Can eDNA be an indicator of tree groundwater use? A perspective

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Abstract. A major challenge of managing groundwater-dependent ecosystems is determining when and where plants are accessing and using groundwater. Addressing this knowledge gap is particularly pertinent where remnant stands of old growth trees reside within areas where groundwater is being used at an unsustainable rate. The aim of this paper is to investigate what it means to find tree DNA in the groundwater and provide a perspective on whether the detection of tree DNA in groundwater could provide an indicator of groundwater use by trees. This idea arose from recent DNA-based surveys that routinely detected tree DNA in groundwater samples, which may be unexpected given the general absence of plants in dark, subsurface environments. We discuss the likely sources and fate of tree DNA in groundwater and the knowledge needed to progress the development of tree DNA as a robust indicator. If successful, such an indicator would help managers better understand the water requirements of groundwater-dependent vegetation, meet legislative obligations for monitoring and assessment, and improve the conservation and management of groundwater-dependent ecosystems.

Introduction

The development of next-generation sequencing and its application to environmental monitoring and assessment over the past decade has increased exponentially the range of biota that can be routinely identified. In many studies, researchers collect data on the presence of taxa that are extraneous to the research or monitoring questions. In some cases, these non-target taxa will be those that, although inhabiting the ecosystem, are not of direct interest, or may be taxa that occur outside the ecosystem and may be accidental, invasive or contaminants (Chariton *et al.* 2015). So, what can we learn from unexpected or 'non-target' taxa in environmental DNA (eDNA) samples?

Environmental-DNA metabarcoding (i.e. the identification of multiple species from a single environmental sample (e.g. soil and water) containing complex and degraded DNA) has become a popular tool for examining biodiversity, because it is cost effective, non-invasive, and has high detection probabilities for low-abundance taxa (Taberlet *et al.* 2012; Bohmann *et al.* 2014; Ruppert *et al.* 2019). Traditional field methods for surveying richness and abundance, such as mark–recapture and transect surveys, are often affected by disturbance, errors in taxonomic identification and destruction of habitat (Deiner *et al.* 2017; Ruppert *et al.* 2019). eDNA can be used to complement traditional sampling because it has the ability to detect the presence of biota through DNA shed in the environment, which provides a method for sampling difficult-to-access habitats (e.g. subterranean and deep-sea environment). Analysing eDNA also increases

the potential of detecting rare and cryptic species as well as increasing taxonomic resolution and accuracy (Jane *et al.* 2015; Deiner *et al.* 2017; Taberlet *et al.* 2018; Ruppert *et al.* 2019; Nørgaard *et al.* 2021). In addition to characterising the structures of communities, eDNA has a wide range of other applications, and can be used for examining population dynamics, such as detecting non-indigenous species, and for wildlife DNA forensics, monitoring ecosystem health, and functional profiling (Dejean *et al.* 2011; Díaz-Ferguson and Moyer 2014; Chariton *et al.* 2015; Goldberg *et al.* 2015; Barnes and Turner 2016; Pawlowski *et al.* 2018; Coble *et al.* 2019; Beng and Corlett 2020).

Despite the technological advances and increases in the use of eDNA for species detection and conservation, many challenges and limitations remain (Stoeckle *et al.* 2017). These may arise during field sampling, laboratory processing and the interpretation and analysis of results (Thomsen and Willerslev 2015; Zinger *et al.* 2016, 2019; Trebitz *et al.* 2017). Specifically, the limitations can include the use of inappropriate bioinformatic pathways, limited reference databases for taxonomic groups, suboptimal primers and polymerase chain reaction (PCR) conditions, contamination from in the field or during laboratory processing and difficulty in estimating longevity of DNA and its persistence in environments (see sections below; Thomsen and Willerslev 2015; Trebitz *et al.* 2017; Ruppert *et al.* 2019; Coble *et al.* 2019; Saccò *et al.* 2022).

Recent surveys of shallow alluvial aquifers at 122 sites across arid and semi-arid western New South Wales (NSW), Australia,

targeting the V7 region of the *18S* rDNA gene have identified diverse eukaryote assemblages in the groundwater (Korbel *et al.* 2017; Nelson 2020; Pollitt 2020). Biota targeted in these studies included invertebrates and fungi, which are key components of groundwater ecosystems. However, as with many eDNA studies using 'universal' primers (i.e. those that amplify very common conserved gene regions that capture a wide range of taxa), samples contained a complex mixture of possibly degraded DNA from various organisms in the ecosystem (Taberlet *et al.* 2018). These samples included several non-target species, i.e. taxa that are not of interest, which are generally removed from the dataset before processing. Of particular interest was the number of plant species detected in these groundwater samples, which may be unexpected given that functioning photosynthetic organisms are typically absent in the dark subsurface environment (Humphreys 2006). However, for numerous plant species, access to groundwater is critical to meet some or all life-cycle stages (Eamus *et al.* 2006) and, consequently, it is reasonable that the DNA from such species is detected in groundwater.

Knowing when and where plants are accessing and using groundwater is key to managing and conserving terrestrial groundwater-dependent ecosystems (GDEs), particularly in the face of increasing competition for groundwater resources in a changing climate (Eamus *et al.* 2006). This is a critical and urgent challenge because groundwater overuse is a major global problem (Wada *et al.* 2010). In Australia, groundwater accounts for one third of the water used and demand is increasing (Harrington and Cook 2014). In arid and semi-arid regions of Australia, groundwater is often the only reliable source of water and the allocation of water to meet agricultural, environmental, industrial and societal needs is a constant point of contention (Leblanc *et al.* 2012).

The Murray–Darling Basin (MDB) is the primary region of agricultural production in Australia. It provides over 50% of the nation's agricultural produce, while using over 70% of Australia's total water usage (Australian Bureau of Statistics 2019). Much of the MDB has been cleared of native trees to accommodate agriculture (Walker *et al.* 1993), and trees that remain may be under stress and populations of some species are in decline (Ngugi *et al.* 2021). Given the immense value of this region, natural resources, such as vegetation and water, need to be carefully monitored. However, the water requirements of the natural environment are often secondary to agricultural and industrial needs (Murray–Darling Basin Authority 2017). The picture in the MDB reflects a global trend, with GDEs being increasingly threatened by groundwater abstraction and declining water tables (Brown *et al.* 2010; Hoogland *et al.* 2010; Tomlinson and Boulton 2010; Eamus *et al.* 2015; Pérez Hoyos *et al.* 2016; van Engelenburg *et al.* 2018; Erostate *et al.* 2020). Tools that provide cost-effective and rapid indication of groundwater dependence are needed globally.

Traditional approaches to determining tree groundwater use include stable isotope analysis of tree and groundwater, sap-flow analysis, and remote sensing, which largely rely on the inference between plant attributes (water flow, greenness) and water use and require specialised equipment or analytical capabilities (Eamus *et al.* 2006; Cook and Eamus 2018; Cleverly *et al.* 2020). In comparison to these traditional measurements, eDNA potentially provides a real indicator of plant interactions

with groundwater. The aim of this discourse is to consider *what does it mean to find tree DNA in the groundwater*, and can this be an indicator that trees are accessing groundwater?

Potential sources of plant DNA in groundwater

Plant DNA detected in samples of groundwater could be derived from (1) contamination during collection as groundwater is drawn from a well or spring, (2) DNA leached from surficial plant material through the soil to the groundwater or (3) DNA released from tree roots that are accessing the groundwater and thus submerged in the groundwater (Fig. 1; Groom *et al.* 2000; Zencich *et al.* 2002; Poté *et al.* 2003, 2009*a*; Bravo *et al.* 2010; Froend and Sommer 2010). It is important to determine the source of DNA in groundwater if we are to indicate plant–groundwater dependency. Typically, non-woody plants rely on soil moisture unless the groundwater is near to the surface, and it is only woody plants (tree species) with extensive root systems that are able to access groundwater that is several metres or more below the surface (Eamus *et al.* 2015). It is the detection of such trees that provides potential for the indication of groundwater dependency.

A contaminant or the real deal?

There are many challenges that arise from analysing the large datasets created from DNA sequencing. One such challenge is interpreting whether DNA is truly originating from the sample or is potentially reflective of contamination. The presence of 'unexpected' DNA in any sample is a possible indicator of contamination and managing and avoiding contamination is important at all stages of the eDNA analysis pipeline (Rees *et al.* 2015; Ruppert *et al.* 2019).

In the case of tree DNA in groundwater, contamination is a possible source, because plant material (particularly pollen) may be blown into sampling containers during sample collection or during downstream processing, but this can be managed by meticulous attention to sampling protocols (Gulden *et al.* 2005; Rosi-Marshall *et al.* 2007; Thomsen and Willerslev 2015). Furthermore, field-derived contamination *should* be infrequent, and regular detection of unexpected taxa can provide evidence to support it being a true occurrence. Sampling protocols such as purging bores before collecting samples can remove the likelihood of localised contamination from within the bore hole (Korbel *et al.* 2017). In the case of vegetation, contamination from sources such as pollen may be seasonal, corresponding with flowering periods or weather conditions at the time of sampling that might increase the likelihood of airborne contamination. Sampling bores on multiple occasions during different seasons may help account for such contamination and would provide further support and greater confidence that the species detection is 'real' (de Vet *et al.* 2009; Ceccherini *et al.* 2009; Zinger *et al.* 2016).

During laboratory and bioinformatic processes, contamination and misidentification may result in the identification of 'unexpected' DNA; however, this can be mitigated through sterile laboratory conditions and the separation of pre- and post-PCR products (Goldberg *et al.* 2016; Furlan *et al.* 2020). Amplicon sequencing errors, poor taxonomic resolution, poor taxonomic match and reference database errors may also
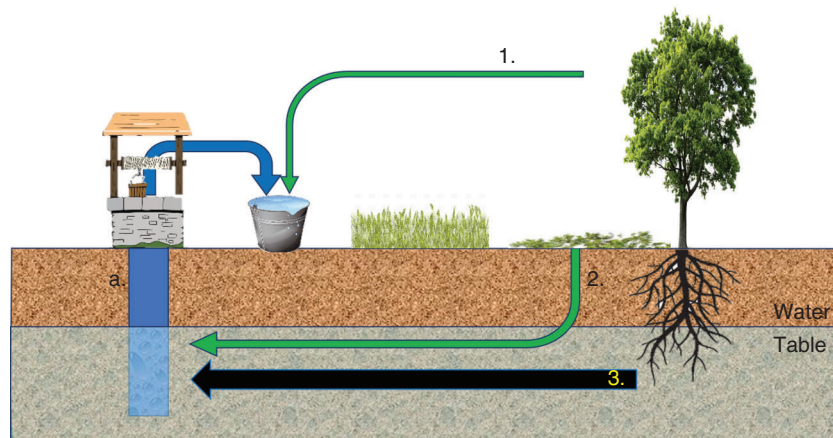
Fig. 1. Sources and pathways of plant DNA in groundwater samples. Groundwater drawn from a well (a) may contain plant DNA that is derived from (1) a contaminant from surface vegetation (such as pollen) during the sampling process, (2) DNA of species that leaches through the soil profile to reach groundwater, or (3) the roots of deep-rooted tree species that are accessing groundwater.

provide contamination, errors and the misidentification of DNA (Coissac *et al.* 2012; Ficetola *et al.* 2015; Furlan *et al.* 2020). However, these can be attenuated during library preparation by various bioinformatic approaches, as well as by filtering data with the aid of positive and negative controls (Alsos *et al.* 2018; Harper *et al.* 2019; Carøe and Bohmann 2020; Furlan *et al.* 2020; Gillmore *et al.* 2021).

Leaching of surface material

The accumulation of plant material on the soil surface, and the subsequent breakdown and leaching of that material through the soil profile to the water table, is also a potential source of plant-derived eDNA in groundwater (Fig. 1). In leaf litter, DNA can degrade rapidly, with persistence in soil likely to be affected by both the species present and water saturation, with terrestrial plant-derived eDNA degrading as quickly as in 2 days (Poté *et al.* 2005), or persisting for up to 1 year (Gulden *et al.* 2005; Meier and Wackernagel, 2003). The fate of eDNA transported to groundwater as a leachate is less well known.

Leaching of DNA through the soil profile has been demonstrated for several plant species (e.g. Poté *et al.* 2007, 2009*a*, 2009*b*). The mobilisation of extracellular DNA or its associated leaf material is then dependent on soil hydraulics, including the rate of water flow through the soil, grain size and porosity, and the mineral and organic composition of the soil (Gulden *et al.* 2005), and also depends on the size (and hence, state of degradation) of the eDNA fragment itself (Turner *et al.* 2014; Barnes and Turner 2016).

The process of leaching, including adsorption of DNA to soil and organic matter and microbial breakdown, means that DNA fragments reaching groundwater will be degraded. Gulden *et al.* (2005) suggested that the degradation of DNA in leachate water is most likely to be enzymatic, with a half-life between 2 and 30 h for corn and soybean plants. However, degradation rates are strongly and positively correlated with temperature, water chemistry and fragment length (Jo and Minamoto 2021; Saito and Doi 2021*a*). The extent of degradation also depends on retention time in the soil, such that groundwater depth is likely to influence the extent of degradation. Groundwater closer to surface or within the topsoil horizon is more likely to contain less degraded DNA than is deeper groundwater, which is reflected in the concentrations of DNA in groundwater often decreasing with depth (Nielsen *et al.* 2007; Eamus 2009).

Degradation of plant-derived eDNA in the soil profile may potentially be correlated with a reduction in the fragment length. If so, this suggests that fragment length of plant DNA retrieved in groundwater samples could be used to infer the DNA source as leached (if short) or being derived from plants accessing vegetation (if long). Overall, the contributions of DNA from surface leaching may be small when considering the vertical soil-leaching pathway and the potential for rapid microbial and chemical degradation of the DNA in that process (Poté *et al.* 2003, 2009*a*; Gulden *et al.* 2005).

DNA from deep plant roots

The most likely source of tree DNA in groundwater is from tree roots that have grown deeper than the water table. In this case, release of root cells or lysis of root material can release plant DNA (either intra- or extracellular) directly into the groundwater. Deep-rooted tree species in this region are dominated by the Myrtaceae and Mimosaceae, including *Eucalyptus camdulensis*, *E. largiflorens*, *E. coolabah*, *Acacia stenophylla*. Other common tree species include *Callitris endlicheri* (Cupressaceae) and *Casuarina cunninghamiana* (Casuarinaceae). *Eucalyptus* is by far the most abundant genus and those trees are known for their fast and deep root growth (Benson 2008; Christina *et al.* 2017), leading to an average rooting depth of ∼10 m, and up to 60 m for some species (Stone and Kalisz 1991). If we assume that trees with roots in groundwater release DNA to that environment, then there should be a relationship between the groundwater depth, tree root depth and the detection of tree DNA. Therefore, the detection of tree DNA should identify sites where the tree root zone is within the groundwater table (Eamus *et al.* 2015).

Fate and persistence of DNA in groundwater

Understanding the longevity, fate and transportation of tree-derived DNA is key to being able to use the presence of that DNA as an indicator of groundwater use. Several factors, including water quality, temperature and UV radiation, are important factors influencing DNA persistence (Zhu 2006; Barnes *et al.* 2014; Strickler *et al.* 2015) as is the length of DNA fragment (Deagle *et al.* 2006). Temperature is considered one of the most critical variables affecting DNA detection and longevity within aquatic environments (Strickler *et al.* 2015), with DNA being markedly more persistent in cold climates (Zhu 2006; Barnes *et al.* 2014). Additionally, salinity and pH affect DNA degradation; DNA longevity is greatest in neutral and slightly alkaline environments (Strickler *et al.* 2015), whereas high salinity increases DNA degradation (Barnes *et al.* 2014; Collins *et al.* 2018; Saito and Doi 2021*b*). Fragment length also appears important for DNA degradation, with shorter fragments usually slower to degrade (Deagle *et al.* 2006). For example, fragments of 300–400 bp persisted in water for up to 1 week in controlled environments (Dejean *et al.* 2011), with smaller fragments detected for up to 1 month (Zhu 2006). However, microbial enzymatic degradation of the DNA fragments is a key mechanism. The breakdown of DNA under sterile conditions or environments with low microbial activity is much slower than under natural conditions in surface waters where the half-life may be as low as 2 h (Matsui *et al.* 2001; Gulden *et al.* 2005; Zhu 2006; Jo and Minamoto 2021; Saito and Doi 2021*a*). Fortunately, the low microbial biomass and activity expected in groundwater (see Griebler and Lueders 2009) suggest that DNA may be more persistent in groundwater than in surface waters.

The degradation of DNA has been shown to be slow in groundwater (Poté *et al.* 2003, 2007, 2009*a*). This may be due to the stability of groundwater ecosystems in terms of temperature and water quality (Humphreys, 2006), lack of UV-induced DNA degradation, and low microbial biomass and activity (Griebler and Lueders 2009). All of these factors potentially increase DNA persistence when compared with surface environments, although studies in groundwater have been limited (e.g. Zhu 2006) and have detected plasmid DNA in groundwater microcosms with average durations of 48–96 h. Preliminary evidence from cave environments suggest DNA persistence for over 1 month (Boulton *et al.*, in press).

DNA longevity in aquatic environments has mainly been studied using animal species in surface waters. Such studies have indicated that species and life stage affect DNA fragment length, longevity and detectability in contemporary environmental DNA samples, ranging from days to weeks (Dejean *et al.* 2011; Thomsen *et al.* 2012*a*, 2012*b*; Barnes *et al.* 2014). However, there have been fewer studies investigating detection and persistence of plant DNA in aquatic environments (Anglès d'Auriac *et al.* 2019), with Bravo *et al.* (2010) indicating that the half-life of plant DNA in aquatic sediments was more than 1 day, whereas DNA of plant pathogens in soils is detectable for over 1 year (Kunadiya *et al.* 2021). The soil and sediment matrix can influence the preservation and absorption of DNA, with the clay and sand content being shown to have a positive effect on the absorption and protection of the DNA from nuclease degradation (Poté *et al.* 2003; Gulden *et al.* 2005). However, large

knowledge gaps remain in the transport, longevity and degradation rates of DNA in groundwaters (Dale *et al.* 2002), with no studies having been conducted on plant DNA within these environments.

Knowledge on DNA transportation and retention within groundwaters is important if DNA from plants is to be used as an indicator. In surface waters, DNA can be detected kilometres from its origin (Barnes and Turner 2016; Wacker *et al.* 2019), although the concentration of the detected DNA decreases with distance (Deiner and Altermatt 2014). This is of benefit in detecting vegetation within groundwater because opportunities to access and collect groundwater are limited to caves, wells and springs, which are often not located immediately adjacent to the sites or vegetation of interest (Larned 2012). Like streams and rivers, groundwaters flow (albeit slowly) such that the water collected at a site reflects its interactions with the environment and biota in areas higher up the hydraulic gradient (i.e. 'upstream'). Consequently, detection of tree-derived eDNA in groundwater requires that the DNA persist in the environment for a sufficient period for it to be transported to the point of collection.

Groundwater flow rates (i.e. hydraulic conductivity), at least in shallow alluvium of the MDB, range from 10^{-4} to 10^2 m day^{-1} (Bioregional Assessment Programme 2016). Given that DNA may remain detectable in aquatic environments for up to several days, DNA released from trees directly into groundwater may be detected in groundwater abstracted from a bore or well within a radius of up to several hundred metres down the hydraulic gradient (i.e. 'downstream') from the tree, but this will depend heavily on the hydrogeology of the aquifer (Jane *et al.* 2015; Pang *et al.* 2020).

What do we need to know to make this a useful indicator?

For eDNA to be an effective and reliable method for inferring plant groundwater use, the detection of tree DNA in the groundwater, when present, needs to be consistent, thus avoiding false negative results. From the 122 sites sampled across western NSW (Pollitt 2020) that were sampled more than once, 63 had plant DNA detected in at least one, but not all samples, which suggests a large amount of heterogeneity in the detection of plant DNA. In part, this heterogeneity of detection may be an issue with primer specificity and variation in primer affinity. Because the DNA detected in the groundwater is likely to be degraded, the use of large generic primers that are not designed to specifically target plants (such as the V7 *18S* rDNA eukaryote primer) may limit the detection of possibly degraded, much shorter DNA fragments and may also influence the taxonomic resolution in the output (Taberlet *et al.* 1991; Hadziavdic *et al.* 2014). The use of a short highly conserved gene region for plants may help limit some of the heterogeneity detected. The *trn*L (UAA) intron, and more specifically the P6 loop of the *trn*L intron, and the second internal transcribed spacer (*ITS2*) were identified as good target regions for degraded plant DNA because they are variable, highly conserved and short (Taberlet *et al.* 2007, 2018; Hollingsworth *et al.* 2011; Moorhouse-Gann *et al.* 2018). Because no single barcode is without drawbacks or fits the needs perfectly, future analyses should consider using multiple coding regions with additional markers or barcodes (Hollingsworth *et al.* 2011). In conjunction with using multiple coding regions, a combination of primers that target short,

degraded DNA and longer regions may be beneficial to capture as much of the heterogeneity as possible (Ruppert *et al.* 2019).

Polymerase chain reaction bias may also contribute to the heterogeneity of the species detected in eDNA samples, in which certain sequences may or may not be amplified. Like other detection technologies, eDNA is not without its imperfections and is susceptible to Type I (false positive) and Type II (false negative) errors (Ficetola *et al.* 2015; Evans *et al.* 2017; Lopes *et al.* 2020). False positives can result from (1) contamination, (2) incorrect detection of nontarget species, or (3) detection of DNA from dead organisms in the ecosystem (Darling and Mahon 2011; Rees *et al.* 2015; Evans *et al.* 2017). False negatives occur when taxa are present in the ecosystems but not detected and can result from (1) failure to collect DNA in the sample, (2) insufficient assay sensitivity, (3) PCR dropout or (4) lack of viable or degraded DNA (Darling and Mahon 2011; Rees *et al.* 2015; Evans *et al.* 2017). The risk of false negatives may be increased in groundwater samples because of the likely degraded state of the DNA (Ficetola *et al.* 2015). Increasing sample volumes and replication of each stage of analysis (i.e. sample collection, extractions per sample, and amplifications per extraction) may moderate this risk (Darling and Mahon 2011; Rees *et al.* 2015; Evans *et al.* 2017). Ficetola *et al.* (2015) suggested the following three steps to reduce the risk of bias and improve the robustness and reliability: run occupancy models to estimate detection probability, false negatives and true occupancy, evaluate current methods for controlling false negatives and remove 'uncertain presences'. The characterisation of DNA collected, including concentration and fragment-size distribution, can begin to shed light on several of these issues.

A better understanding of DNA longevity in soils and groundwater ecosystems will help improve our knowledge and understanding of the origin and fate of eDNA in groundwater and the likelihood of its detection over time (Gulden *et al.* 2005). Further analysis of whether DNA is present in sediments (where it is typically more stable; Poté *et al.* 2003; Gulden *et al.* 2005), or in solution, is needed. The expected short-term persistence of tree-derived eDNA in groundwater means that observations will likely reflect recent conditions, and reduces the likelihood of detecting 'zombie' DNA (i.e. DNA from dead, rather than living individuals), but further confirmation is required (Baird and Hajibabaei 2012). The use of RNA for detection of recent biotic activity may also be of benefit. Because RNA is produced only by living organisms and degrades quickly (Chimento *et al.* 2012; Laroche *et al.* 2017; von Ammon *et al.* 2019; Wood *et al.* 2020), detection of RNA could be used as a reliable marker of plant presence and use of groundwater; however, it is more costly, and difficult to sample and preserve in remote locations.

The limited understanding of local hydrogeology at most sampling locations complicates the interpretation of eDNA detections. Groundwater flow directions are difficult to determine at a local scale and often require interpretation of water levels across a region to determine hydraulic gradients. Often such water-level data are difficult to obtain, and groundwater flows cannot be determined from observations of the site or surface features. Knowledge of groundwater flow paths is critical for determining whether trees are upstream or downstream, and, thus, whether eDNA is likely (or not) to be detected in the groundwater. Even when groundwater flow direction is known, aquifer matrix properties such as porosity and dispersivity determine how eDNA maybe dispersed within the aquifer (e.g. the shape and location of the eDNA 'plume' downstream of the tree) and whether this intersects with observation points (bores and wells).

Before eDNA can be used as an indicator of plant groundwater use, two key steps remain. First, the reliability of detecting tree DNA in groundwater samples needs to be improved. Through the testing and use of plant-specific primers and the characterisation of detected DNA by analysing the concentration and fragment sizes may help improve issues with PCR bias and heterogeneity. A greater understanding of the transport, longevity and persistence of DNA in groundwater environments will also help improve the reliability of eDNA as an indicator. The use of methods such as DNA spiking may help reduce this knowledge gap. Once the reliability of detecting tree-derived DNA in groundwater samples is confirmed, a critical step remains. That is, linking tree DNA detection to groundwater use by the tree. This step will require linking eDNA analysis with traditional measurements of vegetation groundwater use (see Eamus *et al.* 2006), such as measurements of sap flow and transpiration and analysis of water isotopes to validate the approach. Once tested and validated against traditional approaches for assessing tree groundwater use, the use of eDNA would enable rapid and broad-scale assessments of tree water use. Indeed, rapid test kits or mobile PCR systems (e.g. Doi *et al.* 2021) may enable on-site, real-time analyses.

If eDNA can indicate that trees are accessing groundwater, it will provide a new tool for identifying groundwater-dependent vegetation. Further, by confirming that tree DNA in groundwater is sourced from roots means that it can infer the minimum tree root depth, which cannot be determined simply by other means. Targeted testing of eDNA in groundwater as water tables rise and fall (and move in and out of the root zone), or as plants flower or exhibit signs of stress, will also provide detailed knowledge of the timing of groundwater dependence of the vegetation, and identify periods when groundwater resources and levels must be maintained or have reached critical levels. With such knowledge, groundwater abstraction can be more sustainably managed to meet the needs of water users and the environment. Although limitations and challenges still exist, through increased understanding of the longevity and movement of DNA through the groundwater and site-scale hydrology and groundwater flow, eDNA could provide a new insight into tree groundwater use.

Data availability

Data sharing is not applicable as no new data were generated or analysed during this study

Conflicts of interest

Anthony Chariton is an editor for *Marine and Freshwater Research* but did not at any stage have editor-level access to this manuscript while in peer review, as is the standard practice when handling manuscripts submitted by an editor to this journal. *Marine and Freshwater Research* encourages its editors to publish in the journal and they are kept totally separate from the

decision-making processes for their manuscripts. Jodie Dabovic is an employee of the funding organisation, but this relationship had no influence on the outcomes of the work reported in this paper. The authors have no further conflicts of interest to declare.

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