Banerjee, S., Büchi, L., Charles, R., Wächter, D., Martin-Laruent, F., Bucheli, T. D., Walder, F., & van der Heijden, M. G. A. (2021). Widespread occurrence of pesticides in organically managed agricultural soils—The ghost of a conventional agricultural past? *Environmental Science and Technology*, *55*(5), 2919–2928. https://doi.org/10.1021/acs.est.0c06405

Rillig, M. C., & Mummey, D. L. (2006). Mycorrhizas and soil structure. *New Phytologist*, *171*(1), 41–53. https://doi.org/10.1111/j.1469-8137.2006.01750.x

Schroeder, P., Anggraeni, K., & Weber, U. (2019). The relevance of circular economy practices to the sustainable development goals. *Journal of Industrial Ecology*, *Yale University*, *23*(1), 77–95. https://doi.org/10.1111/jiec.12732

Schweiger, P. F., & Jakobsen, I. (1998). Dose-response relationships between four pesticides and phosphorus uptake by hyphae of arbuscular mycorrhizas. *Soil Biology and Biochemistry*, *30*(10–11), 1415–1422. https://doi.org/10.1016/S0038-0717(97)00259-9

Siemens, J., Huschek, G., Siebe, C., & Kaupenjohann, M. (2008). Concentrations and mobility of human pharmaceuticals in the world's largest wastewater irrigation system, Mexico City-Mezquital Valley. *Water Research*, *42*(8–9), 2124–2134. https://doi.org/10.1016/j.watres.2007.11.019

Smith, S. E., & Reed, D. J. (2008). *Mycorrhizal symbiosis*. New York: Academic Press. ISBN 9780123705266

Smith, S. R. (2009). Organic contaminants in sewage sludge (biosolids) and their significance for agricultural recycling. *Philosophical Transactions. Series a, Mathematical, Physical, and Engineering Sciences*, *367*(1904), 4005–4041. https://doi.org/10.1098/rsta.2009.0154

Snelders, E., Camps, S. M., Karawajczyk, A., Schaftenaar, G., Kema, G. H., van der Lee, H. A., Klaassen, C. H., Melchers, W. J., & Verweij, P. E. (2012). Triazole fungicides can induce cross-resistance to medical triazoles in Aspergillus fumigatus. *PLoS ONE*, *7*(3), e31801. https://doi.org/10.1371/journal.pone.0031801

Sun, C., Dudley, S., Trumble, J., & Gan, J. (2018). Pharmaceutical and personal care products-induced stress symptoms and detoxification mechanisms in cucumber plants. *Environmental Pollution*, *234*, 39–47. https://doi.org/10.1016/j.envpol.2017.11.041

Templeton, I. E., Thummel, K. E., Kharasch, E. D., Kunze, K. L., Hoffer, C., Nelson, W. L., & Isoherranen, N. (2008). Contribution of itraconazole metabolites to inhibition of CYP3A4 in vivo. *Clinical Pharmacology and Therapeutics*, *83*(1), 77–85. https://doi.org/10.1038/sj.clpt.6100230

Thirkell, T. J., Campbell, M., Driver, J., Pastok, D., Merry, B., & Field, K. J. (2020). Elevated atmospheric CO_2 elicits cultivar-specific mycorrhizal nutrient exchange responses in barley. *Plants People Planet*. https://doi.org/10.1002/ppp3.10174

Thirkell, T. J., Charters, M., Elliott, A. E., Sait, S. M., & Field, K. J. (2017). Are mycorrhizal fungi our "sustainable saviours"? Considerations for achieving food security. *Journal of Ecology*, *105*, 921–929.

Thirkell, T. J., Pastok, D., & Field, K. J. (2019). Carbon for nutrient exchange between arbuscular mycorrhizal fungi and wheat varies according to cultivar and changes in atmospheric carbon dioxide concentration. *Global Change Biology*, *26*(3), 1725–1738. https://doi.org/10.1111/gcb.14851

Timmerer, U., Lehmann, L., Schnug, E., & Bloem, E. (2020). Toxic effects of single antibiotics and antibiotics in combination on germination and growth of *Sinapis alba* L. *Plants*, *9*(1), 107. https://doi.org/10.3390/plants9010107

Toop, T. A., Ward, S., Oldfield, T., Hull, M., Kirby, M. E., & Theodorou, M. K. (2017). AgroCycle—Developing a circular economy in agriculture. *Energy Procedia*, *123*, 76–80. https://doi.org/10.1016/j.egypro.2017.07.269

Twanabasu, B. R., Smith, C. M., Stevens, K. J., Venables, B. J., & Sears, W. C. (2013). Triclosan inhibits arbuscular mycorrhizal colonization in three wetland plants. *The Science of the Total Environment*, *447*, 450–457. https://doi.org/10.1016/j.scitotenv.2012.12.085

Valipour, M., & Singh, V. P. (2016). Global experiences on wastewater irrigation: Challenges and prospects. In B. Maheshwari, B. Thoradeniya, & V. P. Singh (Eds.), *Balanced urban development: Options and strategies for liveable cities. Water science and technology library* (Vol. 72). Cham: Springer. https://doi.org/10.1007/978-3-319-28112-4_18

Verbruggen, B., Gunnarsson, L., Kristiansson, E., Österlund, T., Owen, S. F., Snape, J. R., & Tyler, C. R. (2018). ECOdrug: A database connecting drugs and conservation of their targets across species. *Nucleic Acids Research*, *46*(D1), D930–D936. https://doi.org/10.1093/nar/gkx1024

Verlicchi, P., Al Aukidy, M., & Zambello, E. (2012). Occurrence of pharmaceutical compounds in urban wastewater: Removal, mass load and environmental risk after a secondary treatment—A review. *The Science of the Total Environment*, *429*, 123–155. https://doi.org/10.1016/j.scitotenv.2012.04.028

Walters, E., McClellan, K., & Halden, R. U. (2010). Occurrence and loss over three years of 72 pharmaceuticals and personal care products from biosolids-soil mixtures in outdoor mesocosms. *Water Research*, *44*(20), 6011–6020. https://doi.org/10.1016/j.watres.2010.07.051

SUPPORTING INFORMATION

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