1-29-2020

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Elizabeth M. Wandrag
University of New England

Christina Birnbaum
Deakin University

Metha M. Klock
San Jose State University, metha.klock@sjsu.edu

Luke G. Barrett
CSIRO Agriculture & Food

Peter H. Thrall
CSIRO Agriculture & Food

Recommended Citation

Elizabeth M. Wandrag, Christina Birnbaum, Metha M. Klock, Luke G. Barrett, and Peter H. Thrall. "Availability of soil mutualists may not limit non-native Acacia invasion but could increase their impact on native soil communities" *Journal of Applied Ecology* (2020): 786-793. https://doi.org/10.1111/1365-2664.13577

Journal of Applied Ecology

DR ELIZABETH MARY WANDRAG (Orcid ID : 0000-0001-8140-539X)

Article type : Commentary

Handling Editor: Lei Cheng

Availability of soil mutualists may not limit non-native *Acacia* invasion but could increase their impact on native soil communities

Elizabeth M. Wandrag[1, 2§], Christina Birnbaum[3§], Metha M. Klock[4], Luke G. Barrett[5], Peter H. Thrall[5]

[1]School of Environmental and Rural Science, University of New England, NSW 2351, Australia.

[2] Institute for Applied Ecology, University of Canberra, ACT 2617, Australia

[3] Centre for Integrative Ecology, Deakin University, Burwood campus, 221 Burwood Hwy, Burwood, VIC 3125 Australia

[4]Department of Environmental Studies, San José State University, One Washington Square, San José, CA 95192, USA

[5]CSIRO Agriculture & Food, Canberra, ACT 2601, Australia

[§]Contributed equally

Corresponding author: Elizabeth M. Wandrag. elizabethwandrag@gmail.com

ABSTRACT

1. The availability of compatible mutualistic soil microbes could influence the invasion success of non-native plant species. Specifically, there may be spatial variation in the distribution of compatible microbes, and species-specific variation in plant host ability to associate with available microbes. Although either or both factors could promote or limit invasion, the scale

This article has been accepted for publication and undergone full peer review but has not been through the copyediting, typesetting, pagination and proofreading process, which may lead to differences between this version and the Version of Record. Please cite this article as doi: 10.1111/1365-2664.13577

over which most studies are conducted makes it difficult to examine these two possibilities simultaneously. However, this is critical to identifying a role of soil microbes in invasion.

2. A series of recent research projects focused on interactions between Australian *Acacia* and nitrogen-fixing bacteria (rhizobia) at multiple spatial scales, from the local to the inter-continental, has allowed us to evaluate this question. Collectively, this research reveals that nodulation, performance and rhizobial community composition are all broadly similar across spatial scales and differentially invasive species.
3. *Synthesis and applications.* We argue that current research provides convincing evidence that interactions with rhizobia do not determine invasion success in *Acacia*, but instead highlights key knowledge gaps that remain unfilled. Importantly, the ease with which non-native *Acacia* species form mutualistic associations with rhizobia, regardless of invasive status, highlights the critical need to understand the impacts of all non-native *Acacia* on native soil communities.

KEYWORDS: Biological invasion; invasional meltdown; mutualism; nitrogen-fixation; plant-soil feedback; symbiosis; wattles.

Improving our understanding of interactions between invasive legumes and rhizobia

Mutualistic interactions with soil microbes can facilitate nutrient acquisition and hence plant establishment in new locations. For non-native species, relying on mutualistic soil microbes for nutrient acquisition may limit invasion if species fail to encounter compatible microbes in non-native locations (Dickie et al., 2017; Simonsen, Dinnage, Barrett, Prober, & Thrall, 2017). Consequently, differences in invasion success (see Table 1 for definitions; Richardson et al., 2000) among non-native species are hypothesized to arise, at least in part, from differences in their ability to access mutualistic soil microbes (Harrison, Simonsen, Stinchcombe, & Frederickson, 2018). However, robust field tests of this idea are lacking because most studies examine only single plant species or do so only in single, non-native locations. Concluding that interactions with soil microbes contribute to invasion success requires evidence that differentially invasive species vary in the extent to which they encounter compatible rhizobia in non-native locations. Recent work on non-native species within a single leguminous taxon (the Australian *Acacia*) has generated insights into interactions between plants and nitrogen-fixing soil bacteria (rhizobia) across multiple ecological scales, providing an opportunity to develop an understanding of the role that soil microbes play in invasion. We highlight consistent patterns that have emerged from this work that provide a partial answer to the role of soil microbes in *Acacia* invasion and discuss clear gaps in current knowledge that serve as focal points for future research.

The availability of rhizobia as a constraint on invasion

Features of both the plant host and rhizobial mutualist determine rhizobial availability for non-native plant species, potentially acting as a sequence of barriers to invasion (Figure 1). The specificity of the mutualism, or the 'promiscuity' of either the plant or microbe is important (Klock, Barrett, Thrall, & Harms, 2015; Thrall, Burdon, & Woods, 2000). For the plant host, promiscuous (i.e. generalist) plant species may be more likely to invade and to do so in multiple locations (Figure 1) because they more frequently encounter compatible rhizobia and thus have a higher probability of establishing effective mutualisms (Keet, Ellis, Hui, & Le Roux, 2017; Klock et al., 2015; Wandrag, Sheppard, Duncan, & Hulme, 2013). For rhizobia, both promiscuity and effectiveness (i.e. the extent to which they promote growth in the host plant) traits are important. Rhizobia that are both promiscuous and effective are more likely to facilitate invasion than those that are specialised or minimally benefit plant growth. Consequently, spatial variation in rhizobial community composition (i.e. the extent to which compatible rhizobia are present locally, Figure 1) may lead to plant species becoming invasive in some locations and not others if rhizobia are differentially compatible and effective in non-native locations (Rodríguez-Echeverría, 2010). Evidence that *Acacia* species that are more promiscuous hosts and more widespread in their native range are more likely to be invasive where introduced (Klock et al., 2015) laid the foundation for tests of the hypothesis that differentially invasive *Acacia* species vary in their ability to associate with rhizobia in non-native locations.

The Australian *Acacia* as a model system

Australian *Acacia* have been introduced globally. Many regions contain species that range from only being recorded casually to being invasive and widespread (Richardson & Rejmánek, 2011). Consequently, *Acacia* have become a model system for studying the mechanisms underlying invasion (Richardson et al., 2011), including the role of soil microbes. This interest has generated a series of studies investigating the ability of non-native *Acacia* to access rhizobia, and the performance implications of doing so. These include: six studies that examine differentially invasive *Acacia* species in both native and non-native locations (Birnbaum, Barrett, Thrall, & Leishman, 2012; Birnbaum, Bissett, Thrall, & Leishman, 2016; Birnbaum & Leishman, 2013; Klock, Barrett, Thrall, & Harms, 2016; Wandrag et al., 2013; Warrington et al., 2019), two that examine only one species but do so in both the native Australian and non-native (Portugal and South Africa) range (Crisóstomo, Rodríguez-Echeverría, & Freitas, 2013; Ndlovu, Richardson, Wilson, & Le Roux, 2013), and one that examines differentially invasive *Acacia* in only the non-native (South Africa) range (Keet et al., 2017). These studies cover 22 *Acacia* species that range from only casually occurring in a few locations, to those that are widespread invaders in almost all non-native locations (Table 1), and five geographic ranges: Australia (both native and non-native ranges), New Zealand, South Africa, the US (California) and Europe (Portugal). We next discuss what these studies have revealed regarding rhizobial availability as a constraint on invasion.

No consistent evidence that rhizobial availability constrains *Acacia* invasion

Effective associations with rhizobia are generally quantified by counting the number and size of effective root nodules (structures produced in plant roots when infected by rhizobia and coloured pink by leghaemoglobin if nitrogen fixation occurs) (Corbin, Brockwell, & Gault, 1977). All 22 species in Table 1 have had nodulation quantified (i.e. the percentage of plants that form nodules or mean number of nodules per plant) in at least one non-native location, with successful nodulation recorded in each location (Table 2). Importantly, studies that examined differentially invasive species failed to find differences in nodulation among species, and the six studies that examined species in non-native relative to native ranges revealed few differences in rhizobial availability between each range (Table 2). Whenever nodulation was examined in soils collected from established *Acacia* populations, nodulation was the same in each range. Differences were only observed when nodulation was tested in novel (not previously colonised by *Acacia*) soils in New Zealand and California: nodulation was reduced in novel soils in both locations (Klock et al., 2016; Wandrag et al., 2013). Although a reduction in rhizobial availability may limit *Acacia* performance when they are first introduced outside Australia, differentially invasive *Acacia* appear equally limited. Collectively, these studies indicate that *Acacia*-compatible rhizobia are globally widespread and available to both invasive and non-invasive *Acacia* species.

Does rhizobial community composition matter?

Co-introduced rhizobia could benefit plant hosts more than novel associations (Le Roux, Hui, Keet, & Ellis, 2017). Consequently, characterising the rhizobial communities associated with differentially invasive *Acacia* in non-native relative to native ranges is another approach to investigating the role of rhizobia in invasion (Figure 1; Table 2). As with nodulation, few clear patterns have emerged. The similarity of rhizobial communities in non-native and native locations

(Table 2) provides overwhelming evidence for the co-introduction of *Acacia* and rhizobia. The consistency of this among species provides no evidence that differentially invasive species vary in the degree of co-introduction. For example, in South Africa there were no differences in the diversity and community composition of rhizobia associated with 19 differentially invasive *Acacia* species (Table 2; Keet et al., 2017), and no differences in the rhizobia associated with four of those species relative to their native Australian range (Table 2; Warrington et al., 2019). Similarly, there were no differences in the richness or community composition of rhizobia associated with differentially invasive *Acacia* in their non-native Californian or native Australian range (Table 2; Klock et al., 2016). Rhizobia associated with *Acacia* in New Zealand (Warrington et al., 2019), Portugal (Crisóstomo et al., 2013; Rodríguez-Echeverría, 2010) and the non-native Australian range (Birnbaum et al., 2016) were all similar to their native Australian range. There is only one clear example of differences between the rhizobial communities in native and non-native locations, with *A. longifolia* forming novel associations with rhizobia in its non-native Australian range (Birnbaum et al., 2012).

Do plant species respond differently to available rhizobia?

While rhizobial availability appears similar for differentially invasive species, the benefit derived by *Acacia* from rhizobia could vary to influence plant performance and hence invasion. However, as with nodulation, there is no clear evidence that the benefit of rhizobia to currently established *Acacia* species varies in non-native locations. Growth or survival differences in response to field soils were quantified in the glasshouse for a total of 11 species in both native and non-native ranges, with no differences among species that could explain invasion (Table 2). Three of four invasive species had equal biomass in non-native and native range soils within Australia (Table 2; Birnbaum et al. 2012). While there were differences in survival among seven species in California, this did not correlate with their variable invasion success (Table 2; Klock et al., 2016). Five species that are differentially invasive in New Zealand and globally (Table 1) showed similar growth performances in non-native New Zealand relative to native Australian soils taken from established *Acacia* populations. Although three species tested in novel soils in New Zealand showed a reduction in growth, this did not vary among species (Table 2; Wandrag et al., 2013).

Synthesis

Examining differentially invasive *Acacia* species at multiple spatial scales and in both native and non-native ranges should address the question of whether interactions with rhizobia influence invasion success. Despite some evidence for an initial reduction in fitness because of limited rhizobial availability in novel soils outside Australia, the magnitude of limitation does not consistently vary among species or locations. Furthermore, rhizobia that are compatible with a range of *Acacia* species and similarly promote growth in those species appear widespread. The consistent finding from nine studies targeting similar questions, and using similar methods, is that differentially invasive species form equally effective associations with rhizobia in non-native locations, most likely due to co-introduction. We argue that this provides convincing evidence that rhizobial availability is rarely a barrier to invasion for *Acacia* species and instead highlights the need to adopt new approaches to understanding interactions between non-native *Acacia* and rhizobia.

The implications for our understanding of the role of rhizobia in plant invasions

Human factors often determine invasion success for non-native *Acacia* (Richardson, Le Roux, & Wilson, 2015). Propagule pressure is considered particularly important because *Acacia* that have become invasive (e.g. Table 1) have generally been planted at higher densities and more widely than non-invasive *Acacia*. Consequently, the finding that non-invasive *Acacia* are equally able to access rhizobia as invasive *Acacia* suggests that rhizobial availability is unlikely to constrain the future spread of non-invasive *Acacia* if human factors, such as propagule pressure, change. To advance understanding in this area, we suggest there are three important questions to resolve: (1) Does reliance on rhizobia explain the failure to establish self-sustaining populations? (2) What is the invasion potential of non-invasive *Acacia*? (3) What are the implications of *Acacia* invasion for native plant and microbial communities? We discuss these below.

Could reliance on rhizobia explain establishment failures?

Many more species of *Acacia* have been introduced than are currently recognised as established or invasive anywhere in the world (e.g. Magona, Richardson, Le Roux, Kritzinger-Klopper, & Wilson, 2018), yet there are few examples of research on species that have been introduced but never established. A recent meta-analysis suggests that the reliance on rhizobia must put some constraint on invasion success among legumes (Simonsen et al., 2017), and at least three lines of evidence suggest that the absence of compatible rhizobia could limit *Acacia* establishment in some locations, including: (1) the need for rhizobial inoculants to improve *Acacia* performance in forestry (Burdon, Gibson, Searle, Woods, & Brockwell, 1999); (2) forestry trials in Asia that found several species of *Acacia* failed to nodulate (Zhang et al., 1997); and (3) the finding in New

Zealand that the performance of even invasive *Acacia* was limited by the rhizobial availability in novel soils (Wandrag et al., 2013). Targeted studies that examine the rhizobial availability for non-established species are needed to rule out the possibility that interactions with rhizobia could constrain invasion success in these species.

Future invasion scenarios

Strong support for the co-introduction of *Acacia* and rhizobia suggests that the broad distribution of many non-native *Acacia* species may be mirrored by an unseen but similarly broad distribution of introduced rhizobia. If so, the co-invasion of *Acacia* and rhizobia could promote the invasion of other *Acacia* species as an example of invasional meltdown (Rodríguez-Echeverría, 2010). Indeed, evidence that non-invasive *Acacia* species perform equally well with the rhizobial communities associated with congeners as they do with those associated with conspecifics (Wandrag et al., 2013; Warrington et al., 2019) suggests that invasive *Acacia* could facilitate the establishment of non-invasive *Acacia* via shared rhizobia. Further testing of non-invasive *Acacia* with the soil communities associated with invasive *Acacia* species would clarify the potential for invasive *Acacia* to facilitate the establishment and spread of non-invasive *Acacia* (or other non-native legumes). Moreover, testing non-invasive *Acacia* in novel soils would reveal the spatial extent of any facilitation.

What are the implications of Acacia *invasion for native plant and microbial communities?*

Emerging evidence suggests that invasive *Acacia* alter native microbial communities by cultivating distinct rhizobia (Barrett, Bever, Bissett, & Thrall, 2015). Since *Acacia* and co-occurring native legumes in Portugal (Rodríguez-Echeverría, 2010), New Zealand (Weir, Turner, Silvester, Park, & Young, 2004) and South Africa (Le Roux, Mavengere, & Ellis, 2016) appear to form distinct rhizobial associations, it is likely that such alterations could disrupt native plant-soil microbe interactions (Le Roux et al., 2018) and thereby impact native plant communities. If so, the broad geographic distribution of non-native *Acacia*, the relatively high success of the genus following introduction into diverse environments, and the ability of even non-invasive *Acacia* species to associate with (and thus cultivate) distinct rhizobial communities, points to an urgent need to understand the impacts of *Acacia* on native soil microbial communities, regardless of invasive status. The impacts of non-invasive plants are generally ignored yet, collectively, non-invasive *Acacia* species span a very large range. Consequently, the broad similarities in *Acacia*-rhizobia interactions among species suggest a much greater potential for non-invasive *Acacia* to impact native plant and microbial communities than is currently recognised. This argues for research on the impact of non-native *Acacia* on native plant and microbial communities to consider all naturalised species, regardless of their current distribution and invasive status.

Conclusions

Convincing evidence suggests that the availability of rhizobia is an unlikely barrier to invasion for *Acacia*, though may still constrain initial establishment in some species. Rather, the consistent finding that nodulation, performance, and rhizobial community composition are similar across species that span the invasion spectrum, and in native and non-native locations globally, highlights an urgent need to shift focus towards a better understanding of disruptions to native plant-soil microbe interactions caused by all non-native *Acacia*, regardless of invasive status.

Acknowledgements

We thank the Council of Australasian Weed Societies and the Weed Society of Western Australia for hosting the symposium 'Wattle we do about wattles?' that led to the first draft of this manuscript and for providing funding to EMW, and two anonymous reviewers for helpful comments on the manuscript.

Author contributions

All authors conceived the idea. EMW and CB led the writing with input from all authors. All authors gave final approval for publication.

Data availability statement

Data have not been archived because this article does not use data.

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Table 1. Invasive status* of each *Acacia* species included in studies that either assessed multiple species, or assessed species in both native and non-native locations, and the non-native ranges examined for each species. Definitions of invasive status (Richardson et al., 2000): **non-native** – plant species introduced to a new location as a result of human activity; **casual** – non-native plant species that do not establish persistent self-sustaining populations; **naturalized** – non-native plant species that establish self-sustaining populations but do not spread far from parent plants; **invasive** – naturalised, non-native plant species that spread away from parent plants.

Species	Number of regions invasive*	Introduced Australian[1, 2, 3]	New Zealand[4,5]	California[6]	South Africa [5,7, 8]	Portugal[9, 10]	Total non-native regions studied
Acacia adunca	0				Naturalized		1
Acacia baileyana	3		Naturalized	Naturalized	Invasive		3
Acacia cultriformis	0			Casual			1
Acacia cyclops	5	Invasive			Invasive		2
Acacia dealbata	7		Invasive	Invasive	Invasive		3
Acacia decurrens	4		Invasive		Invasive		2
Acacia elata	1				Invasive		1
Acacia fimbriata	0				Invasive		1
Acacia implexa	1				Invasive		1
Acacia longifolia	8	Invasive		Naturalized	Invasive	Invasive**	3
Acacia mearnsii	13				Invasive		1
Acacia melanoxylon	11	Invasive	Invasive	Invasive	Invasive		4
Acacia paradoxa	5				Invasive		1
Acacia piligera	0				Naturalized		1
Acacia podalyriifolia	2				Invasive		1
Acacia pravissima	0		Casual				1
Acacia pycnantha	3			Casual	Invasive		2
Acacia retinodes	2				Localized[8]		1
Acacia saligna	5	Invasive			Invasive	Invasive	3
Acacia stricta	1				Invasive		1

Acacia verticillata	2				Casual			1
Acacia viscidula	0					Naturalized		1
		Total	**4**	**5**	**7**	**19**	**2**	

* (Magona, Richardson, Le Roux, Kritzinger-Klopper, & Wilson, 2018; Rejmánek & Richardson, 2013)

** Native range community composition inferred

[1] (Birnbaum, Barrett, Thrall, & Leishman, 2012)

[2] (Birnbaum, Bissett, Thrall, & Leishman, 2016)

[3] (Birnbaum & Leishman, 2013)

[4] (Wandrag, Sheppard, Duncan, & Hulme, 2013)

[5] (Warrington et al., 2019)

[6] (Klock, Barrett, Thrall, & Harms, 2016)

[7] (Ndlovu, Richardson, Wilson, & Le Roux, 2013)

[8] (Keet, Ellis, Hui, & Le Roux, 2017)

[9] (Rodríguez-Echeverría, 2010)

[10] (Crisóstomo, Rodríguez-Echeverría, & Freitas, 2013)

Table 2. Summary of each paper included in Table 1 and which of the putative barriers to invasion highlighted in Figure 1 that they target.

Paper	**Species included**	**Location**	**Stage targeted**	**Outcome**
Birnbaum et al. 2012	*Acacia cyclops* *Acacia longifolia* *Acacia melanoxylon*	Native and non-native Australian range	2. Are compatible rhizobia present locally?	All species nodulated, with no differences in nodulation among species or between ranges

	Acacia saligna		4. Do available rhizobia equally promote performance?	Equal growth for all species except *A. longifolia*, which showed higher growth in non-native soils
Birnbaum & Leishman 2013	As above	As above	2. Are compatible rhizobia present locally?	All species nodulated, with no differences in nodulation among species or between ranges
			4. Do available rhizobia equally promote performance?	Equal growth for all species except *A. longifolia*, which showed higher growth in non-native soils
Birnbaum et al. 2012	As above	As above	3. Are rhizobia co-introduced?	Rhizobial communities were similar across ranges for three of four species (*A. longifolia* was the exception)
Crisóstomo et al. 2013	*Acacia saligna*	Australia and Portugal	3. Are rhizobia co-introduced?	Rhizobial communities were similar in native and non-native locations
Keet et al. 2017	*Acacia adunca* *Acacia baileyana* *Acacia cyclop* *Acacia dealbata* *Acacia decurrens* *Acacia elata* *Acacia fimbriata* *Acacia implexa*	South Africa	1. Invasive species are more promiscuous	No differences in the rhizobial communities associated with species, regardless of invasive status

	Acacia longifolia			
	Acacia mearnsii			
	Acacia melanoxylon			
	Acacia paradoxa			
	Acacia piligera			
	Acacia podalyriifolia			
	Acacia pycnantha			
	Acacia retinodes			
	Acacia saligna			
	Acacia stricta			

Paper	Species included	Location	Stage targeted	Outcome
Klock et al. 2015	*Acacia baileyana*	Australia and California	1. Invasive species are more	No differences in the rhizobial communities associated with species,
	Acacia cultriformis		promiscuous	regardless of invasive status
	Acacia dealbata			
	Acacia longifolia		2. Are compatible rhizobia	All species nodulated in the non-native range, though nodulation was
	Acacia melanoxylon		present locally?	lower in non-native soils and two species failed to nodulate in some
	Acacia pycnantha			soils
	Acacia verticillata			

			4. Do available rhizobia equally promote performance?	No difference in survival among species or between native and non-native range
Ndlovu et al. 2013	*Acacia pynantha*	Australia and South Africa	1. Invasive species are promiscuous	*A. pycnantha* associated with a diverse rhizobial community, with higher diversity in the non-native range
			3. Are rhizobia co-introduced?	Rhizobia of Australian origin present in nodules
Rodríguez-Echeverría 2010	*Acacia longifolia*	Portugal	3. Are rhizobia co-introduced?	Rhizobia of Australian origin present in nodules
Wandrag et al. 2013	*Acacia baileyana* *Acacia dealbata* *Acacia pravissima*	Australia and New Zealand	1. Invasive species are more promiscuous	No difference on nodulation among differentially invasive species in either location
			2. Are compatible rhizobia locally present?	Rhizobia were limiting in novel soils in the non-native but not native range
			4. Do available rhizobia equally promote performance?	No difference in biomass among differentially invasive species and biomass increase per nodule was the same in both native and non-native range

Warrington et al. 2019	*Acacia baileyana* *Acacia dealbata* *Acacia decurrens* *Acacia melanoxylon*	Australia, New Zealand and South Africa	3. Are rhizobia co-introduced?	Rhizobia associated with *Acacia* in New Zealand and South Africa co-introduced from Australia
			4. Do available rhizobia equally	Little variation in performance among species or locations

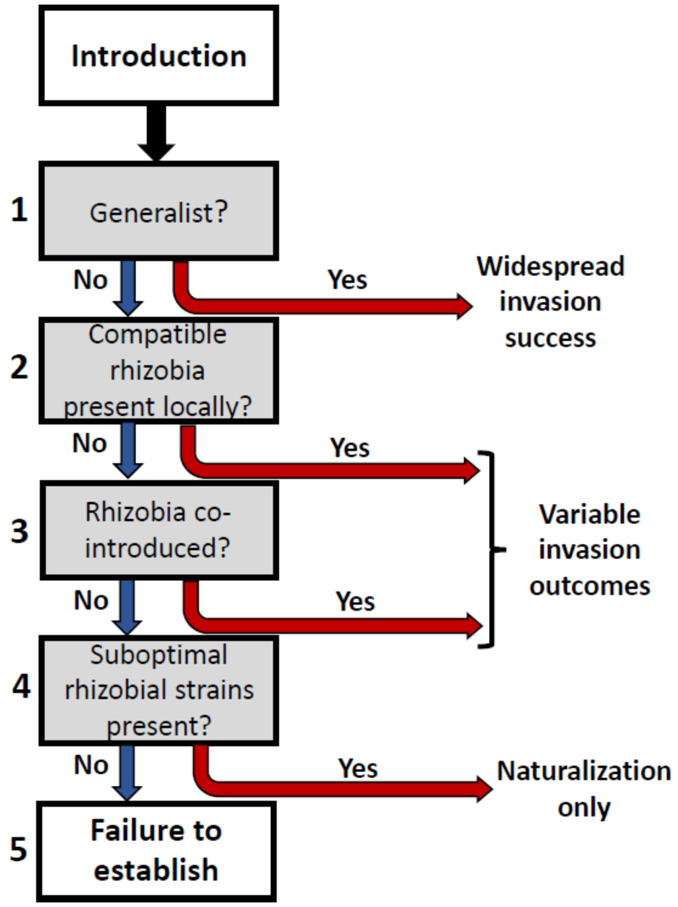

Figure 1. Factors that influence rhizobial availability in non-native locations as a sequence of barriers to invasion. Species that are highly promiscuous (generalist) and able to effectively associate with a range of rhizobia should have the potential for widespread invasion (1). Where species are not generalists, either the local presence of more specialised rhizobia (2) or the co-introduction of rhizobia from species' native ranges (3) should determine whether rhizobial availability is a barrier to invasion, leading to variable invasion outcomes. A final possibility is that species could associate with rhizobial strains that are less specialized and effective, potentially allowing establishment or local naturalization (4). Where species are unable to form any associations with rhizobia, species should fail to establish self-sustaining populations (5).