

Authors:

Ai Kawahara, Gi-Hong An, Sachie Miyakawa, Jun Sonoda & Tatsuhiro Ezawa **Associate Editor:** Gogi Kalka

Abstract

While you are reading this, special alliances are being formed underground... invisible to the human eye. But they are crucial to plants, and help them conquer extreme environments such as acidic soil. We're talking arbuscular mycorrhizal (AM) fungi who help plants improve their nutrient uptake.

But how can these fungi themselves tolerate acidic soil? Are they "acidic soil specialists", who can thrive despite the acidity? We tested this hypothesis by collecting soil containing AM fungi along an acidity gradient in Japan. We also exposed fungi from two soils of different pH (acidic/neutral) to changes in their soils' acidity levels in the lab, and observed their response.

Surprisingly, we discovered that the fungi in the acidic soils also occurred in the less acidic and neutral soils, making them generalists! We believe that being generalists opens up many opportunities for AM fungi (and for their associated plants) to colonize new or quickly changing habitats.

Introduction

It is tough for plants to grow in soil that is acidic. Luckily, plants have evolved a close relationship with certain fungi that help them overcome this problem. These fungi are called *arbuscular mycorrhizal* fungi, or AM fungi for short. (The term *arbuscular refers* to special structures of the fungi, and *mycorrhizal* means root fungus in Greek). AM fungi enter plants' root cells, and at the same time grow into the soil, forming networks that consequently, increases the overall root surface area. This helps the plant capture nutrients such as phosphorus, sulfur, nitrogen and other micronutrients from the soil. In return, the fungus living in the roots gets food from the plants. This collaboration is a "Win-Win" for both partners, or what biologists call a mutualistic symbiosis.

And even though you might have never heard of this before, this collaboration is ancient - fossil records prove it existed for over 460 million years - and extremely helpful and important to plants. It can be found in over 80% of all terrestrial plants alive today.

But let's come back to our original point: acidic soils. We know that not only are they tough on plants, but also on most fungi! So it might take specialized fungi to tolerate such extreme environments. Or, fungi in acidic soils could be more generalists, meaning that they could tolerate a wide range of acidity in their soils, including both neutral *and* acidic ones. No one has successfully answered this question before, so we conducted an experiment to address it.

Methods

To find out whether AM fungi living in acidic soil are generalist or specialist, we collected soil with AM fungi from two areas that differed in soil acidity: One soil was neutral (pH around 7), the other acidic (pH around 3.5). In the lab, we mixed both soils with an equal portion of sterilized soil from the other group, to equalize chemical composition but keep each soil's original fungal communities intact. We then divided each group's soil into 3 planting pots and manipulated their acidity level to *very acidic* (pH 3.4), *medium acidic* (pH 4.0) and *slightly acidic* (pH 5.5). This allowed us to see how fungi from each soil would respond to changes in acidity levels of their respective environment.

To minimize other factors that could influence the fungal communities, we selected a single plant - Chinese Silver grass (*Miscanthus sinensis*) as the host for the fungi, and kept all pots in separate greenhouses (Fig. 1). Silver grass is common and grows in acidic and neutral soils, making it an ideal host for our AM fungal cultures. We sowed Silver grass seeds on each of the soil samples, and identified the associated AM fungi in the plant roots by DNA analysis using PCR technology. (They are too small to be distinguishable by eye or microscope).

Finally, we collected soil from the root areas of Chinese Silver grass from six sites with a pH range from 3 (very acidic) to 7.4 (neutral). We then cultivated AM fungi with the grass on these soils in our greenhouse and compared our results to those of the pH manipulation study.



Figure 1:

One-month-old chinese silver grass (*Miscanthus sinensis*). We collected the soils underneath the grass naturally grown in the field, manipulated the pH, planted the grass seeds and grew them in pots in a greenhouse.

Results

We found a wide diversity of AM fungi in the soil we sampled: all together 52 different types. The analysis of our pH manipulation experiment showed that both pH, the origin of the fungus (acidic or neutral soil) as well as the interaction of the two factors were important for the composition of the fungal community. Changes to the acidity of the soil impacted the fungi originating from the neutral soil, but *not* the ones from the acidic soil. Interestingly, many fungi that lived in the acidic soil also occurred in the neutral soil (Fig. 2).

This means that these fungi seem to be able to tolerate a wide range of soil acidity, making them *generalists*. But we also saw that while these generalists were present in a wide range of soils, they did not always outnumber the more specialist fungal types.

Analysis of our field survey revealed that soil pH largely determined fungal species composition and that fungi in acidic soils also occurred in less acidic soils.

	Habitat Soil pH	Neutral	Acidic	AM fungal communities from
		soil	soil	two different soils after
		55 NO 3A	50 A	pH manipulation.
	•	5. 8. 3.	5. 8. 3:	Chinese Silver grass was
Fungal species	A			sown to the soils with
	В			different pH (5.5 for slightly
	C			acidic, 4.0 for medium acidic,
	D			and 3.4 for very acidic) and
	Ε			grown in our greenhouse.
	F			Two months later, DNA was
	A B C D E F G			extracted from the roots, and
	Н			fungal species compositions
	1			were investigated. The
	J			rows show different fungal
	K			species, and the columns
	L			show what the soil acidity
	М			level has been changed to.
	N			Fungi depicted in blue boxes
	0			originated from the neutral
	P			soil, fungi in red boxes from
	Q			the acidic soil. Darkness of
	R			color describes frequency of
	O P Q R S T			the fungi that colonize the
	T			grass roots –
	U			the darker the color, the
	V			more frequently a particular
	W			fungal species was present.

Figure 2:

Discussion

Our results show that many fungi who live in very acidic conditions (low pH) can also survive in less acidic or even neutral soil. Being able to tolerate a wide range of conditions defines them as generalists. Interestingly, the generalist fungi that live in neutral soil are also as acid tolerant as the ones originating from acidic soil.

Being generalists may allow AM fungi to help plants settle new habits that were created through disturbances. In fact, other studies have shown that the fungi colonize an area first before plants move in. (AM fungi have been around long before the first plants settled land, and more than 100 million of years before the first dinosaurs evolved).

While it is not yet fully understood how exactly certain AM fungi can deal with such extreme conditions as acidic soils, we assume that many of these coping mechanism use a lot of energy and therefore are *more costly* to the acid tolerant fungi. This would explain how "neutral soil specialists" (fungi that cannot NOT tolerate a wide range of soil acidity) dominate in numbers in more neutral soils, despite the occurrence of generalists.

Conclusion

If confronted with an extreme environment – such as acidic soils – is it better to be a generalist or a specialist? Our study shows that both have their advantages. Specialists are better adapted to tolerate a narrow range of certain environmental factors. When in their preferred habitat, specialists do very well, and can outperform species that are

more tolerant (the generalists). Generalists, on the other hand, can survive in a much wider range of environments. Being more flexible might make them less well adapted for a certain (extreme) environment, but allows them to spread further and be pioneers in colonizing changing or new habitats, giving associated plants a huge advantage.

Your Task: Write an informative piece that looks at how survival of the fittest works in the animal kingdom. Use examples from the article to provide evidence and reasoning for this.