FORUM

Unifying facilitation and recruitment networks

Julio M. Alcántara[1,2] | **José L. Garrido**[3,4] | **Alicia Montesinos-Navarro**[5] | **Pedro J. Rey**[1,2] | **Alfonso Valiente-Banuet**[6,7] | **Miguel Verdú**[5]

[1]Departamento de Biología Animal, Biología Vegetal y Ecología, Universidad de Jaén, Jaén, Spain

[2]Instituto Interuniversitario del Sistema Tierra en Andalucía (IISTA), Jaén, Spain

[3]Departamento de Ecología Evolutiva, Estación Biológica de Doñana (EBD-CSIC), Sevilla, Spain

[4]Dept. Microbiología del suelo y sistemas simbióticos, Estación Experimental del Zaidín (EEZ-CSIC), C/ Profesor Albareda, 1, Granada, España

[5]Centro de Investigaciones sobre Desertificación, (CSIC-UVEG-GV), Valencia, Spain

[6]Departamento de Ecología de la Biodiversidad, Instituto de Ecología, Universidad Nacional Autónoma de México,, México D.F., México

[7]Centro de Ciencias de la Complejidad, Ciudad Universitaria, Universidad Nacional Autónoma de México,, México D. F., México

Correspondence
Miguel Verdú, Centro de Investigaciones sobre Desertificación (CSIC-UVEG-GV), Valencia, Spain.
Email: Miguel.Verdu@ext.uv.es

Abstract

Ecological network studies are providing important advances about the organization, stability and dynamics of ecological systems. However, the ecological networks approach is being integrated very slowly in plant community ecology, even though the first studies on plant facilitation networks (FNs) were published more than a decade ago. The study of interaction networks between established plants and plants recruiting beneath them, which we call Recruitment Networks (RNs), can provide new insights on mechanisms driving plant community structure and dynamics. RNs basically describe which plants recruit under which others, so they can be seen as a generalisation of the classic FNs since they do not imply any particular effect (positive, negative or neutral) of the established plants on recruiting ones. RNs summarise information on the structure of sapling banks. More importantly, the information included in RNs can be incorporated into models of replacement dynamics to evaluate how different aspects of network structure, or different mechanisms of network assembly, may affect plant community stability and species coexistence. To allow an efficient development of the study of FNs and RNs, here we unify concepts, synthesise current knowledge, clarify some conceptual issues, and propose basic methodological guidelines to standardise sampling methods that could make future studies of these networks directly comparable.

KEYWORDS
canopy–recruit interactions, ecological networks, facilitation networks, nurse plants, plant–plant interactions, recruitment networks, replacement dynamics, replacement networks, sapling bank, strongly connected components

1 | INTRODUCTION

… plant shrubs and bushes which can break the force of the wind, diminish that of frost, and moderate the inclemency of the seasons (…) are the shelter which guards the young trees, and protects them against heat and cold. (…) after young trees passed the first few years in the shade and shelter of the others, they quickly stretch up, and suppress all the surrounding plants

(Buffon 1742, as cited by Egerton, 2015:428).

The recruitment phase spans all the stages along a plant's life cycle except adulthood. Understanding the ecological interactions that drive the success of plants during this phase (e.g., seed dispersal, seed predation, intra- and inter-specific competition, facilitation, herbivory,

mutualistic interactions with mycorrhizal fungi) is of major importance to understand plant population and community dynamics (Grubb, 1977; Harper, 1977). How these different types of interactions affect recruitment and shape the composition and structure of plant communities is a key question in plant community ecology (see, for example, Comita, Aguilar, Pérez, Lao, and Hubbell 2007 on the effect of plant–plant competition, Montesinos-Navarro, Segarra-Moragues, Valiente-Banuet, and Verdú 2012 on the effect of the plant–mycorrhizal fungi mutualism, Terborgh et al. 2008 on the effect of seed dispersers, Wolf et al. 2018 on the effect of seed predators, and van Zonneveld, Gutiérrez, and Holmgren 2012 on the effect of plant–plant facilitation).

Plant facilitation is a well-known interaction assembling ecological communities through its effects on different aspects of plant performance, including recruitment (Callaway, 2007). Detailed studies on the functional mechanisms underlying facilitation between plants were accumulating at the beginning of this century (Brooker et al., 2008). It was in this context that Verdú and Valiente-Banuet (2008) pioneered the analysis of plant facilitative interactions as ecological networks at the community level: at the scale of the whole community, plant–plant facilitative interactions can be approached as a network of interactions between nurse plants and facilitated plants recruiting under their canopy. However, the complex nature of plant–plant facilitation offers other possibilities to build facilitation networks (FNs) besides recruitment interactions. Thus, one can envisage FNs where links would represent, for example, the effect of shared pollinators on plant reproduction (Moeller, 2004) or the effect of shared mutualistic fungi on plant nutrition (Montesinos-Navarro, Verdú, Querejeta, & Valiente-Banuet, 2017). These alternatives would increase our knowledge on the mechanisms of facilitation and how facilitation integrates within a broader context of higher-order interactions (Levine, Bascompte, Adler, & Allesina, 2017). Notwithstanding the importance of these other aspects of facilitation, focusing on recruitment has three important advantages: (a) recruitment informs on population level (demographic) effects of the interaction, which is a direct way of connecting interaction networks with the analysis of plant community dynamics; (b) recruitment is the integrated outcome at the population level of multiple interactions (pollination, seed dispersal, seed predation, pathogen and herbivore attack); and (c) sapling banks contain several cohorts, so their structure is less biased by particularly good or bad years for the interactions (e.g., caused by extreme weather conditions) or by population cycles of certain species (e.g., masting events, pest outbreaks).
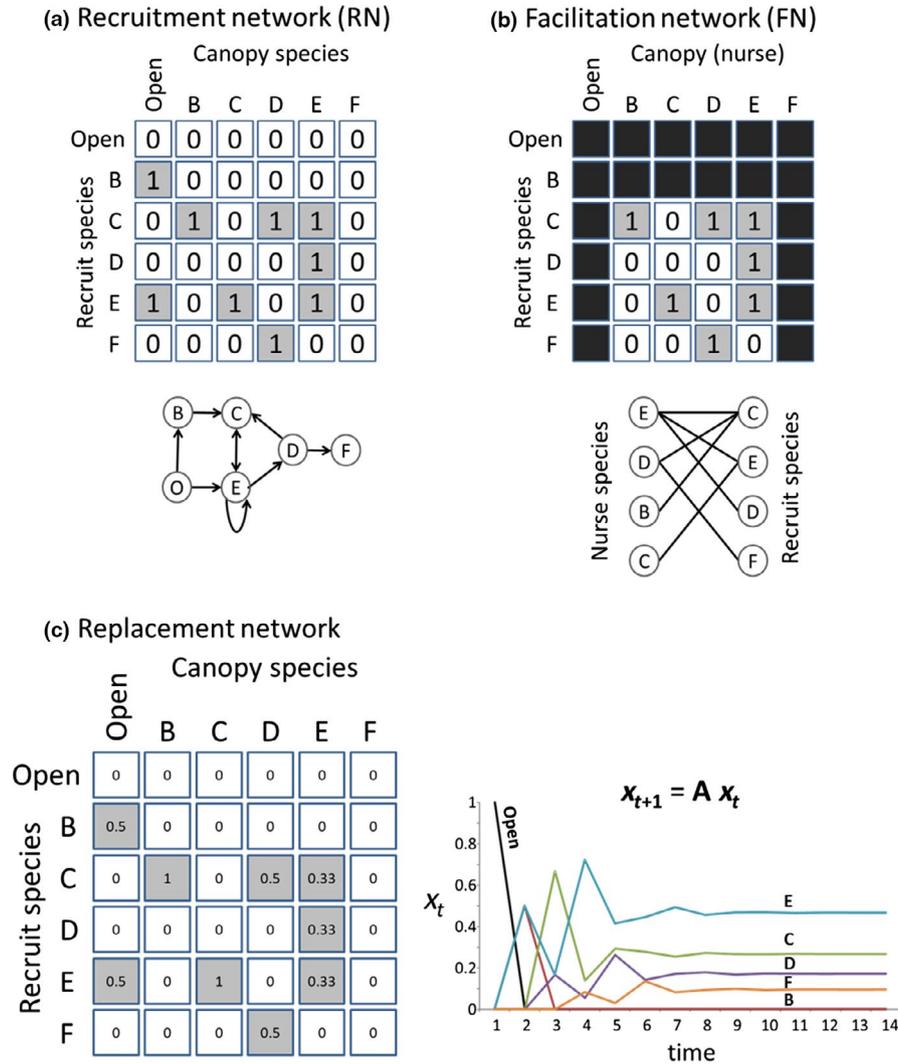
As in food webs and mutualistic networks, incorporating the complex network approach to plant community ecology can open new research avenues and opportunities to address classic and far-reaching questions. For example, these studies are advancing our understanding on the conditions necessary for the coexistence of competing species (Godoy, Bartomeus, Rohr, & Saavedra, 2018; Levine et al., 2017), or on the ways in which plant–plant facilitation contributes to the assembly of plant–pollinator interactions (Losapio et al., 2019). Surprisingly, and in clear contrast with studies on other ecological interactions, after a decade since the pioneering work of Verdú & Valiente-Banuet, very few studies have been published on

networks describing all (or most of) the recruitment interactions occurring in a plant community (Alcántara & Rey, 2012; Fodor, Haruta, & Dorog, 2018; Marcilio-Silva et al., 2015; Pulgar, Alcántara, & Rey, 2017; Verdú, Jordano, & Valiente-Banuet, 2010; Verdú & Valiente-Banuet, 2008, 2011). Consequently, a huge gap of knowledge on plant recruitment networks (RNs) exists. For example, we still have not agreed on a common framework to study plant RNs (should they describe facilitation, competition, co-occurrence, spatial repulsion–attraction; which measure of interaction strength should we use to address each question?); we do not know whether these networks have any particular structure predominantly (nestedness, modularity, motifs, hubs, intransitive cycles, asymmetry); whether, how and why their structure varies across ecosystems, environmental gradients and during succession; whether it is resistant against different types of biotic and abiotic disturbance; which mechanisms are more or less important drivers of network structure; and how network structure affects the stability of the community, species coexistence, and species relative abundances. Moreover, although there are many theoretical studies addressing these last topics in networks of competing species (Alcántara, Pulgar, & Rey, 2017; Allesina & Levine, 2011; Godoy et al., 2018; Laird & Schamp, 2006; Levine et al., 2017), we still do not know whether real plant RNs have the types of topologies and the distribution of interaction strengths that are explored in these studies.

In this forum, we present the idea of combining FNs with RNs under a unified framework. To this end we (a) briefly describe the key concepts of the approach and the main findings so far; (b) address the possible conceptual and methodological causes of the still limited use of this approach; and finally (c) propose basic guidelines to standardise sampling methods that could be used in any type of ecosystem and that could make future studies characterizing plant facilitation/RNs directly comparable.

1.1 | Facilitation, recruitment and replacement networks

Before proceeding any further, it is worth clarifying the meaning of the terms FNs, RNs and replacement networks. First of all, the three networks have fundamental aspects in common: (a) the three networks are aimed at understanding the organization and functioning of plant communities (excluding small herbaceous species), so their nodes represent populations of different species and their links indicate the qualitative (binary presence/absence) or quantitative magnitude of the effect of the population of one species on the population of the other; (b) they use the frequency of recruitment of one species under another as a surrogate for interaction strength; and (c) since they explore community-level questions, they consider all the possible pairwise interactions between as many species as possible within a given local community. Many studies of plant–plant interactions have focused on one or a few nurse plant species, so they studied a few nodes and links, what cannot be considered as representative of a community-level interaction network (Losapio, Pugnaire, O'Brien, & Schöb, 2018; Soliveres, Torices, & Maestre, 2012). Other studies have explored the outcome of many pairwise

FIGURE 1 Comparison of recruitment networks, facilitation networks and replacement networks. All networks represent the interactions between established plants (canopy or nurse plants) and plants recruiting beneath them. (a) Recruitment networks (RNs) contain information on the recruitment interactions between all possible pairs of species, and also between recruiting species and open interspaces that are represented by their own node. Every species potentially participates as canopy and as recruit, so the network is unipartite. RNs are directed networks, so interactions in the graph representation are indicated as arrows pointing from the canopy to the recruit species. (b) Facilitation networks (FNs) focus on the subset of species whose recruitment is facilitated by some of the canopy species (black cells in the matrix are those present in the RN but not in the FN, as we explain next). FNs are bipartite networks, with a group of species playing the role of nurses and another group with the role of recruits; some species can occur in both groups, but this is not a necessary condition. The group of nurses does not include open ground as a node, nor those species under which no other plant was found recruiting (species F in the example). Similarly, FNs exclude from the group of recruit species those that recruit only in open ground (species B in this example), like pioneer and shade-intolerant plants. Thus, FNs are subnetworks of RNs. (c) Replacement networks are functions of RNs. In this toy example, the entries of the RN have been transformed into transition probabilities so that the matrix can be incorporated in a simple Markov model of community dynamics like the one projected in the left figure [Colour figure can be viewed at wileyonlinelibrary.com]

interactions within communities but did not combine them into a network representative of the whole community (Cavieres et al., 2014; Rey, Alcántara, Manzaneda, & Sánchez-Lafuente, 2016; Soliveres, Maestre, Berdugo, & Allan, 2015). Although these studies are fundamental to improve our knowledge on plant–plant interactions, they cannot be qualified as studies of community networks, so they are out of the focus of the present study.

The first studies depicting plant RNs (Verdú & Valiente-Banuet, 2008; Verdú et al., 2010) focused on the importance of the facilitative effect of nurse plants on the 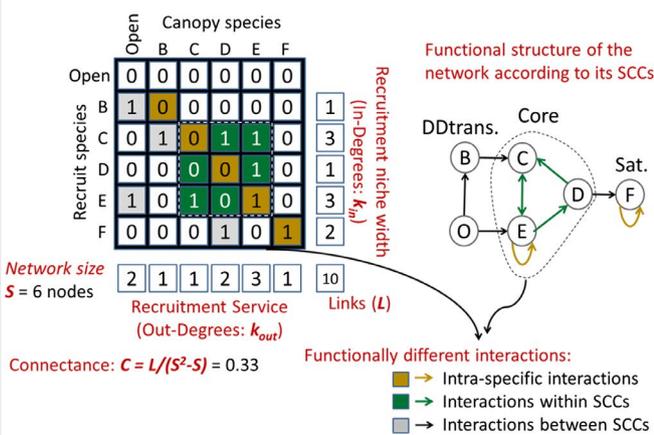recruitment of other plants in drought-prone environments; thence the term FNs. However, the concept can be generalised to include any recruitment interaction, not only those involving facilitation. Thus, a recruitment network can be defined as a network depicting the interactions between established (canopy) plants and plants recruiting beneath them (Figure 1a). In this context, we use the term "canopy plant" instead of "nurse plant" because the first does not assume any positive or negative effect of the established plant on the recruiting one. Accordingly, from a graph theoretical perspective, FNs are a subnetwork of RNs (Figure 1b).

Box 1 **Basic components and some analytical possibilities of recruitment networks.**
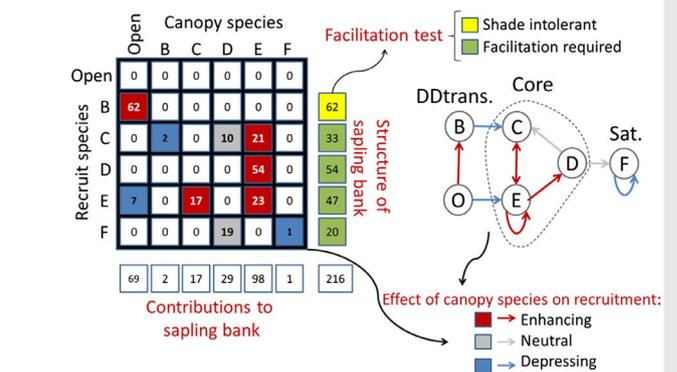
(A) The most basic analyses are based on the adjacency matrix of the network. The adjacency matrix is binary (0/1) and represents simply the existence or not of recruitment of individuals of the recruit species (rows of the matrix) under individuals of the canopy species (columns of the matrix). It allows qualitative analyses. In the context of RNs, we can define the recruitment niche of a species as the set of canopy species (including open interspaces and/or open gaps) under which it is able to recruit. The sum across rows (known as species in-degree: k_{in}) indicates the number of species under which each species recruits, so it can be interpreted as the width of its recruitment niche. The sum along columns (out-degree: k_{out}) indicates the number of species that recruit under a canopy species, so it can be interpreted as the recruitment service provided by the canopy species in the community. The main diagonal informs on the number of species that allow conspecific recruitment. When the columns and rows are ordered in Lower Triangular Block Form (as in this example), there are large blocks in the main diagonal (indicated with dashed white line) corresponding to the so-called non-trivial Strongly Connected Components (SCCs: groups of species formed so that one can reach any species of the group from any other species of the group, and return following the direction of the arrows, as shown by the core in the left graph). The SCCs and their relationships in the graph inform on the functional structure of the network as follows: RNs are typically formed by a core of species (the, usually unique, largest SCC; species C, D and E in this example), satellite species (those that recruit under some core species but do not allow any core species to recruit beneath them; species F in this example), strictly transient species (those that do not recruit in the plot; i.e., there are only adult individuals; none in this example) and disturbance-dependent transients (DDTrans; that recruit only in open interspaces or gaps; like species B in this example). In replacement dynamics models, core and satellite species have a higher probability of persisting in the long term (see the dynamics of the example model in Figure 1c), transient species will become locally extinct unless local environmental conditions change, and disturbance-dependent species can only persist if new open interspaces or gaps are created.

Panel B. The weighted network usually contains information on the frequency of canopy–recruit interactions (number or density of recruits of one species under the other). The sum across rows informs on the structure of the sapling bank, while the sum along the columns informs on the contribution of canopy species to the sapling bank. Combined with information on species abundance it can be used to test observed frequencies against frequencies expected from a null model that assumes random assembly of interactions. For example, it can be used to test the existence of facilitation (comparing the expected frequency of recruitment in open vs under canopy species). Similarly, the frequency of each interaction can be tested against the frequency expected from the abundance of the canopy species to determine whether the canopy species enhances, depresses or has neutral effects on the recruitment of the recruit species. The predominance along the main diagonal of zeroes and depressing interactions can be used as an estimate of the prevalence of Janzen–Connell effects in the community.

(a) Adjacency (unweighted) matrix

(b) Frequency (weighted) matrix

Alcántara and Rey (2012) firstly showed that RNs also contain valuable information on the functional role that each species plays in community dynamics and coexistence using models of replacement dynamics; thence the term "replacement networks". The concept of replacement network derives from the concept of replacement dynamics used in most theoretical models of plant community dynamics (from Buffon to Tilman or Hubbell): when a plant dies, another of the same or different species takes its place. The mechanisms involved in this process drive the temporal changes in relative species abundance that we call "community dynamics". In the absence of a strong disturbance, replacement events are usually too slow to be recorded observationally; alternatively, we can assume that when a plant dies it will be replaced by some of the plants that recruited under its canopy. Thus, replacement networks are a function of RNs, what allows incorporating the information contained in the latter in theoretical models of plant community dynamics, as explained

in Figure 1c. For example, to build a replacement network we can transform recruitment frequencies to replacement probabilities for use in Markov Chain models (Horn, 1976; Siles, Rey, Alcántara, & Ramírez, 2008) or to recruitment densities for use in compartmental models (Alcántara, Rey, & Manzaneda, 2015).

Hereafter, for generality and simplicity, we will unify all these networks under the term RNs as inclusive of facilitation and replacement networks.

1.2 | What do we know about RNs?

The basic concepts in the field of ecological networks are already well established and in widespread use (Bascompte & Jordano, 2013). Nevertheless, each different use implies its own interpretations of network elements and descriptors, so we have synthesised the basic description and analysis of RNs in Box 1. In this section, we will not focus on network description but on what is most important about ecological networks: the inferences about community and interaction properties that can be obtained from their analysis (Poisot, Stouffer, & Kéfi, 2016).

Connectance (Box 1A) is the ratio of the realized number of interactions to the maximum potential number of interactions that could occur in the network. Most RNs studied to date show connectance below 30%. This implies that more than 70% of the potential interactions in a local community are unobserved interactions. In the communities studied by Verdú and Valiente-Banuet (2011), Marcilio-Silva et al. (2015) and Alcántara, Pulgar, Trøjelsgaard, Garrido, and Rey (2018), the frequency of canopy–recruit interactions was positively related with the abundance of the interacting species, so the prevalence of unobserved interactions could be a consequence of the low abundance of many species which may severely limit their chances of interacting (i.e., they are "neutral forbidden" interactions as defined by Canard et al., 2012). However, Alcántara et al. (2018) found that nearly 1/3 of the unobserved interactions were consistently unobserved even though the participating species were abundant enough to interact at least by chance, what indicates that an important part of the potential interactions are impeded for some ecological reason (i.e., they are "forbidden links" as defined by Olesen et al., 2011). Among the realized interactions, interspecific interactions had more frequently neutral or enhancing than depressing effects on recruitment, while intraspecific interactions had more frequently depressing effects (but some intraspecific enhancing effects were also found). Although the implications of these patterns for community dynamics are yet to be explored explicitly, they agree with two properties of ecological networks known to confer them stability: (a) that networks containing many weak and few strong interactions are more stable (McCann, Hastings, & Huxel, 1998; Wootton & Stouffer, 2016); and (b) that the coexistence of species that differ in fitness requires stronger intra- than interspecific limitation of population growth (Chesson, 2000). At least in the RNs analysed by Verdú and Valiente-Banuet (2011) and Alcántara, Garrido, and Rey (2019) there is evidence suggesting that the second property may actually be acting in these communities, creating

a community compensatory trend whereby more abundant species have lower rates of recruitment than rarer ones (Comita et al., 2007; Connell, Tracey, & Webb, 1984; Soliveres et al., 2015).

There can be multiple factors determining the frequency of individual canopy–recruit interactions (like seed dispersal vectors, seed predators, herbivores, pathogens, mycorrhizal fungi, symbiotic bacteria, competitors and pure chance). Indeed, this is the reason why the outcome of canopy–recruit interactions can be depressing, neutral or enhancing for recruitment, since it results from the balance between multiple positive and negative interactions. We have barely started to disentangle the relative contributions of these interactions. Besides species abundance effects, Verdú and Valiente-Banuet (2008, 2011) and Alcántara et al. (2018) found that the frequency of canopy–recruit interactions increases with the phylogenetic distance between species. This phylogenetic pattern could be expected if closely related species were functionally similar so that competitive exclusion would take place, or if third interactants, such as pathogens, affected closely related species more strongly. In contrast, distantly related species could be functionally different and would not only compete less strongly but could also help each other through complementary effects (Montesinos-Navarro et al., 2017).

For phylogeny to be used as a good proxy of interaction strength and RN structure, the key traits determining such interactions must be evolutionarily conserved, but this is not always the case. For example, Marcilio-Silva et al. (2015) found that the best model explaining an observed facilitation network in subtropical Brazilian forest–grasslands ecotones included species abundance but not phylogeny. Despite the relevance of plant traits determining plant–plant interactions (Losapio, de la Cruz, Escudero, Schmid, & Schöb, 2018; Soliveres et al., 2014), so far, no study has assessed the phenotypic structure of RNs to test to which extent the relevant plant traits structuring the networks are evolutionarily conserved. There has been some attempt to explain the frequency of recruitment in RNs based on the phenotype of the plants involved, showing that facilitation is stronger between species with contrasting communities of associated arbuscular mycorrhizal fungi (AMF) (Montesinos-Navarro et al., 2012). However, this relationship is not explained by the phylogenetic distance between plants, as closely related species do not necessarily have similar AMF communities, so it may be part of the species-specific effects.

Knowing how recruitment interactions are structured in the network also allows predicting its stability (Levine et al., 2017; Wootton & Stouffer, 2016). The structure of RNs confers plant communities with a high resistance to species loss and allows the long-term coexistence of many species. When a species disappears from a local community its interactions disappear too, which can affect other species and may unleash a cascade of secondary extinctions. Verdú and Valiente-Banuet (2008), Alcántara and Rey (2012) and Pulgar et al. (2017) have found that the structure of RNs can make plant communities very resistant to the removal of species, but the extinction of a few highly connected species could be fatal for a large number of other species (Valiente-Banuet & Verdú, 2013). These studies also found that RNs contain a core of highly interconnected species.

Alcántara et al. (2017) showed that this core is formed by a set of species interacting intransitively among them (i.e., A replaces B, B replaces C, C replaces A). Under replacement dynamics, this core would allow the long-term coexistence not only of the group of species involved, but also of their satellite species (Box 1), thus having a disproportionate effect on species richness and community stability.

2 | CONCEPTUAL OBSTACLES THAT MAY HAVE HINDERED RESEARCH ON RECRUITMENT NETWORKS

While the findings described in the previous section are very stimulating, the scarcity of studies published to date does not allow determining their generality, or whether they vary idiosyncratically or deterministically across communities and environmental gradients. During these years we have found that some of the basic concepts behind the RNs approach are frequently misunderstood, potentially handicapping the development of research in this field. In this section, we discuss some of these conceptual misunderstandings about RNs and show how moving the focus from facilitation to a more general approach to recruitment, with no a priori assumption regarding the outcome of plant–plant interactions, can help avoiding these problems.

2.1 | Focus on facilitation

Facilitation is thought to be the dominant plant–plant interaction in stressful environments while competition seems to predominate under milder conditions (Bertness & Callaway, 1994; Maestre, Callaway, Valladares, & Lortie, 2009; Rey et al., 2016). These patterns may have discouraged the study of FNs in plant communities from many temperate and tropical environments. However, as argued above, the recruitment of one species can be facilitated by some species, depressed by others, and unaffected by others in any community (Alcántara et al., 2018). Moreover, the predominance of one type of interaction in some ecosystems must not lead to uncritically assume that all recruitment associations between plants have the same outcome.

2.2 | Relevant organization level

One of the most frequent sources of confusion concerns the organization level at which the outcome of canopy–recruit interactions must be considered in network studies. Ecological interactions are frequently defined in terms of the effect that individuals of one species have on the performance (survival, growth, reproduction) of individuals of another species. Accordingly, plant–plant facilitation is commonly conceived as an interaction where one individual is benefited (the recruited plant) by another (the canopy plant), so experimental approaches measure recruit individuals' performance in the presence and in the absence of canopy plants (Callaway et al., 2002). The individual level is fundamental in the context of evolutionary studies of facilitation (Bronstein, 2009). But facilitation in the context of RNs belongs to the community level, so RNs represent

interactions between populations, not between individuals (Martorell & Freckleton, 2014). At this level, two species interact if one of them has an effect on the population dynamics of the second (Abrams, 1987). Thus, the presence of saplings of the recruit species under individuals of the canopy species is an indication that the canopy species is making a positive contribution to the sapling bank of the recruit population, even if individual saplings experience competition from the canopy plant (e.g., achieving lower growth rates).

2.3 | RNs are not classic co-occurrence networks

A third misconception that is important to clarify is the difference between the "classic" co-occurrence networks and RNs. Observational data for RNs are based on the co-occurrence of a plant (the recruit) growing underneath another (the canopy), but the network structure obtained focusing on canopy–recruit interactions can differ from the one obtained using "classic" co-occurrence methods (Delalandre & Montesinos-Navarro, 2018; Freilich, Wieters, Broitman, Marquet, & Navarrete, 2018). There are very important differences. Interactions in classic co-occurrence networks are inferred from the existence of statistically significant spatial covariation in the abundance (or presence) of two species across samples (for plant–plant co-occurrence networks see Saiz, Alados, & Pueyo, 2014; Saiz, Gómez-Gardeñes, Borda, & Maestre, 2018). One important consequence is that interaction matrices from co-occurrence networks are symmetric (undirected): if species A "interacts" (or, more properly, covaries spatially) with species B, then B necessarily "interacts" with A. Moreover, the matrix behind co-occurrence networks is signed because each link has an associated sign (positive or negative covariation). However, in RNs the existence of an interaction does not depend on the existence and power of statistical tests, only on the observation that there exists recruitment of one species under the other. Indeed, two species can covary negatively in space and nevertheless one may recruit under the other. Let's consider the example from Buffon in the epigraph of this work. At the adult stage (which is the focus of classic co-occurrence studies) a pioneer species (e.g., shrubs and bushes) and a shade-tolerant species (e.g., a tree) are likely to covary negatively in abundance, but individuals of the shade-tolerant species may recruit under individuals of the pioneer species. The classic co-occurrence approach applied to this example would conclude that the two species "interact" negatively, what is not true: their requirements for recruitment simply covary in opposite directions with the same environmental factor. Under the RN approach, we would say that the recruitment of the shade-tolerant species is not precluded by the pioneer species (it might even be enhanced), but the recruitment of the pioneer species can be impeded by the shade-tolerant species. In this way, interaction matrices from RNs are not necessarily symmetric: the fact that species A recruits under B does not necessarily imply that B recruits under A. Besides, the matrix behind RNs does not have an associated sign; it is non-negative because it can have only zero or positive entries (this makes RNs mathematically similar to matrices describing the flow of carbon or energy in food webs). These different properties are not merely semantic but have important implications for the descriptive study of the networks and for their use in theoretical models of community dynamics.

3 | METHODOLOGICAL OBSTACLES THAT MAY HAVE HINDERED RESEARCH ON RNS AND A STANDARDISATION PROTOCOL TO PREVENT THEM

Studies of plant–plant facilitation at the community level have used different methodological criteria to choose their nurse species. For example, while some studies surveyed whole plots considering that all plants can potentially contribute as nurses and recruits (Alcántara & Rey, 2012; Pulgar et al., 2017; Verdú & Valiente-Banuet, 2008), others used some randomisation procedure to choose individual plants to sample (Marcilio-Silva et al., 2015), and others sampled only under a predefined subset of species in their study site (Losapio & Schöb, 2017). These differences prevent proper comparisons of the network structures between studies, hindering the possibility of exploring the existence of geographical, temporal or environmental patterns in the structure of RNs. To solidly base the empirical study of RNs we need to set operational definitions of the fundamental components of the RN and a minimally standardised set of sampling procedures.

3.1 | Definition of the study case

The first fundamental component to consider is the study case, which is typically a local assemblage of plants present in a given spatial location. RNs represent which species recruit under which others in a local assemblage of a given plant community. Operationally, a community can be defined more or less loosely (e.g., "Mediterranean mixed forest of *Pinus halepensis* and *Quercus faginea*" or "Creosote bush scrub"), depending on the general objective of the study. In turn, a local assemblage of the community would be a group of plants of different species that co-occur in a spatially delimited area and that can be considered as a representative example of the community. A study site is a delimited area that contains a local assemblage of a given community.

There is no pre-determined spatial extent for a study site. The only requirement is that it contains an area (or areas) occupied by the local assemblage of the community large enough to allow an appropriate sampling effort (see below). On the other hand, local assemblages of the same community can be sampled in different study sites if these are sufficiently distant from each other so that seed flow between sites can be assumed negligible.

3.2 | Species to include in the study

The methods we propose are designed for studies that will focus on communities of woody species, and maybe including some long-lived perennial herbs large enough to act as canopy plants (e.g., tussock grasses). Within these types, only those species should be included for which the researcher is reasonably sure that most of the recruits present in the plots can be found and identified to species level. For example, the very tiny recruits of some species (e.g., many chamaephytes in forests) can make them hard to find and har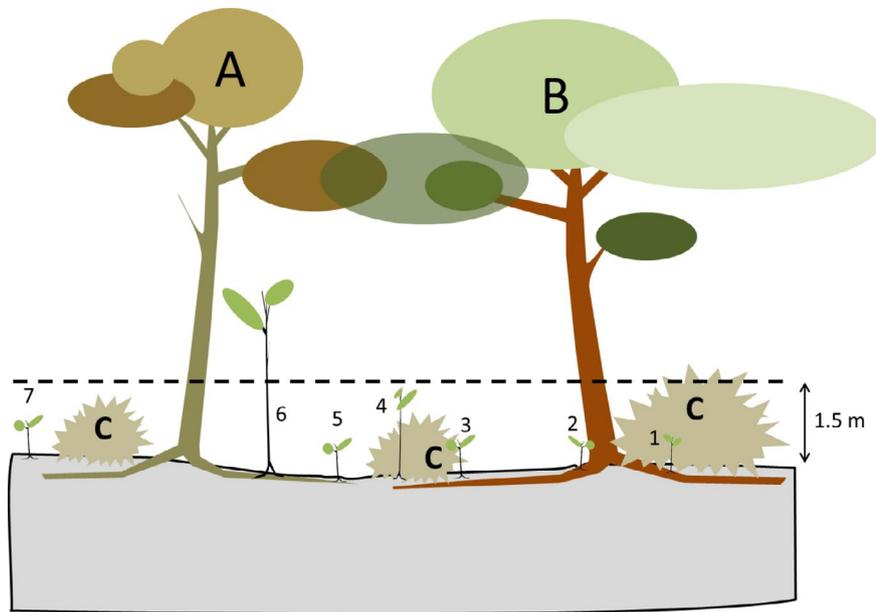d to identify to the species level. These species must not be included in the study because they could be erroneously classified as strongly recruitment-limited species that participate in very few interactions. It is important to recall that the nodes in the networks are species, so it is necessary to identify to the species level all canopy and recruit plants in the study.

3.3 | What is a recruit?

We will define a "recruit" as a >1-year-old plant, that has not reached the reproductive stage or has signs of having set a negligible number of flowers or fruits compared to the crop produced by a fully grown adult of the species. In general, the size of a recruit should be ≪¼ the size of a fully grown adult of the species (this size can be determined from published Floras or expert knowledge) but this can be highly dependent on species' life history and growth rate, so each researcher can tune this size-criteria to each species in the studied community. In the study of RNs only free-standing recruits must be considered, so care must be taken not to count vegetative sprouts as recruits in the case of species with sprouting ability. In case of doubt on whether an individual should be considered as a recruit, it is good practice annotating the reasons causing this doubt so one can decide later on whether to keep or exclude this individual from the dataset.

3.4 | Which is the canopy plant for a recruit?

This question involves also thinking about when we would expect that a recruiting individual is interacting with a given canopy individual. Interactions between sessile organisms are strongly dependent on the distance between them. Studies on the Janzen–Connell hypothesis have shown that the effect of canopy plants on conspecific recruiting plants decreases fast with distance from the trunk (Comita et al., 2014; Swamy et al., 2011). Similarly, sapling growth and survival are influenced by the density of neighbouring plants a few metres around (Condit, Hubbell, & Foster, 1994; Queenborough, Burslem, Garwood, & Valencia, 2007). But the distances relevant for an interaction in plants must also include underground and aboveground dimensions. Since each local assemblage can contain species of many different sizes (from small shrubs to large trees), it is not possible to use a common interaction distance. Instead, we propose two alternatives. For canopy plants with branches growing less than 1.5 m above the ground, their canopy will most likely contact physically, or have a strong direct influence on any plants recruiting beneath, so if a recruit is growing there, we will consider that it interacts with this canopy species (recruits 1, 3 and 4 in Figure 2). For a plant whose first branches grow above 1.5 m, we will consider that a recruit is interacting with it if it contacts the trunk or if it grows less than a distance d from the trunk (recruit 2 in Figure 2). In our studies of Mediterranean pine–oak forests we have used $d = 0.5$ m, but d can be adjusted to each species based on previous knowledge. This distance should be short enough so that it is reasonable to expect that the roots of the recruit should be contacting the roots of the

FIGURE 2 Standardization of what should be considered as canopy, recruit, open interspaces and gaps in RN sampling protocols. In this scheme, there are seven possible recruits growing in an assemblage formed by three species (A, B and C). The accompanying table explains the criteria used to determine the canopy assigned to each recruit [Colour figure can be viewed at wileyonlinelibrary.com]

Recruit	Canopy plant	Why?
1	C	Because C is the plant more directly conditioning the microenvironment of the recruit both above and belowground.
2	B	Because the sapling contacts the trunk, and likely also the roots, of the tree. We consider that this contact is highly probable up to 0.5 m from the tree trunk (this is an arbitrary cut point, so each researcher can tune this distance to each species according to her/his knowledge).
3	C	Because the sapling is contacting the branches of C.
4	C	As in 1.
5	Open Interspaces	Because it does not have a canopy plant less than 1.5 m above, and its distance from A or C suggests that the probability that it touches their roots is very small.
6	----	It is too large to be a considered a recruit. It could be considered a canopy plant.
7	Open gap	Because it has no plant parts above it at all, not even above the 1.5 m limit.

canopy plant, what is more likely the closer it grows to the canopy plant's trunk. These two criteria allow us to define open interspaces as areas far enough from canopy plants so that we can assume that a plant recruiting there will have a negligible interaction with any canopy plant. A recruit in an open interspace would be located more than d from the trunk of any plant and will have no canopy branches less than 1.5 m above it (recruit 5 in Figure 2). Finally, if the recruit is more than d from any trunk and there are not branches above it at any height (e.g., it grows in a gap or in a sparsely vegetated area), then we consider that it is growing in an open gap (recruit 7 in Figure 2). The need to define open interspaces responds to the presence of recruits far from established plants but under the cover of canopies located very high above the recruit. When this happens, the recruit most often can grow to reach maturity without having to replace any of the established plants, so it is better considered as a recruit

that might eventually replace an open space than any canopy plant in particular. Thus, from the perspective of replacement dynamics, recruits in open interspaces and those in open gaps play the same role, so we can assign a single node to open in the RN. Nevertheless, keeping track of whether a recruit is in an open interspace or in an open gap is important to allow investigation of recruitment niches. The cut values of 1.5 m above ground and $d = 0.5$ m are largely arbitrary, but they are necessary in order to standardize protocols, just like tree size is commonly, and arbitrarily, measured as diameter at breast height (dbh).

3.5 | Sampling design

To obtain the information to build a RN it is better to distribute a series of plots within the study site rather than using a single large plot.

Initially, the number, size, and shape of the plots must be determined by the researcher based on experience and the particularities of the study site. It is important to keep in mind that the objective is detecting all the interactions between all the possible pairs of species present in the local assemblage, so sampling effort must be higher than what would be necessary to estimate only species richness. From our results in Mediterranean pine–oak forests, we recommend sampling at least 20 plots of 500 m^2 per site, so that the total area sampled would be 1 ha. However, this obviously depends on the type of plant community studied, so the best way to decide when the number of plots sampled is good enough is through species and link accumulation curves (Pulgar et al., 2017). That is the main reason why we recommend surveying many small plots rather than a few larger, because data from multiple plots allows constructing accumulation curves for network properties.

For each plot, we need a matrix with the number of recruits of each species under each canopy species and in open interspaces/gaps, and the relative abundance (cover within 1.5 m above the ground) of each canopy species and of open interspaces/gaps. The entire ground surface in each plot must be systematically "scanned" searching for all recruiting plants; in this way, each canopy species is sampled proportionally to its abundance. Both the number of recruits and the abundance of each canopy species and open interspaces/gaps within 1.5 m above the ground in the plot must be estimated through some standard procedure (for example the line-intercept method, or visual estimates through the whole plot). This information allows using null models or conducting tests of observed versus expected frequencies of interactions based on species cover.

4 | CONCLUDING REMARKS

The ability of the RN approach to synthesize information relevant to plant community dynamics makes it a very promising tool to incorporate in the field of plant community ecology. Having a unified conceptual structure and a common sampling procedure may help to construct a worldwide public database of RNs (http://elabs.ebd.csic.es/web/213936) that would provide valuable information in the search for general patterns on species coexistence, community stability, competition–facilitation balance, phylogenetic predilections, evolutionary persistence of lineages, recruitment niche conservatism, and environmental correlates of network properties with different drivers of global change. The protocol we propose is flexible enough to allow sampling RNs in many different ecosystems. We hope this paper will pave the way to incorporate plant communities into the growing field of ecological networks, ultimately allowing a more complete understanding of how plant–plant interactions contribute to the functioning and conservation of ecological communities.

AUTHOR CONTRIBUTIONS

JMA and MV wrote the first draft and all the authors contributed to the final version.

ORCID

Julio M. Alcántara https://orcid.org/0000-0002-8003-7844

José L. Garrido https://orcid.org/0000-0002-6859-4234

Alicia Montesinos-Navarro https://orcid.org/0000-0003-4656-0321

Pedro J. Rey https://orcid.org/0000-0001-5550-0393

Alfonso Valiente-Banuet https://orcid.org/0000-0002-7533-6671

Miguel Verdú https://orcid.org/0000-0002-9778-7692

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