Author/s:
Lukinykh, M;Lyell, C;Jayasekara, C;Lane, P;Sheridan, G

Title:
Moisture and Species Effect on Water Colour Generation in an Australian Temperate Forested Catchment

Date:
2025-09-01

Citation:
Lukinykh, M., Lyell, C., Jayasekara, C., Lane, P. & Sheridan, G. (2025). Moisture and Species Effect on Water Colour Generation in an Australian Temperate Forested Catchment. Ecohydrology, 18 (6), https://doi.org/10.1002/eco.70106.

Persistent Link:
https://hdl.handle.net/11343/362670

*Ecohydrology*

WILEY

RESEARCH ARTICLE OPEN ACCESS

# Moisture and Species Effect on Water Colour Generation in an Australian Temperate Forested Catchment

Mariia Lukinykh | Christopher Sean Lyell | Charuni Jayasekara | Patrick Lane | Gary Sheridan

School of Agriculture, Food and Ecosystem Sciences (SAFES), Faculty of Science, The University of Melbourne, Parkville, Victoria, Australia

**Correspondence:** Mariia Lukinykh (m.lukinykh@unimelb.edu.au)

**Received:** 23 May 2025 | **Revised:** 31 July 2025 | **Accepted:** 21 August 2025

**Funding:** This research was funded by the Melbourne Water Corporation–funded research project 'Securing Melbourne's Water Quantity and Quality in a Changing Climate' (TA37690) and by the Victorian Government, Department of Energy, Environment and Climate Action (DEECA) as part of the Integrated Forest Ecosystem Research (IFER) program (TA301102).

**Keywords:** *Acacia* | catchment | decomposition | *Eucalyptus* | laboratory leaching experiment | true colour | water discolouration

## ABSTRACT

South-eastern Australian forested catchments supply most of Melbourne's drinking water, and discoloured water poses significant challenges for authorities because of the need for complex and costly treatment procedures. Identifying the sources and mechanisms of colour production is therefore critical. We investigated the role of leaf litter from five common catchment tree species in generating water colour under three moisture conditions (Saturated, Moist and Air-dry) by conducting a laboratory leaching experiment. Results showed that leaf litter under Moist conditions (2871.2 ± 383.24 PCU) produced significantly higher cumulative colour compared with Saturated (496 ± 98.34 PCU) and Air-dry (452.2 ± 62.67 PCU) conditions. This is likely due to optimal microbial decomposition under Moist conditions, where both oxygen and water availability are sufficient. In contrast, Saturated samples exhibited a faster initial rate of colour production that peaked earlier (Week 1) than Moist (Week 2) and Air-dry (Week 4) samples, likely driven by a combination of leaching and decomposition processes. In Moist and Saturated samples, decomposition was strongly correlated with the colour generation process. When species effects are considered, *Eucalyptus radiata* had the highest cumulative colour production (1711.33 ± 892.61 PCU), whereas *Eucalyptus regnans* had the lowest (730 ± 320.44 PCU). Our findings highlight the critical role of litter moisture in driving colour generation in drinking water catchments. Future research should focus on understanding how changes in rainfall patterns and subsequent litter moisture levels may influence colour production. Such insights could inform management strategies to mitigate water discolouration and reduce treatment costs.

## 1 | Introduction

Discoloured surface waters are a common issue faced by catchment management authorities around the world. Depending on the type of light-absorbing material present in water, colour can be referred to as 'true colour', which is caused by dissolved components only, and 'apparent colour', which is caused by both dissolved and suspended components (Zobkov and Zobkova 2020). Although increased water colour can be caused by a range of anthropogenic activities, including industrial waste discharge and agricultural runoff, fresh waters may exhibit a yellow, brown or even black true colour as a result of natural processes. Natural water discolouration may partially occur because of excessive algal growth and dissolved metals like iron and manganese; however, high true water colour is believed to be largely caused by coloured dissolved organic matter (CDOM) commonly referred to as 'gilvin', 'gelbstoff' or 'yellow substance' (Kirk 1976; Luo et al. 2020; Qiu et al. 2005). These coloured organic compounds, primarily polyphenols like tannins and humic substances such as humic and fulvic acids, are leached into the rivers and lakes from soils, aquatic vegetation and

https://doi.org/10.1002/eco.70106

decomposing terrestrial vegetation, particularly from litter, bark and tree roots (Christman and Ghassemi 1966; Cuassolo et al. 2021; Das et al. 2020; Mitchell 1990; Novak et al. 1975; Yang et al. 2019; Zobkov and Zobkova 2020).

High concentrations of dissolved organic carbon (DOC) that tint the water may have significant implications for water quality and stream ecology. Intense water colour is caused by the DOC compounds absorbing light in the UV and short wavelength visible regions of the spectrum, which limits light and heat penetration (Novak et al. 1975; Solomon et al. 2015). This, in turn, negatively affects autotrophic productivity and predator–prey interactions that are dependent on light availability in freshwater ecosystems (Solomon et al. 2015). However, as DOC also serves as an excess carbon source in streams, weak discolouration of waterways might have the opposite effect on primary productivity in some water bodies and promote algal growth, potentially leading to eutrophication (Boyd 1972; Liu et al. 2022; Novak et al. 1975). Apart from attenuating light and supplying carbon, CDOM lowers the pH of water, which may also alter solubility, mobility and bioavailability of nutrients and contaminants, e.g., heavy metals, thus indirectly affecting aquatic organisms (Novak et al. 1975; Qiu et al. 2005; Solomon et al. 2015). Moreover, humic substances and tannins may possess direct toxicity effects on aquatic invertebrates and fish, as well as interfere with their reproduction processes, potentially decreasing the physical health of individuals and the ability of populations to persist in highly coloured freshwater environments (Morrongiello et al. 2011; Yang et al. 2019).

Continuous water discolouration can alter not only the biological activity of affected streams but also the acceptability of harvested water for domestic and industrial water supply (Bennett and Drikas 1993; Mitchell 1990; Novak et al. 1975). Although water with high CDOM content is mostly an aesthetic issue in water supplies because of its colour and potentially unpleasant odour and taste, it may become harmful for human consumption if the water colour is not removed prior to disinfection during the water treatment procedure. As increased true colour of water is largely associated with the presence of coloured organic compounds, the residual disinfection of water with chlorine ions can result in the formation of disinfection by-products or DBPs (e.g., trihalomethanes [THMs] and haloacetic acids [HAAs]) that are potentially linked to cancer and adverse reproductive/developmental effects in humans (Dayarathne et al. 2021; Ghernaout 2020; Hongve and Åkesson 1996; Krasner 2009; Mitchell 1990). The presence of organic matter in water was also found to lower the efficiency of water treatment procedures by clogging filter membranes, corroding pipes and fittings, which may elevate copper and lead concentrations in water, as well as interfering with the UV disinfection and promoting the formation of biofilms in the distribution system, which encourages the growth of pathogens (Ghernaout 2020).

There are multiple effective methods of water colour removal currently in use (e.g., coagulation and flocculation, adsorption to activated carbon/chitosan/resins/minerals, chemical oxidation with ozone, membrane filtration and biological treatments like biofiltration), but the majority of the existing methods are expensive (Dayarathne et al. 2021; Zhang et al. 2015). As a result, using less coloured water sources for big cities with a growing drinking water demand is usually preferred by the catchment management authorities (Pikaev et al. 1996). It is predicted that the changing climate in the future will likely further increase natural water colour generation in forested catchments because of enhanced primary productivity and changes in the forest structure and composition that result from increasing temperature, changing precipitation regimes and more frequent bushfire events (Ghernaout 2020; Solomon et al. 2015). This highlights the need to study the sources of natural water discolouration in forested catchments in an attempt to avoid increasing colour generation and reduce the operational costs in water treatment plants in the future.

Although it is broadly known that water colour comes from decaying vegetation, its exact chemical composition and mechanisms of colour generation are still understudied as both can vary spatially and temporally because of differences in decomposition conditions and decomposing material (Christman and Ghassemi 1966; Hensgens et al. 2021; Kirk 1976; Mitchell 1990; Novak et al. 1975; Qiu et al. 2005; Solomon et al. 2015). As the process of decomposition heavily relies on microbial activity, optimal environmental conditions must be met to facilitate the rate of organic material breakdown. In general, maximum decomposition rate and litter leaching can be achieved under warmer temperatures, higher moisture levels and sufficient oxygen access (Giweta 2020; Liu et al. 2022; Nykvist 1959). However, decomposition rates are also dependent on litter quality, which is determined by the C:N ratio and total nutrient content in litter, i.e., the rate of decomposition increases with lower carbon (lignin and cellulose) and higher nutrient (nitrogen and phosphorus) content (Cuassolo et al. 2021; Giweta 2020; Grootemaat et al. 2017; Liu et al. 2022; Nykvist 1959; Prescott 2010; Steart et al. 2009). As these vary greatly between different plant species and material types (e.g., leaves and bark), the potential for CDOM generation also tends to differ (Giweta 2020; Grootemaat et al. 2017; Hensgens et al. 2021; Nykvist 1959).

Despite understanding that the process of organic matter decomposition is likely the major driver of colour generation in forested catchments, exact environmental conditions favouring excessive colour production and the relative contribution of different plant species and material types to the extent of released colour are still largely unknown. Most of the existing research focuses on measuring DOC content and/or colour in field water samples (e.g., Bennett and Drikas 1993 and Kirk 1976 in Australia, Timperley 1985 in New Zealand, Watts et al. 2001 in the United Kingdom, Leenheer 1980 in Brazil and Luo et al. 2020 in China), and only a couple of studies were found to experimentally investigate the drivers of DOC release and colour generation in water (e.g., Francis and Sheldon 2002 and Qiu et al. 2005 in Australia, Christman and Ghassemi 1966 and Novak et al. 1975 in the United States and Hongve 1999 in Norway). Moreover, only one study by Qiu et al. (2005) attempted to investigate the sources of colour, i.e., a light-absorbing fraction of DOC generation rather than overall DOC production, in the context of Australian freshwater streams. Therefore, our study aimed to further explore and quantify the relationship between decomposition conditions, particularly moisture and oxygen availability, and colour generation, as well as between different plant species/decomposing material types and colour generation in Australian temperate forests.

## 2 | Methods

### 2.1 | Study Area and Sample Collection

The true colour generation potential of decomposing leaf and bark materials was studied in a leaching experiment. The study was conducted on litter collected from the McMahons Creek catchment, one of the major tributaries of the Upper Yarra River in north-eastern Victoria, Australia (Figure 1). The upper part of the Yarra River catchment is situated in the forested, mountainous, high-rainfall region, which has been protected from public access for over 100 years and currently supplies more than 65% of the city of Melbourne's drinking water (Barua et al. 2013; Melbourne Water 2023). McMahon's Creek is a topographically diverse catchment with elevations ranging from 300 to 900 m (Figure 1). This leads to highly variable rainfall and temperature patterns, but the average annual precipitation in the area is 1081 mm (Bureau of Meteorology 2025a), and the average annual daily temperatures vary between a maximum of 25°C in January and a minimum of 5°C in July (Meteoblue 2025).

We selected the five most commonly found (that grow in close proximity to waterways) overstorey plant species (VicFlora 2025) in the McMahons Creek catchment, including *Acacia dealbata*, *Acacia melanoxylon*, *Eucalyptus radiata*, *Eucalyptus regnans* and *Nothofagus cunninghamii* for our study. The collected samples included fresh leaves from all five plant species and two types of bark from *E. regnans* (Figure 1). Bark sample 1 included *E. regnans* shedding smooth bark from the upper part of the tree trunk, and bark sample 2 included rough, fibrous bark from the lower part of the tree trunk. Extension poles were used to collect mature leaves and bark from the tall *Eucalyptus* trees (with a maximum collected height of 7–8 m), whereas leaves of smaller *Acacia* and *Nothofagus* trees were hand-picked at 1- to 2-m height. Sampling heights were determined based on the lowest height at which mature leaves typically develop in each species.

### 2.2 | Litter and Bark Leaching Experiment

The colour was extracted from leaves and bark by submerging 15 g (dry weight) of samples in polyester mesh bags in 1 L of deionised water for 15 min in a temperature-controlled laboratory (23°C). To ensure that all generated colour has been extracted, this procedure was performed twice on each measurement day. The effect of different moisture and oxygen levels on the amount of colour generated from decomposing organic material was tested by incubating litter/bark samples in three types of conditions (‘Air-dry’, ‘Moist’ and ‘Saturated’), adding water to the samples and measuring water colour. Since different parts of the catchment are exposed to variable rainfall and drainage patterns because of the high topographic variability, different elevation levels were used as a proxy for general moisture content in litter. ‘Air-dry’ conditions were created by draining the water after colour measurements and keeping the containers with the samples exposed to air at all times, thus generating a low-moisture, high-oxygen environment that represents drier conditions of lower elevation areas. ‘Moist’ conditions were created by draining the water after colour measurements and keeping the containers airtight, thus generating a sufficient moisture and oxygen environment that represents the more humid conditions of upper elevation areas. The ‘Saturated’ conditions were created by keeping the samples in closed containers filled with water at all times, thus generating a high-moisture and low-oxygen environment that represents in-stream conditions. The duration of the experiment was 26 weeks for all samples. However, the frequency

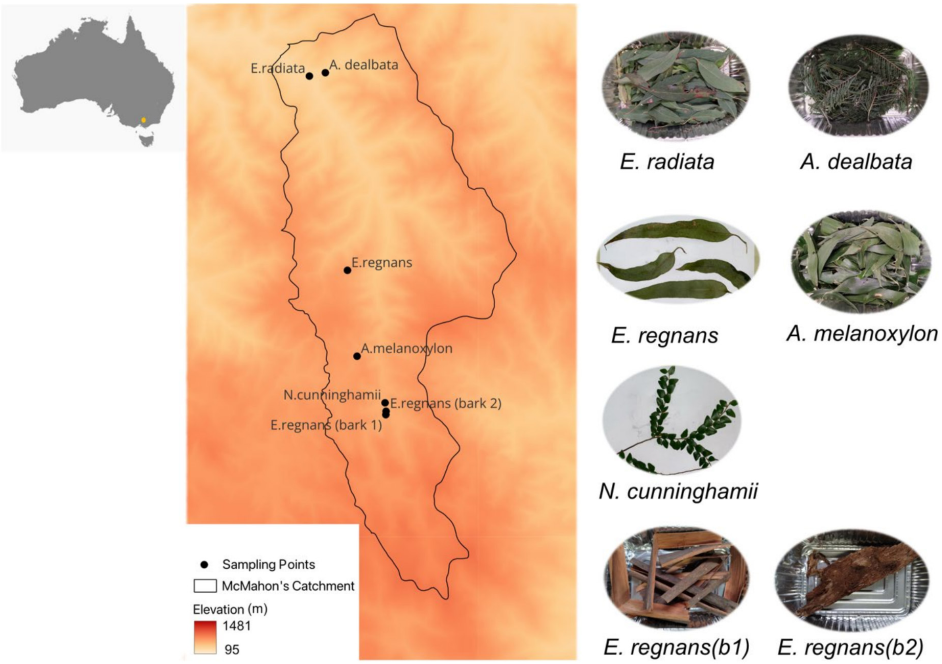

**FIGURE 1** | The McMahons Creek catchment and the locations where each leaf and bark samples were collected. Images of collected samples are also displayed in the figure.

of water additions, followed by colour measurements, varied throughout the experiment: weekly for the first 6 weeks, then fortnightly for the next 6 weeks and finally monthly for the last 14 weeks. In addition to apparent and true colour measurements, turbidity (NTU), electrical conductivity (µS/cm), pH and temperature of coloured water were recorded.

### 2.3 | Colour Measurements

All colour measurements were performed using the Portable Photometer–HI97727 (Hanna, USA) that measures colour in platinum-cobalt units (PCU) at 420 nm. This method was chosen because of the simplicity of measurements and data interpretation, fast colour reading times and low operational costs. The apparent colour of water was measured by analysing raw samples, and the true colour of water was measured by analysing samples that were filtered using the 0.45-µm syringe filter (Figure S1). To avoid potential instrumental errors and water colour degradation with time, all colour measurements were performed at room temperature on the day of water additions. Since 1 PCU is equivalent to 1 mg/L of Pt in Pt-Co solution, and all water additions were 1 L, the cumulative colour generation results were calculated by adding up colour values from each separate measurement and expressed in mg of Pt-Co eq. units. To ensure the accuracy of photometric measurements, UV–Vis spectrophotometric measurements (Analytik Jena SPECORD 250 PLUS, Germany) were done for 216 samples and compared with values measured by Hanna photometer, resulting in a strong correlation of 0.92–0.94 between the two methods (Figure S2).

### 2.4 | Dry Mass Loss Measurements of Incubated Samples

The dry mass loss was calculated by oven-drying leaf and bark samples at 60°C–70°C for 48 h. The initial dry mass of the samples was determined by separating representative 15 g of subsamples and oven-drying them. The final dry mass of the samples was calculated by oven-drying all the samples that were used in the experiment after the final colour measurement (Figures S3 and S4). The percentage dry mass loss was then calculated using the following equation:

$$\text{Dry mass loss}\,(\%) = \left(\frac{W_0 - W_1}{W_0}\right) \times 100$$

where $W_0$ is the initial dry mass of the litter and bark subsamples (g) and $W_1$ is the final dry mass of the litter and bark samples used in the experiment (g).

### 2.5 | Statistical Analysis

Although both apparent and true colour were measured in this experiment (Figures S5 and S6), only true colour was used in subsequent analyses, as apparent colour measurements are influenced by sample turbidity. PERMANOVA (multivariate permutational ANOVA) was used to investigate the effects of moisture status (Air-dry vs. Moist vs. Saturated, fixed effect) on the true colour generation of the leaf samples. Bark samples were excluded from the statistical analysis because of an insufficient sample size. PERMANOVA was chosen because of the right-skewed nature of the true colour dataset, even after log-transforming the data. Euclidean distance was used for the resemblance matrices, running 999 permutations with Type III (partial) sums of squares. All the statistical analysis results were reported at a confidence interval of 0.05. Furthermore, a non-linear least squares (NLS) model was fitted to cumulative true colour generation in order to identify asymptotic points of true colour generation under three moisture conditions, as well as for different species. The fitted NLS model is as follows:

$$\text{True Colour} = a\left(1 - e^{(-b \times \mathit{time\ since\ start})}\right)$$

where $a$ is the asymptote (the maximum value that true colour will approach as time increases), $b$ is the rate constant (controls how fast the curve approaches the asymptote) and *time since start* is the time variable (weeks).

Furthermore, a linear regression model was fitted to investigate the correlation between the total dry mass loss of the leaf samples and the total cumulative colour generation. The regression model was fitted to the mass loss for all samples and under three different moisture statuses. From the two bark samples that were used for this study, only the bark sample 1 data were used for further analysis because of the uncertainty of bark sample 2 colour measurements.

## 3 | Results

### 3.1 | Effects of Moisture Content on True Colour Generation From Leaves

The true colour generation of leaves was higher under Moist conditions than under Air-dry and Saturated conditions throughout the experimental period (Figure 2). The colour generation of Moist samples peaked in Week 2 (432.8 ± 24.95 PCU), followed by a gradual decline in colour production until 105.4 ± 48.89 PCU in Week 26 (Figure 2a). The colour generation of Saturated samples was higher than that of Air-dry samples until Week 3. Thereafter, Air-dry samples produced more colour than Saturated samples for the remainder of the study period. The colour generation of Saturated samples peaked before Moist samples (Week 1, 111.8 ± 11.8 PCU), whereas at the end of the experiment, Saturated samples only produced 15.2 ± 2.06 PCU of colour. However, Air-dry samples showed a delay in reaching a peak in colour generation, which was observed in Week 4 (59.8 ± 48.14 PCU). Furthermore, the lowest colour generation of the Air-dry samples was observed in Week 10 (25.0 ± 36.62 PCU), whereas after that, a slow increase in colour generation could be observed until Week 26 (35.8 ± 26.37 PCU). The results of the PERMANOVA analysis also indicated that moisture status has a significant effect on true colour generation in leaf samples ($p < 0.001$). According to the pair-wise analysis, leaf samples under Moist conditions emitted significantly higher true colour than leaf samples under Saturated and Air-dry conditions ($p = 0.002$ on both occasions). However, the results indicated that the true colour generation between Air-dry and Saturated samples was not significantly different from each other ($p = 0.41$).

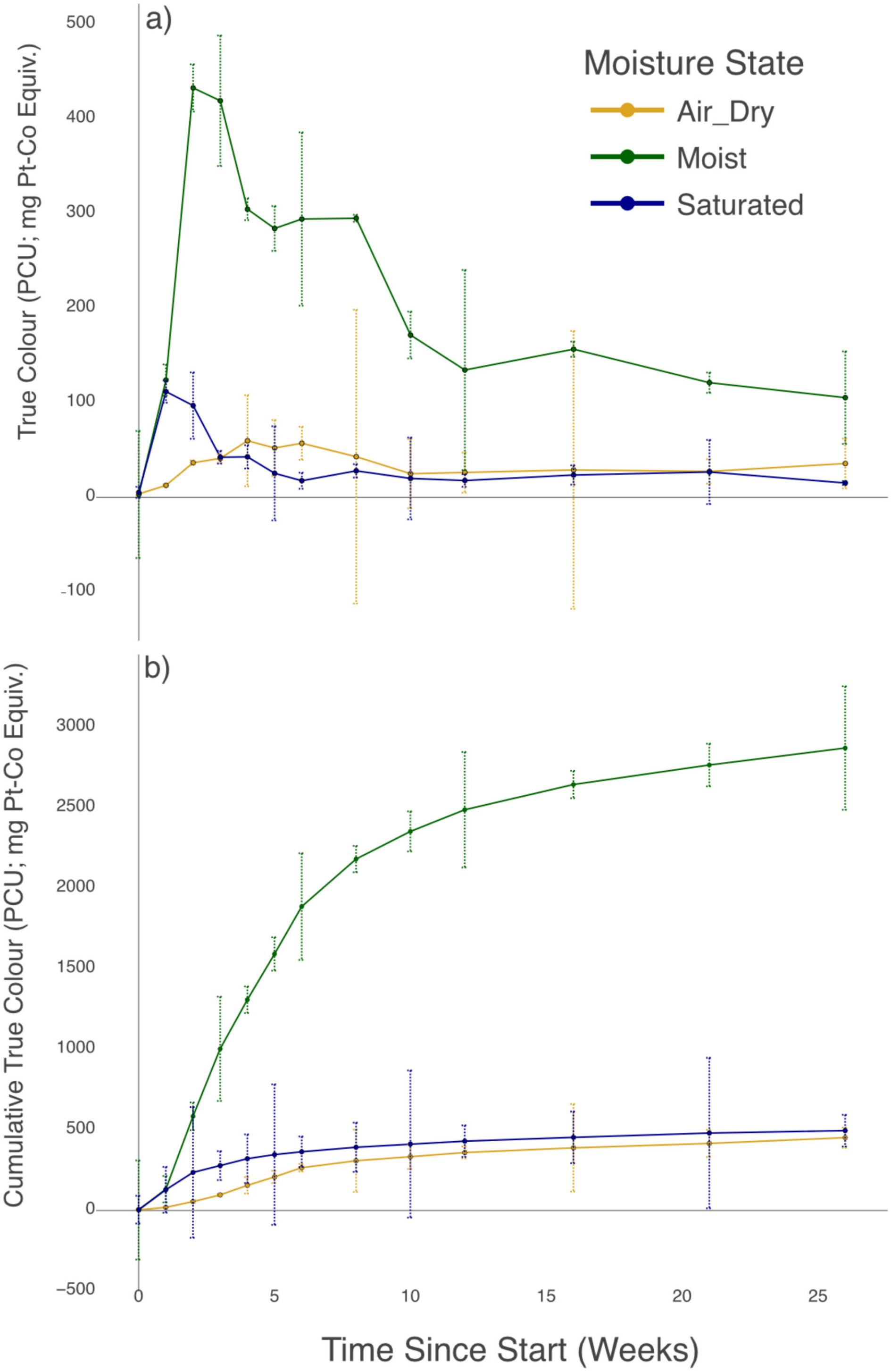

**FIGURE 2** | True colour generation on each measurement occasion (a) and cumulative true colour values (b) of leaf samples that were incubated under Moist, Air-dry and Saturated conditions over the 26-week experimental period.

When considering the cumulative true colour generation (Figure 2b), Moist leaf samples generated a significantly higher true colour amount by Week 26 (2871.2 ± 383.24 PCU) than both Air-dry and Saturated samples ($p = 0.002$ on both occasions). In contrast to true colour generation each week, Saturated samples always had higher cumulative true colour than Air-dry samples, which was also significantly different ($p = 0.03$). The cumulative true colour generation of Saturated and Air-dry leaf samples at the end of the experiment period was 496 ± 98.34 PCU and 452.2 ± 62.67 PCU, respectively.

The fitted asymptotic model revealed that both the asymptote and the rate were significant across all moisture levels ($p < 0.001$), indicating that the model provides a good fit to our data set (Figure 3). The model outputs indicated that the Moist leaf samples had the highest asymptote (2952.0 PCU), followed

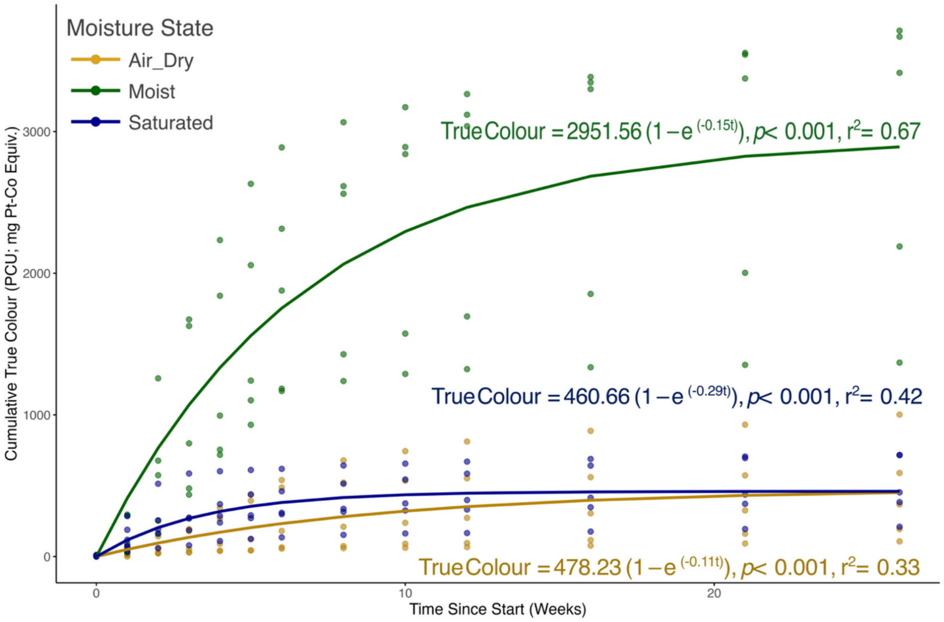

**FIGURE 3** | Fitted asymptotic models for the cumulative true colour generation of leaves under Moist, Saturated and Air-dry conditions. The exponential value of the equation provides the rate of colour generation.

by Air-dry (478.23 PCU) and then Saturated leaf samples (460.66 PCU). Furthermore, our analysis showed that leaf samples under Saturated conditions reached the asymptote state before Moist and Air-dry samples. In addition, our model explained 67% of the true colour variability in Moist samples, whereas in Air-dry and Saturated samples, the correlations were 33% and 42%, respectively.

### 3.2 | Effects of Species on True Colour Generation From Leaves

The true colour generation of different plant species varied between a minimum of 0 (all species at Week 0) and a maximum of 430.67 ± 87.2 PCU (*A. dealbata*) when averaged across all three moisture statuses (Figure 4a). The colour generation of *A. dealbata* peaked in Week 2, whereas *E. radiata* showed the second-highest peak of 397.33 ± 46.69 PCU in Week 3. Furthermore, *E. regnans* reached a peak in colour generation in Week 1 (135 ± 7.45 PCU), which was faster than all other species. *N. cunninghamii* took 8 weeks to reach its peak colour generation (236.67 ± 51.17 PCU), whereas *A. melanoxylon* showed no clear trend but reached peak colour generation in Week 6 (90 ± 74.51 PCU).

At the end of the experiment, *E. radiata* leaves had the highest cumulative true colour generated (1711.33 ± 892.61 PCU), whereas *A. dealbata* produced the second-highest cumulative true colour (1673 ± 386.69 PCU) (Figure 4b). *A. melanoxylon* released 893.33 ± 824.54 PCU of cumulative true colour, which was lower than *N. cunninghamii* (1358 ± 75.84 PCU) ($p = 0.01$) but was higher than *E. regnans* (730 ± 320.44 PCU), which generated the lowest cumulative true colour.

An asymptotic function was also fitted to the cumulative colour generation of each species. The asymptotic function indicated that the true colour generation rate and the asymptotic values were not significantly different from each other (Table S1). However, *E. radiata* showed the highest asymptotic value of 1744 ± 333 PCU, whereas both *N. cunninghamii* and *A. dealbata* reached an asymptote around 1622 PCU. Furthermore, *A. melanoxylon* asymptoted at 906 ± 308 PCU, whereas *E. regnans* asymptoted at 715 ± 132 PCU.

### 3.3 | True Colour Generation From Barks

The *E. regnans* bark samples (bark sample 1) that were incubated under the same three moisture conditions showed slightly different results from those of the leaves. All bark samples produced colour during the first wash, whereas the highest colour generation peak was observed in Moist samples in Week 2 (478 PCU) (Figure S7a). The second-highest colour peak was observed in Saturated samples in Week 1 (393 PCU). However, the colour generation of Saturated bark samples was lower than that of both Air-dry and Moist samples by Week 6, with a value of 16 PCU. Even though Air-dry samples did not show any clear peaks in colour release, the highest colour generation was observed in Week 6, with a 62 PCU value. By the end of the experimental period, Moist samples were producing more colour than both Air-dry and Saturated samples.

The cumulative colour generation was the lowest in Air-dry samples, with a total cumulative colour of 424 PCU by the end of the experimental period (Figure S7b). The cumulative colour generation of Saturated samples was higher than that of Moist samples at the beginning of the experiment, but the colour generation of Moist samples exceeded that of Saturated samples after Week 2. Moist samples produced 1808 PCU of cumulative true colour by the end of the experiment, whereas Saturated samples only produced 1061 PCU of cumulative true colour.

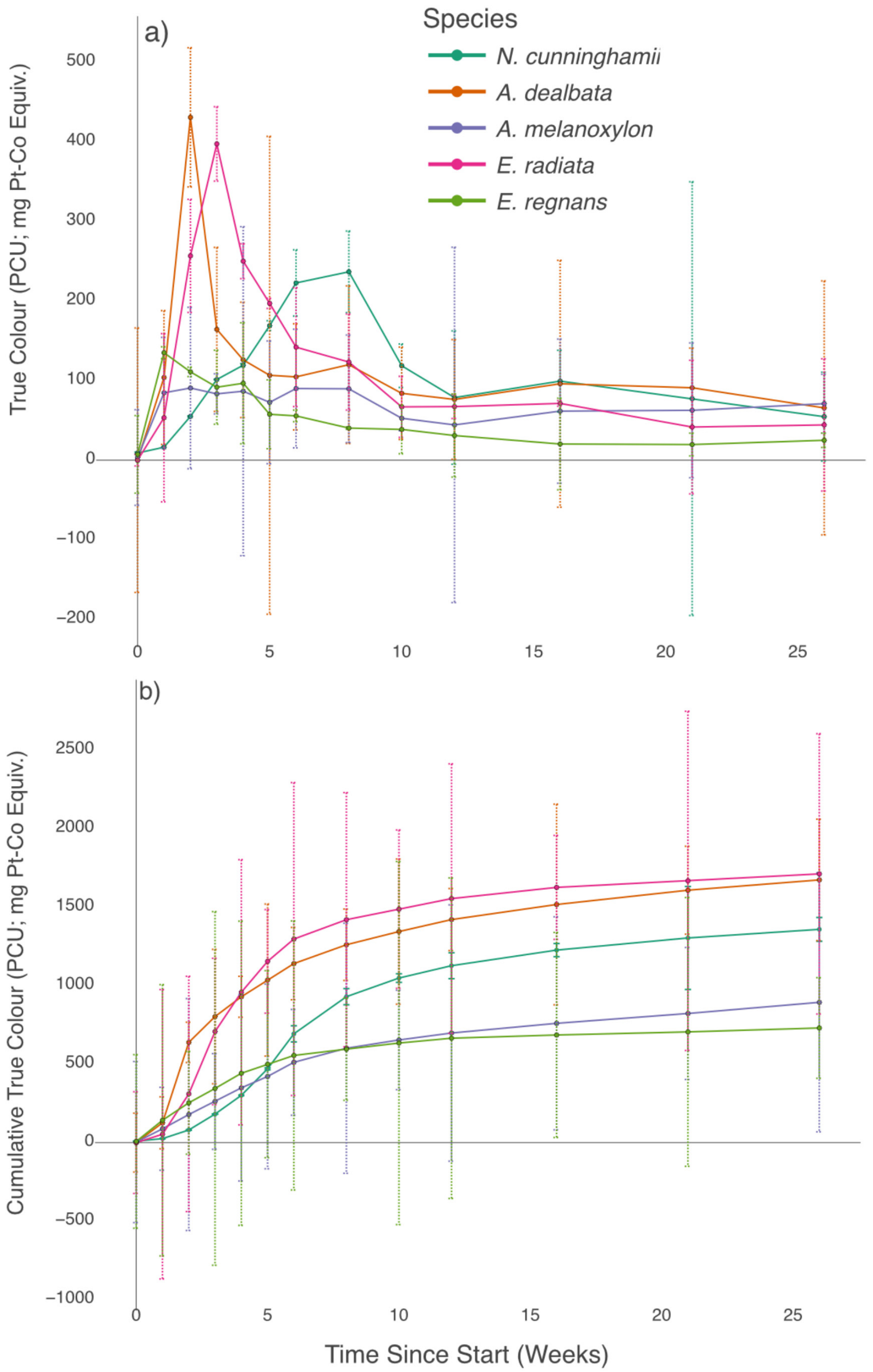

**FIGURE 4** | True colour generation averaged across all moisture states on each measurement occasion (a) and cumulative true colour values (b) of leaf samples from different plant species over the 26-week experimental period.

### 3.4 | True Colour Generation From *E. regnans* Leaves and Barks

True colour generation of *E. regnans* barks (bark sample 1) was higher than that of leaves under all three moisture statuses (Figure S8a). Both leaf and bark samples that were incubated under Air-dry and Saturated conditions showed peak colour generation in Week 6 and Week 1, respectively (Figure S7a). Although the peak values were not considerably different between the Air-dry leaf (57 PCU) and bark (62 PCU) samples, bark samples (393 PCU) incubated under Saturated conditions produced considerably more colour than leaf samples (107 PCU) under the same conditions. In contrast, leaf samples incubated under Moist conditions peaked in Week 1, whereas bark samples peaked at colour generation in Week 2, with peak colour values of 292 and 478 PCU, respectively.

The highest cumulative colour at the end of the experiment was produced by Moist bark samples, whereas the second-highest cumulative colour was produced by Moist leaf samples (Figure S8b). However, Saturated bark samples (1061 PCU) produced considerably higher cumulative colour than Saturated leaf samples (453 PCU), whereas both bark and leaf samples incubated under Air-dry conditions produced a similar amount of cumulative true colour (424 and 368 PCU, respectively).

### 3.5 | Dry Mass Loss From Leaf and Bark Samples

The leaf samples that were incubated under Moist conditions showed higher dry mass loss than all other leaf and bark samples (Figure 5). From the Moist leaf samples, the highest dry mass loss was observed in *A. dealbata* (49.4%), whereas the lowest dry mass loss was observed in *E. regnans* (28.33%), which was also compatible with the highest and lowest cumulative colour generation. When considering the Air-dry and Saturated leaf samples, Air-dry samples of *E. radiata* and *A. dealbata* showed higher dry mass loss than Saturated samples. In contrast, *A. melanoxylon* and *E. regnans* showed higher dry mass loss in Saturated samples than in Air-dry samples, whereas *N. cunninghamii* showed a similar dry mass loss in both Air-dry and Saturated samples. Air-dry *E. regnans* leaves showed similar dry mass loss to Air-dry bark samples, which had the lowest dry mass loss out of all samples. Moist *E. regnans* leaves showed higher dry mass loss than Moist *E. regnans* barks, whereas the cumulative true colour generation was higher for one type of *E. regnans* bark than that for *E. regnans* leaves.

Our linear regression analysis indicated that the dry mass loss of leaf samples can explain 64% of the total cumulative true colour generation (Figure 5). However, when the linear regression model was fitted under three different moisture statuses

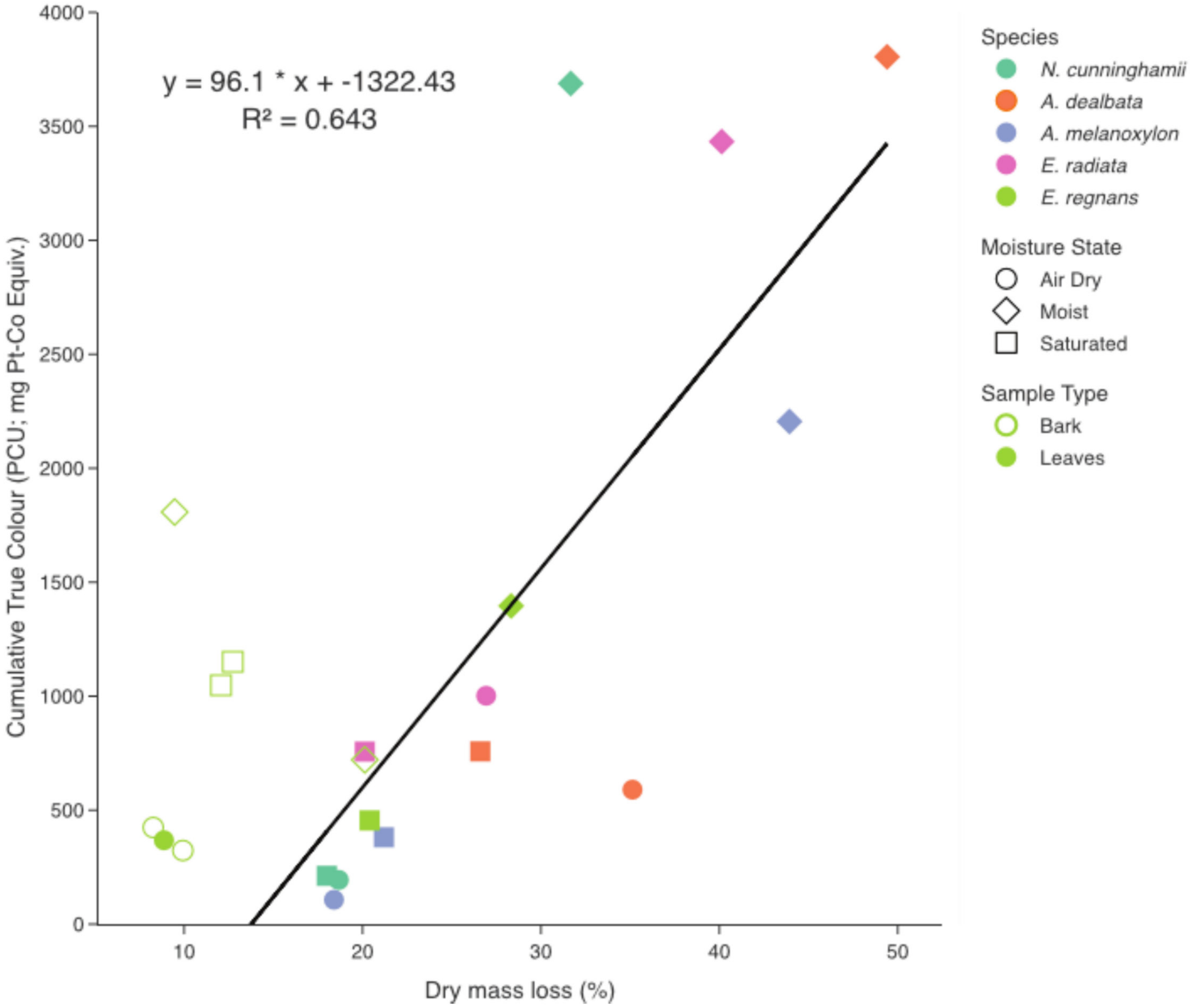

**FIGURE 5** | Correlation between cumulative true colour generation and percentage mass loss for leaf litter samples of all species at all moisture statuses. Bark samples (nonfilled symbols) are included in the figure but excluded from the regression analysis.

(Figure S9), the highest correlation between dry mass loss and true colour generation was observed in Saturated samples ($r^2 = 0.45$). The second-highest correlation was observed in Air-dry leaf samples ($r^2 = 0.3$), whereas the lowest correlation was observed in Moist samples ($r^2 = 0.21$).

## 4 | Discussion

### 4.1 | The Effect of Moisture Status on Cumulative Colour Generation

True colour generation in the streams of forested catchments is intrinsically linked to the organic matter decomposition process. However, there is still only a limited number of published resources linking colour generation to litter decomposition and quantifying the produced colour, especially in the context of Australian forested catchments. Although the effect of moisture availability on the rate of organic material decomposition is a well-researched topic, there is still a lack of clear understanding of how moisture conditions affect the amount of colour produced and the timing of colour release in water as a result of litter decomposition. The present study directly linked the quantity of generated colour and its release dynamics to litter decomposed at different moisture levels over a 26-week period.

Leaf litter samples kept in the Moist conditions produced significantly higher cumulative colour than samples kept in the Air-dry and Saturated conditions, which may be explained by the increased decomposition under Moist conditions due to sufficient moisture and oxygen availability. The complex interaction between moisture and oxygen access primarily controls the decomposition rate of organic material (Sierra et al. 2017), which subsequently affects colour generation. As discussed by Giweta (2020), organic matter decomposition and associated mass losses are accelerated in the rainy season because of increased moisture levels and microbial loads in the soil as a consequence of high rainfall. Soil moisture facilitates the decomposition process by making substrates more soluble, thereby increasing their availability to microbes (Sierra et al. 2017; Tang et al. 2017; Zhou et al. 2014). Under limited moisture conditions, the movement of soluble substrate to active microbial sites is restricted, which slows down organic material decomposition (Zhou et al. 2014). However, at high moisture levels, oxygen accessibility becomes the limiting factor for the rate of decomposition as aerobic microorganisms rely on an adequate oxygen supply to decompose organic compounds (Sierra et al. 2017; Zhou et al. 2014). Although decomposition also occurs under anaerobic conditions, the production of CDOM is primarily an oxidative process (Du and Li 2017; Nykvist 1959). On the forest floor, an intermediate moisture level where soil macropores within the substrate are filled with sufficient amounts of water and air that promote the diffusion of both oxygen and soluble substrates is therefore considered optimal for organic material decomposition and true colour generation (Bunnell et al. 1977; Tang et al. 2017; Zhou et al. 2014). This correlates with the findings of our experiment, where samples kept in Moist conditions produced higher colour than the samples that were under Air-dry and Saturated conditions (Figure 3). According to Cotrufo et al. (2010), microorganisms can become inactive under unfavourable environmental conditions and can continue decomposing activity when the environmental conditions improve, e.g., when moisture is supplied. In our study, there may not be sufficient moisture provided in the Air-dry conditions to promote litter decomposition and colour generation, and in the Saturated conditions, most of the decomposition occurred under anaerobic conditions, resulting in lower quantities of colour released.

Although there was no significant difference in the cumulative colour generation between Air-dry and Saturated leaf samples, the rate of colour production was higher in Saturated samples than in both Air-dry and Moist samples (Figure 3). The litter decomposition process generally occurs in two stages: When adequate water is supplied, soluble organic compounds are leached out of litter first, followed by a long-lasting process of microbially driven decomposition. Although leaching is generally recognised as the first step of litter decomposition (Cuassolo et al. 2021; He et al. 2018; Nykvist 1959), these processes can also occur simultaneously (Gessner et al. 1999; Gessner and Schwoerbel 1989; Tennakoon et al. 2021). The high rate of colour production, which led to colour release peaking in Week 1, and the high correlation between the dry mass loss and cumulative colour generation in Saturated samples could be due to both leaching and decomposition contributing to colour generation. It was previously believed that leaching always happens within the first 24 h of litter being submerged in water (Webster and Benfield 1986); however, recent evidence suggests that this is the case only for dry litter, and fresh litter tends to release its soluble components over the span of several days without substantial initial mass losses (Gessner et al. 1999; Lind et al. 2022). This means that leaching may continue happening after the decomposition process is initiated, which would further explain the highest colour generation from fresh litter contained in the Moist conditions in our experiment since both processes release dissolved organic compounds including CDOM (Gessner et al. 1999; He et al. 2018; Qiu et al. 2005). Similar dynamics of organic colour generation from Saturated and Moist litter samples were observed by Qiu et al. (2005) in their field leaching experiment where flooded (Saturated) litter reached its peak gilvin generation within the first 7 days and nonflooded (Moist) litter continued to release gilvin for the first 40 days of the experiment, resulting in 13% more colour produced compared with flooded conditions.

### 4.2 | The Effect of Species on Colour Generation

The leaf samples from different species produced variable amounts of cumulative colour, with colour release peaking on different measurement occasions. For example, *E. regnans* leaves peaked earlier (Week 1) in colour generation than all other species but produced the lowest cumulative true colour (Figure 4) and had the smallest dry mass loss (Figure 5). The observed differences in decomposition-induced colour production between species may be due to species-specific phenological and substrate quality differences, i.e., structural properties and chemical composition of leaves (Qiu et al. 2005; Steart et al. 2009). If the environmental conditions are unfavourable for decomposition, the rate of organic material breakdown is mostly controlled by the litter quality (Cortez et al. 1996). When litter is decomposed, labile carbon (e.g., nonlignified cellulose and hemicellulose) is quickly consumed by microorganisms first, and then, the recalcitrant fraction of the carbon (lignocellulose, i.e., structural carbohydrates that are bonded to lignin) is slowly decomposed (Cortez et al. 1996; Cuassolo et al. 2021; Ostrofsky 1997; Webster and Benfield 1986). Lignin concentration is believed to be the most important factor determining the decay rates of litter (Cortez et al. 1996; Cuassolo et al. 2021) and the amount of coloured humic substances released. *Eucalyptus* species have higher phenolic content than *Acacia* species (Bernhard-Reversat 1999), and most polyphenols leach out of leaves within the first 7 days of incubation (Alvim et al. 2015; Bernhard-Reversat 1999), which would explain early peaks in colour release for *E. regnans* and *E. radiata* leaves in our experiment. The litter of *Acacia* species, while having lower polyphenol content contributing to colour generation, generally has a higher decomposition rate than the litter of *Eucalyptus* species because of *Acacia*'s N-fixing properties, resulting in lower C/N (Carnovale et al. 2021; Ferreira et al. 2021). Snowdon et al. (2005) reported the C/N in *A. dealbata* and *E. regnans* leaves to be 13–21 and 26–31, respectively, whereas Ashton (1975) found the C/N of *E. regnans* leaves to be 31–42. *A. melanoxylon*, however, has tougher leaves than *A. dealbata* because of higher lignin content, which results in quicker decomposition of *A. dealbata* leaves and earlier colour release from this species (Ferreira et al. 2021), which matches our findings. Steart et al. (2009) compared decomposition rates of *A. melanoxylon*, *E. regnans* and *N. cunninghamii* leaves in a field experiment and, contrary to our results, found that *N. cunninghamii* and *A. melanoxylon* leaves decay at similar rates, slower than *E. regnans* leaves due to *N. cunninghamii* leaves having a higher C/N ratio than the other two species (25.0 compared with 14.3 for *E. regnans* and 11.8 for *A. melanoxylon*). A high C/N ratio in *E. regnans* leaves, as reported by Ashton (1975) and Snowdon et al. (2005), would explain the small mass losses and the slowest decomposition rate of this litter in our experiment. Although the decomposition rates were not measured in our study, mass losses throughout the experiment could be used as a proxy for decomposition and leaching (Ostrofsky 1997; Qiu et al. 2005). According to Alvim et al. (2015), smaller leaf size also increases litter decomposition rates, which would explain the high colour generation potential of *N. cunninghamii* leaves despite having a high C/N ratio as measured by Steart et al. (2009). Multiple researchers have also observed noticeable differences in litter decomposition rates (e.g., Alvim et al. 2015; Cortez et al. 1996; Cuassolo et al. 2021; Ostrofsky 1997; Steart et al. 2009; Webster and Benfield 1986) and the amount of true colour produced as a result of leaching and decomposition (e.g., Qiu et al. 2005) between different plant species.

### 4.3 | Colour Generation of Barks Compared With Leaves

The true colour generation of *E. regnans* bark was considerably higher than that of leaves under all three moisture states, especially for Saturated samples (Figure S7b). Although the mechanisms of leaf litter decomposition in forested streams are a well-researched topic, there is still limited knowledge about the rates of woody tissue breakdown in freshwater (Bunn 1986; Webster and Benfield 1986). Barks and woods generally tend to have much slower decomposition rates than leaves because of their high lignin content (Bunn 1986; Grootemaat et al. 2017; Neumann et al. 2021; Snowdon et al. 2005; Webster and Benfield 1986). Ashton (1975) looked specifically at decomposition rates of *E. regnans* leaves and barks and found that the C/N of bark is double the C/N of leaves (67.4 vs. 31.3–42.1). This correlates with the results of our experiment, where bark samples had lower decomposition rates compared with leaf samples, as indicated by lower dry mass losses in the bark samples (Figure 5). Higher colour generation from bark samples would then primarily be attributed to the leaching of tannins rather than coloured decomposition compounds, especially in the first few weeks of the experiment. Although tannins may be present in all parts of a plant, woody lignified tissues like bark tend to have a higher content of polyphenolic compounds than leaves (Antwi-Boasiako and Animapauh 2012; Bernays et al. 1989; Das et al. 2020).

### 4.4 | Implications for Management

The results of our study indicated that moisture availability and the type of dominant plant species could affect the amount of water colour generated in forested catchments. For example, *E. radiata* leaves in Moist conditions (2294.9 ± 352.9 PCU) produced 43 times the amount of cumulative true colour released from *A. melanoxylon* leaves in Air-dry conditions (52.5 ± 8.2 PCU) (Figure S5b). Therefore, it is expected to see higher water colour generation in forested catchments located in the high-rainfall, low-evapotranspiration areas of south-eastern Australia, which are dominated by *A. dealbata*, *N. cunninghamii* and *E. radiata* species rather than *A. melanoxylon* or *E. regnans* species. The comparison of the true colour generation potential of *E. regnans* bark and leaves also indicated that it is possible to see elevated water colour in the forests where bark shedding is a prominent feature. These assumptions, however, may not be applicable to every forested catchment, and the amounts of generated colour will likely depend on many factors including the climatic conditions of the area, litter generation quantities and times and the ratio of leaves to bark in standing litter apart from the actual species composition of the forest. For example, *E. regnans* produces fewer leaves for a given stem basal area compared with other species such as *N. cunninghamii*, which often leads to the accumulation of *N. cunninghamii* litter in gullies and streams, despite *Eucalyptus* species dominating these catchments (Steart et al. 2005). Although *E. regnans* leaf litter is rarely found in rainforest depositional settings near streams because of its poor dispersive ability (Steart et al. 2002; Steart et al. 2005), Australian Eucalyptus forests are known for their increased smooth bark shedding in summers and accumulation of bark on the forest floor (Grootemaat et al. 2017) and in forested streams (Webster and Benfield 1986). Neumann et al. (2021) found that twigs and barks contribute approximately 38% to litterfall but comprise 60% of the standing litter in Australian forests. Snowdon et al. (2005) identified that leafy components of litter persist only for a couple of years in Australian forests, but woods, barks and branches are more persistent through time, acting as a longer term source of colour than leaves. A declining rainfall trend has been observed in the Yarra River catchment over the past 50 years (Barua et al. 2013), and this trend is expected to continue in the future. The approximate rainfall reduction of 20.6 mm per decade in south-eastern Australia

(Barua et al. 2013) may have notable implications for the forest's structure and functionality. These may involve changes in species distribution, litterfall loads and timing of litterfall (Keenan 2017), leading to a different composition and quantity of standing litter in Australian forests. Such vegetational shifts induced by climate change may, in turn, lead to considerably different colour generation patterns in Yarra River catchments, providing ground for future research.

### 4.5 | Limitations of the Study

Although fresh leaves were used in our experiment, litterfall in the natural environment typically occurs after leaves have aged and desiccated on the tree. Leaves can also shed before they reach senescence because of mechanical damage from heavy rainfalls, strong winds or herbivory (Fonte and Schowalter 2004; Giweta 2020; Li et al. 2021), creating a litter layer composed of leaves in different stages of decomposition with variable C/N ratios and decay rates. Green leaves have double the amount of nutrients compared with dry leaves (Fonte and Schowalter 2004), resulting in faster decay of fresh leaves due to enhanced microbial activity (Feng et al. 2018). Although the use of fresh leaves in this study may not be fully representative of the natural conditions in the catchment, this approach enabled us to standardise the initial condition of litter across all species and treatments, allowing us to isolate and examine the effects of moisture availability on colour release during leaching and decomposition. Future studies could benefit from exploring the effect of leaf senescence stage on decomposition and associated colour generation to better represent field conditions. In addition, leaves were periodically wetted in the 'Air-dry' treatment in our experiment, which does not fully represent the prolonged drought conditions typical for the study area (Barua et al. 2013; Bureau of Meteorology 2025b). Given the aim of the experiment, water additions of the same frequency and amount were necessary to extract the colour from leaves and to ensure the comparability between Air-dry, Moist and Saturated treatments. This experimental limitation should be considered when interpreting the results, where prolonged drought conditions in the catchments may lead to slower decomposition and reduced colour generation compared with the controlled laboratory conditions.

## 5 | Conclusions

The results of our experiment indicated that the moisture condition of the leaf and bark plays a major role in colour generation. Leaf samples incubated in Moist conditions produced significantly higher colour than the leaf samples incubated in Saturated and Air-dry conditions. However, leaf samples under Saturated conditions produced colour at a high rate, and colour generation peaked earlier than in Moist and Air-dry conditions, possibly because of the combined effects of leaching and decomposition. Leaves from different species produced different cumulative colour amounts, whereas in *E. regnans*, barks produced more colour than leaves. Therefore, we concluded that the moisture condition of leaf litter plays a significant role in the water colour generation in forested catchments of south-eastern Australia. We suggest that future research should focus on the colour generation potential of these catchments under future possible climate change scenarios, including reduced precipitation, increased temperature and subsequent litter moisture reduction conditions.

---

### Author Contributions

**Mariia Lukinykh:** investigation, formal analysis, methodology, validation, visualisation, writing – original draft, writing – review and editing. **Christopher Sean Lyell:** data curation, formal analysis, methodology, resources, validation. **Charuni Jayasekara:** formal analysis, visualisation, writing – original draft, writing – review and editing. **Patrick Lane:** conceptualisation, funding acquisition, supervision, writing – review and editing. **Gary Sheridan:** conceptualisation, funding acquisition, project administration, supervision, writing – review and editing. All authors have read and agreed to the published version of the manuscript.

### Acknowledgements

This research was funded by the Melbourne Water Corporation funded research project 'Securing Melbourne's Water Quantity and Quality in a Changing Climate' (TA37690) and by the Victorian Government, Department of Energy, Environment and Climate Action (DEECA) as part of the Integrated Forest Ecosystem Research (IFER) program (TA301102). Open access publishing facilitated by The University of Melbourne, as part of the Wiley - The University of Melbourne agreement via the Council of Australian University Librarians.

### Conflicts of Interest

The authors declare no conflicts of interest.

### Data Availability Statement

The data that support the findings of this study are available from the corresponding author upon reasonable request.

### References

Alvim, E. A. C. C., A. de Oliveira Medeiros, R. S. Rezende, and J. F. Gonçalves. 2015. "Small Leaf Breakdown in a Savannah Headwater Stream." *Limnologica* 51: 131–138. https://doi.org/10.1016/j.limno.2014.10.005.

Antwi-Boasiako, C., and S. O. Animapauh. 2012. "Tannin Extraction From the Barks of Three Tropical Hardwoods for the Production of Adhesives." *Journal of Applied Sciences Research* 8, no. 6: 2959–2965.

Ashton, D. H. 1975. "Studies of Litter in *Eucalyptus regnans* Forests." *Australian Journal of Botany* 23, no. 3: 413–433. https://doi.org/10.1071/bt9750413.

Barua, S., N. Muttil, A. W. M. Ng, and B. J. C. Perera. 2013. "Rainfall Trend and Its Implications for Water Resource Management Within the Yarra River Catchment, Australia." *Hydrological Processes* 27, no. 12: 1727–1738. https://doi.org/10.1002/hyp.9311.

Bennett, L. E., and M. Drikas. 1993. "The Evaluation of Colour in Natural Waters." *Water Research* 27, no. 7: 1209–1218. https://doi.org/10.1016/0043-1354(93)90013-8.

Bernays, E. A., G. C. Driver, and M. Bilgener. 1989. "Herbivores and Plant Tannins." In *Advances in Ecological Research*, edited by M. Begon, A. H. Fitter, E. D. Ford, and A. MacFadyen, vol. 19, 263–302. Academic Press. https://doi.org/10.1016/S0065-2504(08)60160-9.

Bernhard-Reversat, F. 1999. "The Leaching of Eucalyptus Hybrids and Acacia Auriculiformis Leaf Litter: Laboratory Experiments on Early Decomposition and Ecological Implications in Congolese Tree Plantations." *Applied Soil Ecology* 12, no. 3: 251–261. https://doi.org/10.1016/S0929-1393(99)00005-0.

Boyd, C. E. 1972. “Sources of $CO_2$ for Nuisance Blooms of Algae.” *Weed Science* 20, no. 5: 492–497.

Bunn, S. E. 1986. “Origin and Fate of Organic Matter in Australian Upland Streams.” In *Limnology in Australia*, edited by P. De Deckker and W. D. Williams, vol. 61, 277–291. Springer. https://doi.org/10.1007/978-94-009-4820-4_17.

Bunnell, F. L., D. E. N. Tait, P. W. Flanagan, and K. Van Clever. 1977. “Microbial Respiration and Substrate Weight Loss—I: A General Model of the Influences of Abiotic Variables.” *Soil Biology and Biochemistry* 9, no. 1: 33–40. https://doi.org/10.1016/0038-0717(77)90058-X.

Bureau of Meteorology. 2025a. Monthly Rainfall—Reefton (Upper Yarra Dam). http://www.bom.gov.au/jsp/ncc/cdio/weatherData/av?p_nccObsCode=139&p_display_type=dataFile&p_startYear=&p_c=&p_stn_num=086271.

Bureau of Meteorology. 2025b. Previous Droughts. http://www.bom.gov.au/climate/drought/knowledge-centre/previous-droughts.shtml.

Carnovale, D., A. E. Richardson, P. H. Thrall, A. Bissett, and G. Baker. 2021. “Litter Decomposition and Microbial Communities Alters Depending on Litter Type and Overstory Species in Revegetated Agricultural Land.” *Pedobiologia* 84: 150702. https://doi.org/10.1016/j.pedobi.2020.150702.

Christman, R. F., and M. Ghassemi. 1966. “Chemical Nature of Organic Color in Water.” *Journal AWWA* 58, no. 6: 723–741. https://doi.org/10.1002/j.1551-8833.1966.tb01631.x.

Cortez, J., J. M. Demard, P. Bottner, and L. Jocteur Monrozier. 1996. “Decomposition of Mediterranean Leaf Litters: A Microcosm Experiment Investigating Relationships Between Decomposition Rates and Litter Quality.” *Soil Biology and Biochemistry* 28, no. 4: 443–452. https://doi.org/10.1016/0038-0717(96)00005-3.

Cotrufo, M. F., I. D. Galdo, and D. Piermatteo. 2010. “Litter Decomposition: Concepts, Methods and Future Perspectives.” In *Soil Carbon Dynamics: An Integrated Methodology*, edited by A. Heinemeyer, M. Bahn, and W. L. Kutsch, 76–90. Cambridge University Press. https://doi.org/10.1017/CBO9780511711794.006.

Cuassolo, F., V. Díaz Villanueva, and B. Modenutti. 2021. “Low-Decomposition Rates of Riparian Litter in a North Patagonian Ultraoligotrophic Lake.” *Limnologica* 90: 125906. https://doi.org/10.1016/j.limno.2021.125906.

Das, A. K., M. N. Islam, M. O. Faruk, M. Ashaduzzaman, and R. Dungani. 2020. “Review on Tannins: Extraction Processes, Applications and Possibilities.” *South African Journal of Botany* 135: 58–70. https://doi.org/10.1016/j.sajb.2020.08.008.

Dayarathne, H. N. P., M. J. Angove, R. Aryal, H. Abuel-Naga, and B. Mainali. 2021. “Removal of Natural Organic Matter From Source Water: Review on Coagulants, Dual Coagulation, Alternative Coagulants, and Mechanisms.” *Journal of Water Process Engineering* 40: 101820. https://doi.org/10.1016/j.jwpe.2020.101820.

Du, H., and F. Li. 2017. “Characteristics of Dissolved Organic Matter Formed in Aerobic and Anaerobic Digestion of Excess Activated Sludge.” *Chemosphere* 168: 1022–1031. https://doi.org/10.1016/j.chemosphere.2016.10.108.

Feng, H., L. Xue, and H. Chen. 2018. “Responses of Decomposition of Green Leaves and Leaf Litter to Stand Density, N and P Additions in *Acacia auriculaeformis* Stands.” *European Journal of Forest Research* 137, no. 6: 819–830. https://doi.org/10.1007/s10342-018-1142-z.

Ferreira, V., A. Figueiredo, M. A. S. Graça, E. Marchante, and A. Pereira. 2021. “Invasion of Temperate Deciduous Broadleaf Forests by N-Fixing Tree Species—Consequences for Stream Ecosystems.” *Biological Reviews* 96, no. 3: 877–902. https://doi.org/10.1111/brv.12682.

Fonte, S. J., and T. D. Schowalter. 2004. “Decomposition of Greenfall vs. Senescent Foliage in a Tropical Forest Ecosystem in Puerto Rico.” *Biotropica* 36, no. 4: 474–482.

Francis, C., and F. Sheldon. 2002. “River Red Gum (*Eucalyptus camaldulensis* Dehnh.) Organic Matter as a Carbon Source in the Lower Darling River, Australia.” *Hydrobiologia* 481, no. 1: 113–124. https://doi.org/10.1023/A:1021269205983.

Gessner, M. O., E. Chauvet, and M. Dobson. 1999. “A Perspective on Leaf Litter Breakdown in Streams.” *Oikos* 85, no. 2: 377. https://doi.org/10.2307/3546505.

Gessner, M. O., and J. Schwoerbel. 1989. “Leaching Kinetics of Fresh Leaf-Litter With Implications for the Current Concept of Leaf-Processing in Streams.” *Archiv für Hydrobiologie*: 81–90. https://doi.org/10.1127/archiv-hydrobiol/115/1989/81.

Ghernaout, D. 2020. “Natural Organic Matter Removal in the Context of the Performance of Drinking Water Treatment Processes—Technical Notes.” *Open Access Library Journal* 7, no. 9: 9. https://doi.org/10.4236/oalib.1106751.

Giweta, M. 2020. “Role of Litter Production and Its Decomposition, and Factors Affecting the Processes in a Tropical Forest Ecosystem: A Review.” *Journal of Ecology and Environment* 44, no. 1: 11. https://doi.org/10.1186/s41610-020-0151-2.

Grootemaat, S., I. J. Wright, P. M. Van Bodegom, J. H. C. Cornelissen, and V. Shaw. 2017. “Bark Traits, Decomposition and Flammability of Australian Forest Trees.” *Australian Journal of Botany* 65, no. 4: 327. https://doi.org/10.1071/BT16258.

He, Y., N. Song, and H.-L. Jiang. 2018. “Effects of Dissolved Organic Matter Leaching From Macrophyte Litter on Black Water Events in Shallow Lakes.” *Environmental Science and Pollution Research* 25, no. 10: 9928–9939. https://doi.org/10.1007/s11356-018-1267-0.

Hensgens, G., O. J. Lechtenfeld, F. Guillemette, H. Laudon, and M. Berggren. 2021. “Impacts of Litter Decay on Organic Leachate Composition and Reactivity.” *Biogeochemistry* 154, no. 1: 99–117. https://doi.org/10.1007/s10533-021-00799-3.

Hongve, D. 1999. “Production of Dissolved Organic Carbon in Forested Catchments.” *Journal of Hydrology* 224, no. 3–4: 91–99. https://doi.org/10.1016/S0022-1694(99)00132-8.

Hongve, D., and G. Åkesson. 1996. “Spectrophotometric Determination of Water Colour in Hazen Units.” *Water Research* 30, no. 11: 2771–2775. https://doi.org/10.1016/S0043-1354(96)00163-7.

Keenan, R. J. 2017. “Climate Change and Australian Production Forests: Impacts and Adaptation.” *Australian Forestry* 80, no. 4: 197–207. https://doi.org/10.1080/00049158.2017.1360170.

Kirk, J. 1976. “Yellow Substance (Gelbstoff) and Its Contribution to the Attenuation of Photosynthetically Active Radiation in Some Inland and Coastal South-Eastern Australian Waters.” *Marine and Freshwater Research* 27, no. 1: 61. https://doi.org/10.1071/MF9760061.

Krasner, S. W. 2009. “The Formation and Control of Emerging Disinfection By-Products of Health Concern.” *Philosophical Transactions of the Royal Society A: Mathematical, Physical and Engineering Sciences* 367, no. 1904: 4077–4095. https://doi.org/10.1098/rsta.2009.0108.

Leenheer, J. A. 1980. “Origin and Nature of Humic Substances in the Waters of the Amazon River Basin.” *Acta Amazonica* 10, no. 3: 513–526. https://doi.org/10.1590/1809-43921980103513.

Li, R., Y. Zhang, D. Yu, et al. 2021. “The Decomposition of Green Leaf Litter Is Less Temperature Sensitive Than That of Senescent Leaf Litter: An Incubation Study.” *Geoderma* 381: 114691. https://doi.org/10.1016/j.geoderma.2020.114691.

Lind, L., A. Harbicht, E. Bergman, J. Edwartz, and R. L. Eckstein. 2022. “Effects of Initial Leaching for Estimates of Mass Loss and Microbial Decomposition—Call for an Increased Nuance.” *Ecology and Evolution* 12, no. 8: e9118. https://doi.org/10.1002/ece3.9118.

Liu, S., G. He, H. Fang, S. Xu, and S. Bai. 2022. “Effects of Dissolved Oxygen on the Decomposers and Decomposition of Plant Litter in Lake

Ecosystem." *Journal of Cleaner Production* 372: 133837. https://doi.org/10.1016/j.jclepro.2022.133837.

Luo, F., Y. Li, E. Norgbey, et al. 2020. "A Study on the Occurrence of Black Water in Reservoirs in Eucalyptus Plantation Region." *Environmental Science and Pollution Research* 27, no. 28: 34927–34940. https://doi.org/10.1007/s11356-020-09613-3.

Melbourne Water. 2023. Water Catchments. https://www.melbournewater.com.au/water-and-environment/water-management/water-quality/water-catchments.

Meteoblue. 2025. Simulated Historical Climate & Weather Data for McMahons Creek. https://www.meteoblue.com/en/weather/historyclimate/climatemodelled/mcmahons-creek_australia_2158279.

Mitchell, G. N. 1990. "Natural Discoloration of Freshwater: Chemical Composition and Environmental Genesis." *Progress in Physical Geography: Earth and Environment* 14, no. 3: 317–334. https://doi.org/10.1177/030913339001400302.

Morrongiello, J. R., N. R. Bond, D. A. Crook, and B. B. M. Wong. 2011. "Eucalyptus Leachate Inhibits Reproduction in a Freshwater Fish." *Freshwater Biology* 56, no. 9: 1736–1745. https://doi.org/10.1111/j.1365-2427.2011.02605.x.

Neumann, M., J. Turner, T. Lewis, L. McCaw, G. Cook, and M. A. Adams. 2021. "Dynamics of Necromass in Woody Australian Ecosystems." *Ecosphere* 12, no. 8: e03693. https://doi.org/10.1002/ecs2.3693.

Novak, J. T., A. S. Goodman, and D. L. King. 1975. "Aquatic-Weed Decay and Color Production." *Journal AWWA* 67, no. 3: 134–139. https://doi.org/10.1002/j.1551-8833.1975.tb02176.x.

Nykvist, N. 1959. "Leaching and Decomposition of Litter I. Experiments on Leaf Litter of *Fraxinus excelsior*." *Oikos* 10, no. 2: 190–211. https://doi.org/10.2307/3565146.

Ostrofsky, M. L. 1997. "Relationship Between Chemical Characteristics of Autumn-Shed Leaves and Aquatic Processing Rates." *Journal of the North American Benthological Society* 16, no. 4: 750–759. https://doi.org/10.2307/1468168.

Pikaev, A. K., A. V. Bludenko, I. E. Makarov, et al. 1996. "Electron-Beam Treatment of Highly-Coloured River Water." *Radiation Physics and Chemistry* 48, no. 1: 75–80. https://doi.org/10.1016/0969-806X(95)00425-W.

Prescott, C. E. 2010. "Litter Decomposition: What Controls It and How Can We Alter It to Sequester More Carbon in Forest Soils?" *Biogeochemistry* 101, no. 1: 133–149. https://doi.org/10.1007/s10533-010-9439-0.

Qiu, S., A. J. McComb, R. W. Bell, and J. A. Davis. 2005. "Estimating Production of Gilvin From Catchment Leaf Litter During Seasonal Rains." *Marine and Freshwater Research* 56, no. 6: 843. https://doi.org/10.1071/MF04297.

Sierra, C. A., S. Malghani, and H. W. Loescher. 2017. "Interactions Among Temperature, Moisture, and Oxygen Concentrations in Controlling Decomposition Rates in a Boreal Forest Soil." *Biogeosciences* 14, no. 3: 703–710. https://doi.org/10.5194/bg-14-703-2017.

Snowdon, P., P. Ryan, and J. Raison. 2005. "Review of C:N Ratios in Vegetation, Litter and Soil Under Australian Native Forests and Plantations—National Carbon Accounting System Technical Report No. 45". Australian Government—Department of the Environment and Heritage, Australian Greenhouse Office. https://www.dcceew.gov.au/themes/custom/awe/fullcam/Help-FullCAM2020/reps/TR45%20Review%20of%20CN%20Ratios%20in%20Vegetation,%20Litter%20and%20Soil%20under%20Australian%20Native%20Forests%20and%20Plantations.pdf.

Solomon, C. T., S. E. Jones, B. C. Weidel, et al. 2015. "Ecosystem Consequences of Changing Inputs of Terrestrial Dissolved Organic Matter to Lakes: Current Knowledge and Future Challenges." *Ecosystems* 18, no. 3: 376–389. https://doi.org/10.1007/s10021-015-9848-y.

Steart, D. C., P. I. Boon, D. R. Greenwood, and N. T. Diamond. 2002. "Transport of Leaf Litter in Upland Streams of Eucalyptus and Nothofagus Forests in South-Eastern Australia." *Archiv für Hydrobiologie* 156, no. 1: 43–61. https://doi.org/10.1127/0003-9136/2002/0156-0043.

Steart, D. C., D. R. Greenwood, and P. I. Boon. 2005. "Paleoecological Implications of Differential Biomass and Litter Production in Canopy Trees in Australian Nothofagus and Eucalyptus Forests." *PALAIOS* 20, no. 5: 452–462. https://doi.org/10.2110/palo.2004.P04-57.

Steart, D. C., D. R. Greenwood, and P. I. Boon. 2009. "The Chemical Constraints Upon Leaf Decay Rates: Taphonomic Implications Among Leaf Species in Australian Terrestrial and Aquatic Environments." *Review of Palaeobotany and Palynology* 157, no. 3: 358–374. https://doi.org/10.1016/j.revpalbo.2009.06.008.

Tang, S., W. Cheng, R. Hu, et al. 2017. "Decomposition of Soil Organic Carbon Influenced by Soil Temperature and Moisture in Andisol and Inceptisol Paddy Soils in a Cold Temperate Region of Japan." *Journal of Soils and Sediments* 17, no. 7: 1843–1851. https://doi.org/10.1007/s11368-016-1607-y.

Tennakoon, D. S., E. Gentekaki, R. Jeewon, C. H. Kuo, I. Promputtha, and K. D. Hyde. 2021. "Life in Leaf Litter: Fungal Community Succession During Decomposition." *Mycosphere* 12, no. 1: 406–429. https://doi.org/10.5943/mycosphere/12/1/5.

Timperley, M. H. 1985. "Dissolved Coloured Compounds and Suspended Matter in the Waters of the Middle Waikato River." *New Zealand Journal of Marine and Freshwater Research* 19, no. 1: 63–70. https://doi.org/10.1080/00288330.1985.9516075.

VicFlora. 2025. Flora of Victoria, Royal Botanic Gardens Victoria: Eastern Highlands. https://vicflora.rbg.vic.gov.au/pages/eastern-highlands#riparian-forest.

Watts, C. D., P. S. Naden, J. Machell, and J. Banks. 2001. "Long Term Variation in Water Colour From Yorkshire Catchments." *Science of the Total Environment* 278, no. 1–3: 57–72. https://doi.org/10.1016/S0048-9697(00)00888-3.

Webster, J. R., and E. F. Benfield. 1986. "Vascular Plant Breakdown in Freshwater Ecosystems." *Annual Review of Ecology and Systematics* 17, no. 1: 567–594. https://doi.org/10.1146/annurev.es.17.110186.003031.

Yang, G., M. Wen, Y. Deng, et al. 2019. "Occurrence Patterns of Black Water and Its Impact on Fish in Cutover Areas of *Eucalyptus* Plantations." *Science of the Total Environment* 693: 133393. https://doi.org/10.1016/j.scitotenv.2019.07.199.

Zhang, Y., X. Zhao, X. Zhang, and S. Peng. 2015. "A Review of Different Drinking Water Treatments for Natural Organic Matter Removal." *Water Supply* 15, no. 3: 442–455. https://doi.org/10.2166/ws.2015.011.

Zhou, W., D. Hui, and W. Shen. 2014. "Effects of Soil Moisture on the Temperature Sensitivity of Soil Heterotrophic Respiration: A Laboratory Incubation Study." *PLoS ONE* 9, no. 3: e92531. https://doi.org/10.1371/journal.pone.0092531.

Zobkov, M. B., and M. V. Zobkova. 2020. "New Spectroscopic Method for True Color Determination in Natural Water With High Agreement With Visual Methods." *Water Research* 177: 115773. https://doi.org/10.1016/j.watres.2020.115773.

## Supporting Information

Additional supporting information can be found online in the Supporting Information section. **Table S1:** The fitted asymptotic function for true colour generation from leaf samples of each species, significance and correlation. **Figure S1:** Photos of the most coloured unfiltered water samples for all species with true colour (T.C.) and apparent colour (A.C.) values provided. **Figure S2:** Linear regression graphs plotting true colour (PCU) vs. absorbance measurements for 216 samples at 395-, 436- and 456-nm wavelengths. **Figure S3:** Decomposition extent of *Eucalyptus regnans* leaves in Weeks 0, 12 and 26 of the experiment.

**Figure S4:** Decomposition extent of *N. cunninghamii* leaves in Weeks 0, 12 and 26 of the experiment. **Figure S5:** Apparent colour generation on each measurement occasion (a) and cumulative apparent colour values (b) of leaf samples from different plant species over the 26-week experimental period. **Figure S6:** True colour generation on each measurement occasion (a) and cumulative true colour values (b) of leaf samples from different plant species over the 26-week experimental period. **Figure S7:** True colour generation on each measurement occasion (a) and cumulative true colour values (b) of *E. regnans* bark 1 samples under different moisture conditions over the 26-week experimental period. **Figure S8:** True colour generation on each measurement occasion (a) and cumulative true colour values (b) of leaf and bark 1 samples of *E. regnans* under different moisture conditions over the 26-week experimental period. **Figure S9:** Correlation between cumulative true colour generation and percentage mass loss for leaf litter samples of all species at three different moisture statuses.