School of Molecular and Life Sciences

Drone-based remote sensing as a novel tool to assess restoration trajectory at fine-scale by identifying and monitoring seedling emergence and performance

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Declaration

To the best of my knowledge and belief this thesis contains no material previously published by any other person except where due acknowledgment has been made.

This thesis contains no material which has been accepted for the award of any other degree or diploma in any university.

Signature:

Date: 10/02/2020

Summary

Unmanned Aerial Vehicles (UAVs) are a recent addition to the remote sensing toolkit, yet are underused in restoration monitoring, particularly in comparison to other fields such as agriculture. A lack of translational research greatly hinders efforts to apply lessons learned in agriculture to a restoration context. This work presents an overview of the current state of UAV-based monitoring in ecological restoration, identifies several key gaps in UAV monitoring studies worldwide, and seeks to address these gaps through targeted studies. An exhaustive review of current literature identified several key knowledge gaps that are addressed in this work, most pertinently the lack of studies targeting extremely high resolution imagery, and the lack of studies focusing on the use of multi-sensor assemblages. This study shows for the first time that UAVs can be used to classify and track objects as small as individual seedlings and even seeds. Furthermore, additional study showed that the decline and mortality of seedlings over a simulated droughting period can be tracked with the use of multispectral and visible vegetation indices, and that individual seedlings can be indentified and tracked through time. The results are presented in a global context, and build towards the proposal of a one-pass solution for restoration monitoring, giving particular focus to the engineering requirements of multi-sensor pods the proposed solution would require. However, future work is still needed to identify A: ideal sensor choices to gain optimal outcomes with minimal financial and technical requirements, B: the best vegetation indices for tracking plant decline, particularly in identifying specific causes behind the decline, and C: optimal methods for automated classification of seedlings. While there are still questions to be answered before a UAV-based one-pass solution becomes an easily applied solution for restoration monitoring, it nonetheless represents the future of the field.

Contents

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Foreword

This thesis has been prepared as a series of self-contained papers. The status of each chapter in relation to their publication in peer reviewed journals is as follows:

-The general introduction has been published in *Remote Sensing* as a review article.

-Chapter one has been published in *Drones* as an experimental paper.

-Chapter two has been published in *Drones* as an experimental paper.

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Statement of candidate contribution

This thesis contains published papers and manuscripts in preparation for publication that are co-authored. The citations and breakdown of authorship contribution are listed below.

- Buters, T.M.; Bateman, P.W.; Robinson, T.; Belton, D.; Dixon, K.W.; Cross, A.T. Methodological Ambiguity and Inconsistency Constrain Unmanned Aerial Vehicles as A Silver Bullet for Monitoring Ecological Restoration. *Remote Sensing* **2019**, *11*, doi:ARTN 118010.3390/rs11101180.

I contributed 70% to this paper, including conception of the idea, methodology, analysis, investigation, data curation, writing, revision, and visualisation. A.T.C. contributed to methodology and analysis, A.T.C. and D.B. contributed to conceptualisation, A.T.C. and P.W.B. contributed to original draft preparation, A.T.C., P.WB, D.B, T.R, & K.W.D. contributed to review and editing, and A.T.C. and K.W.D. contributed to visualization.

-Buters; Belton; Cross. Seed and Seedling Detection Using Unmanned Aerial Vehicles and Automated Image Classification in the Monitoring of Ecological Recovery. *Drones* **2019**, *3*, doi:10.3390/drones3030053.

I contributed 80% to this paper, including conception of the idea, methodology, analysis, investigation, data curation, writing, revision, and analysis. A.T.C. contributed 15% to this paper including conceptualisation of the idea, editing, and analysis. D.B. contributed 5% to this paper in editing.

-Buters, T.; Belton, D.; Cross, A. Multi-Sensor UAV Tracking of Individual Seedlings and Seedling Communities at Millimetre Accuracy. *Drones* **2019**, *3*, doi:10.3390/drones3040081.

I contributed 75% to this paper, including conception of the idea, methodology, analysis, investigation, data curation, writing, revision, and analysis. A.T.C. contributed 20% to this paper including conceptualisation of the idea, editing, and analysis. D.B. contributed 5% to this paper in editing.

To whom it may concern,

I, Todd Michael Buters, contributed in conception of the idea, methodology, analysis, investigation, data curation, writing, revision, and visualisation for the paper Methodological Ambiguity and Inconsistency Constrain Unmanned Aerial Vehicles as A Silver Bullet for Monitoring Ecological Restoration. *Remote Sensing* **2019**, *11*, doi:ARTN 118010.3390/rs11101180.

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Rationale and outline of the thesis

While the use of UAVs in agricultural and forestry monitoring is well established, there has been little work done to bring UAV monitoring into ecological restoration, and what studies have been conducted have failed to develop the conceptual framework established by previous work. This thesis confronts significant knowledge gaps in UAV based restoration monitoring, specifically focusing on the ability of UAVs to conduct low level flights, and to carry a variety of sensors. I examined the utility of UAVs in fine-scale restoration monitoring by testing several hypotheses:

-UAV-based restoration monitoring is being conducted frequently around the world, but methods and results vary widely from study to study.

-UAVs operating at low altitude provide a high enough resolution to allow for both manual and automated identification of seeds and seedlings across a variety of substrates.

-Declining seedling health during a simulated drought can be monitored with visible and non-visible multispectral indices.

This thesis presents a unique approach to UAV-based monitoring of ecological restoration. The study first builds an understanding of how and why UAVs are used in restoration monitoring, and identifies several key gaps in their use, before proposing a "one-pass" solution for restoration monitoring. Secondly, a world-first study shows that UAVs can be used to classify and monitor objects as small as individual seeds and seedlings despite the presence of non-target grasses, and automated classification and counting can be conducted with commercially available computer programs. Thirdly, I show that the declining health of target and non-target seedlings can be monitored with visible and non-visible multispectral indices over the course of a simulated drought, and that individual seedlings can be identified and tracked over time. Finally, I provide an in-depth analysis of the proposed one-pass solution, covering potential uses and build requirements.

Review

Methodological Ambiguity and Inconsistency Constrain Unmanned Aerial Vehicles as A Silver Bullet for Monitoring Ecological Restoration

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Abstract: The last decade has seen an exponential increase in the application of unmanned aerial vehicles (UAVs) to ecological monitoring research, though with little standardisation or comparability in methodological approaches and research aims. We reviewed the international peer-reviewed literature in order to explore the potential limitations on the feasibility of UAV-use in the monitoring of ecological restoration, and examined how they might be mitigated to maximise the quality, reliability and comparability of UAV-generated data. We found little evidence of translational research applying UAV-based approaches to ecological restoration, with less than 7% of 2133 published UAV monitoring studies centred around ecological restoration. Of the 48 studies, > 65% had been published in the three years preceding this study. Where studies utilised UAVs for rehabilitation or restoration applications, there was a strong propensity for single-sensor monitoring using commercially available RPAs fitted with the modest-resolution RGB sensors available. There was a strong positive correlation between the use of complex and expensive sensors (e.g., LiDAR, thermal cameras, hyperspectral sensors) and the complexity of chosen image classification techniques (e.g., machine learning), suggesting that cost remains a primary constraint to the wide application of multiple or complex sensors in UAV-based research. We propose that if UAV-acquired data are to represent the future of ecological monitoring, research requires a) consistency in the proven application of different platforms and sensors to the monitoring of target landforms, organisms and ecosystems, underpinned by clearly articulated monitoring goals and outcomes; b) optimization of data analysis techniques and the manner in which data are reported, undertaken in cross-disciplinary partnership with fields such as bioinformatics and machine learning; and c) the development of sound, reasonable and multi-laterally homogenous regulatory and policy framework supporting the application of UAVs to the large-scale and potentially trans-disciplinary ecological applications of the future.

Keywords: Ecological restoration; Drone; UAS; Rehabilitation; Revegetation

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1. Introduction

Despite the common public perception of unmanned aerial vehicles (UAVs, drones) as a recent innovation predominantly for military application [1] or for photography [2], surveying or mapping [3], UAVs have been utilised as tools for biological management and environmental monitoring for nearly four decades [4]. Unmanned helicopters were employed for crop spraying in agricultural systems as early as 1990, and this method now accounts for >90% of all crop spraying in Japan [5]. UAVs are commonly used to conduct broad-acre monitoring of crop health and yield in Europe and the United States [6–9]. Additionally, they have been used for atmospheric monitoring projects such as the measurement of trace compounds since the early 1990s [10,11]. While cost and availability have been a broad constraint to the use of UAVs in research projects, the last decade has seen an exponential increase in their application to ecological research.

Rapid advances in technology have produced increasingly smaller, cheaper drones capable of mounting a wider variety of sensors that are able to more rapidly collect a greater diversity of data [12–14]. In addition, improved battery technology has greatly improved the endurance offered by electric models [9,15]. Although highly specialised UAVs require significant financial and infrastructure investment, entry-level drones are commercially available at low cost and are increasingly capable of capturing meaningful data—for example, estimates have been provided of UAV platform and sensor costs ranging from 2000 euros for less advanced systems, up to 120,000 euros for large UAVs with hyperspectral sensors [13]. While even low-cost UAVs may represent a significant expenditure for smaller projects, the price is still low in comparison to obtaining remotely sensed data from manned aircraft or satellites. Correspondingly, the last decade in particular has seen UAVs employed with increasing novelty as tools to address complex ecological questions [16–19]. One field that has benefited from this application in particular is the monitoring of environmental rehabilitation and ecological restoration [20].

Traditional monitoring of ecological restoration is often undertaken manually and can involve transects or quadrats over large areas and frequently on sandy or rocky substrates in remote regions [21,22]. UAVs on the other hand are able to traverse large areas in very short periods of time [23,24], are unaffected by the difficulty of the terrain [25], and have negligible impact upon ecologically sensitive areas or species of interest [16,26,27]. UAVs can collect large amounts of high-resolution images even during short flights [28], allowing scientists to conduct virtual site surveys. In addition to factory-standard digital Red-Green-Blue (RGB) cameras carried by most commercial UAVs, sensors also include multispectral and hyperspectral cameras, thermal imaging, and LiDAR units [9]. Although the accessibility and capability of both UAVs and UAV-mounted sensors continue to improve [13,25], this improvement has occurred asynchronously in relation to translation research and development for the effective, replicable and accurate use of UAVs in the monitoring of ecological restoration.

Historically, robots have been employed to undertake 'the three D's'; tasks that are considered too dirty, dangerous or dull for humans [29]. Although this also reflects the focus of early UAV application and research [4], we propose the future direction of UAV work is another four D's: the collection of detailed data over difficult or delicate terrain. However, full realization of the potential of UAV use in ecological monitoring requires more than simple technological innovation and improvement. The effective implementation of UAV technology by ecologists at the required scales and replicability is likely to be constrained by inconsistency in the application of different sensors to the monitoring of target landforms, organisms and ecosystems, a degree of uncertainty around the optimization of data analysis techniques and reporting to meet project-specific monitoring goals and objectives, and the absence of sound, reasonable and multi-laterally homogenous regulatory and policy framework development in alignment with increasing societal and scientific expectations and aspirations for ecological recovery. We discuss the potential implications of these limitations on the feasibility of UAV use in the monitoring of ecological restoration, and recommend how they may be addressed in order to maximise the quality, reliability and comparability of UAV-generated data.

2. Materials and Methods

We compiled a database of peer-reviewed literature composed of studies employing UAVs in the monitoring of ecological recovery. Ecological recovery projects employ a wide range of terminology depending upon the particular goals of individual projects [30]. For the purposes of this review, we use the term 'restoration' following the widely accepted terminology of McDonald et al., defined as 'the process of assisting the recovery of an ecosystem that has been damaged, degraded or destroyed' [31,32]. Additional search terms were 'UAV', 'UAS', 'RPAS', 'drone' AND 'monitoring' AND 'restoration', 'rehabilitation', 'recovery', 'revegetation', or 'remediation'. Three databases were included in compiling the literature, including Google Scholar (date range from 1950–2019), Web of Science (all databases, date range from 1950–2019), and Scopus (all documents, all years). The results from literature searches are presented in a PRISMA 2009 flow diagram (Figure S1).

Searches returned 121 results on Web of Science, 235 results on Scopus, and 61,553 results on Google Scholar. The extreme magnitude of the difference between databases seems to be a result of how searches were conducted. While Google Scholar returned numerous papers that only contained keywords within the references section, this did not occur in searches conducted on Web of Science or Scopus. Removal of duplicates and out-of-scope papers (e.g., UAV-use in monitoring the restoration of building facades, or articles on the recovery of UAVs that had lost signal) resulted in 2133 articles relating to UAV-use in monitoring broadly. These predominantly comprised articles of silvicultural or agricultural monitoring application (e.g., 210 articles returned by "UAV forest monitoring", 206 articles returned by "UAV agriculture monitoring", and 238 articles returned by "UAV crop monitoring"). In total 56 papers related to UAV-use in ecological recovery monitoring, of which 48 studies presenting experimental data were included in analyses (Table S1).

Publications were sorted into categorical variables based on publication type (e.g., studies presenting experimental data, theoretical development, literature reviews); ecological recovery terminology (restoration, rehabilitation, recovery, revegetation or remediation); pre-recovery land use (agricultural, natural); UAV type (e.g., multirotor, helicopter, fixed-wing or not stated); maximum number of sensors employed in a single flight (one, two, three, or four); type of sensor(s) employed (RGB, modified-RGB (here referring to commercial RGB cameras that have been modified to detect near-infrared (NIR) light), Multispectral, Hyperspectral, Thermal, LiDAR); and method of analysing captured data (manual, object-based, supervised machine-learning). For the purposes of analysis, sensors were classified into four categories of increasing technological complexity. The classification increased in complexity from 'Non-complex' sensors including RGB-alone (category 1) or modified-RGB cameras (category 2), to 'complex sensors', defined here as those designed to capture non-visible light, including multispectral sensors (category 3) and other (Hyperspectral, thermal, LiDAR; category 4). Similarly, analytical techniques were classified in increasing complexity from manual classification (category 1), to Object-Based Image Analysis (OBIA; category 2), and machine learning (category 3). While automated classification methods can be performed at either pixel level or on image objects, OBIA was commonly used throughout the reviewed papers in order to denote the use of a manually generated rulesets being used to classify images that were segmented into image objects, most commonly through the program eCognition. While OBIA is technically an approach classifying images-based upon created image objects rather than on a per-pixel basis that can be used with any means of classification, we adopt this terminology when referring to any instance of user-defined image-object classification for consistency with the published literature.

Pearson's Chi-square tests were undertaken to compare differences between all categorical variables (SPSS Statistics 25, IBM, USA), with statistical significance determined by $P \leq 0.05$. Multinomial logistic regression (SPSS Statistics 25, IBM, USA) was undertaken to assess the relationship between sensor complexity (categorized) and the complexity of analytics (categorised) in published studies, including year of study publication as a control variable.

3. Results

3.1. Date and Origin of Studies

Two thirds of the studied literature had been published since 2017 (Figure 1), and only one publication predated 2007 (published in 1996). Studies were significantly more likely to originate from Europe (31% of papers) or North America (29%) than other regions ($\chi 2 = 23.46$, d.f. = 5, $P = 0.001$). Far fewer studies were returned from regions such as China (13%), Australia and Central and South America (10% each), southeast Asian countries (6%), and Africa (2%).

3.2. Terminology

'Restoration' was the most commonly employed terminology in the literature analysed (> 70% of published studies) followed by 'recovery' (ca. 30%), while 'rehabilitation', 'revegetation', and 'remediation' were infrequently used (8–13% in each case; Figure 1). Studies frequently employed multiple terms in relation to the same ecological monitoring project, with a third of studies using two or more terms (most commonly 'restoration' and 'recovery').

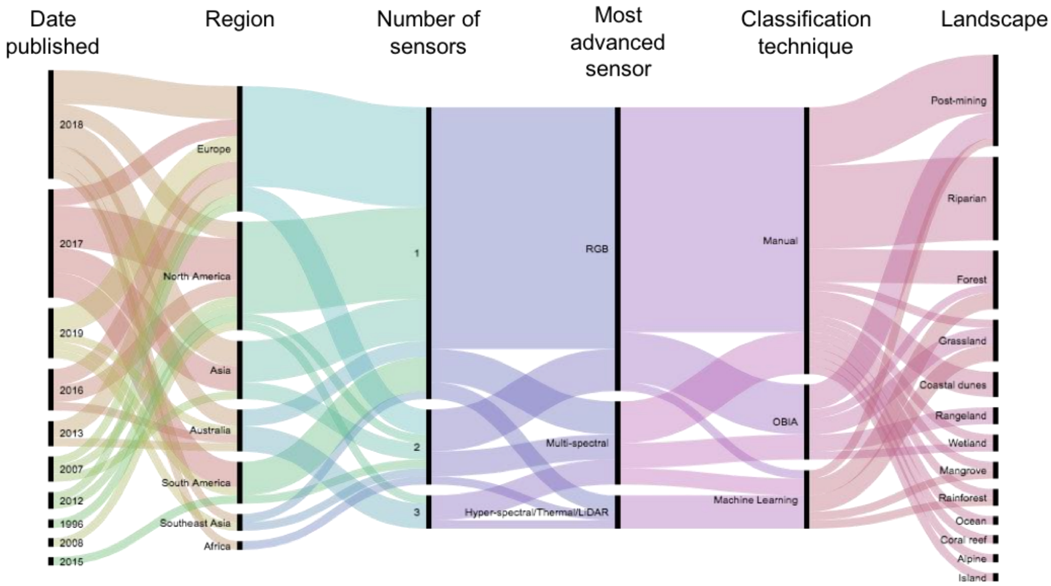

Figure 1. Alluvial diagram illustrating the proportion of research publications from 1996 to 2019 from a literature search over Scopus, Web of Science and Google Scholar providing empirical data on the use of UAVs in the monitoring of ecological recovery in terms of including date of publication, region of study interest, ecological recovery terminology utilised, maximum number of sensors utilised in a single flight, most advanced sensor type utilised during the study, captured imagery classification technique, and landscape of study interest.

3.3. Platform and Sensors

Studies employed a relatively even use of fixed-wing (42%) and multirotor (38%) UAVs (including two articles that utilised both), and these platforms were significantly more common in monitoring than other platforms ($\chi2 = 49.38$, d.f. = 5, $P < 0.001$). Additional studies also employed unmanned helicopters (two studies) or paramotors (one study), while ten percent of studies presented no information about the type of UAV utilised (Figure 1).

Studies overwhelmingly employed only a single sensor (73%; $\chi2 = 34.63$, d.f. = 2, $P < 0.001$). Although 13 studies utilised multiple sensors in data capture, only five employed two sensors simultaneously on flights and only a single study demonstrated the use of more than two sensors simultaneously (however, this comprised two duplicate sensor pairs). RGB cameras were by far the most commonly utilised sensors in the monitoring of ecological recovery (employed in nearly 85% of studies; $\chi2 = 49.38$, d.f. = 5, $P < 0.001$), with more complex non-RGB sensors (e.g., LiDAR, hyperspectral sensors) uncommonly employed (29% of studies). Where more complex non-RGB sensors were used, they were often employed in combination with RGB cameras (half of the 14 studies). RGB cameras were the only sensor employed in nearly 60% of the literature, while true multispectral sensors were employed in 11 studies (22% of studies), hyperspectral sensors in three studies, and thermal cameras and LiDAR sensors in one study each (Figure 2).

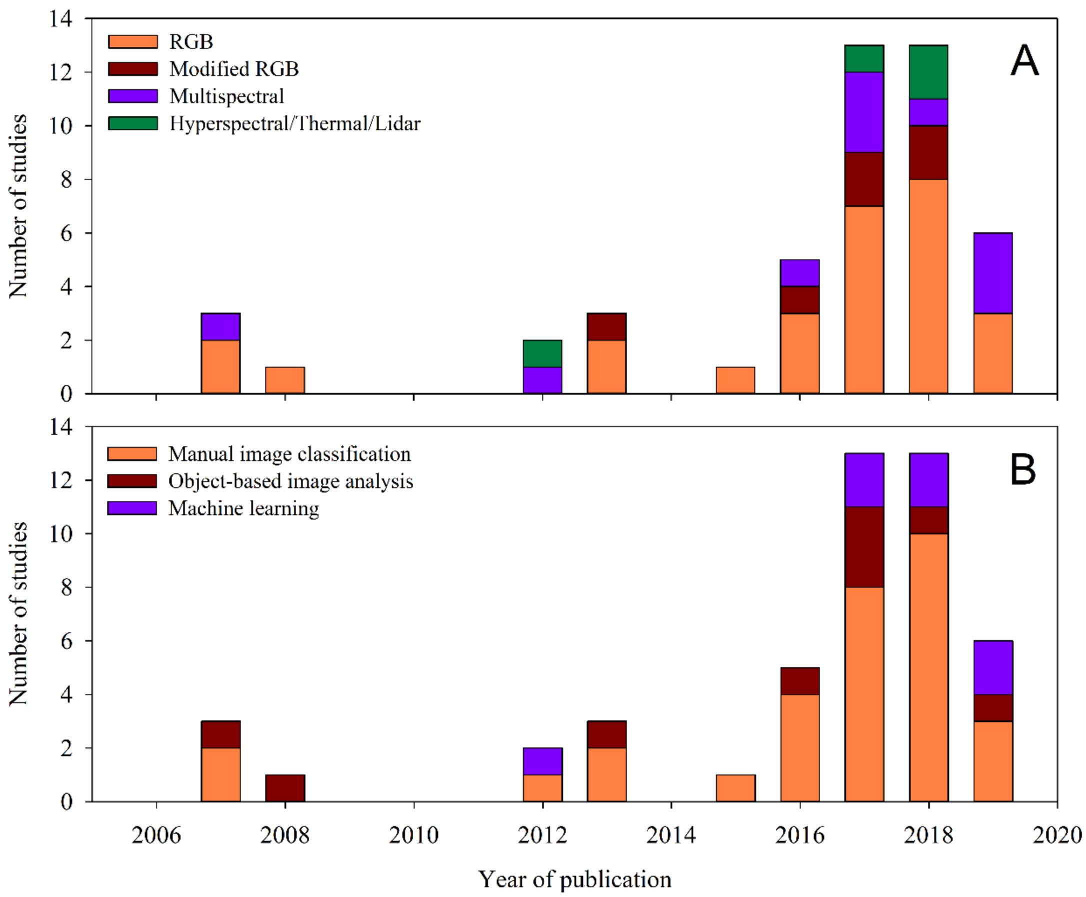

Figure 2. Number of analysed studies employing sensors of various complexity (**A**) and captured image classification techniques of varying complexity (**B**) in relation to year of publication for UAV-based studies monitoring ecological recovery. * Data were collected up until March 2019, thus data are presented for only the first quarter of 2019.

3.4. Classification and Processing of Captured Data

While all studies utilised manual image classification, the majority (67% of studies; $\chi2 = 24.12$, d.f. = 3, $P < 0.001$) used solely manual classification, without utilising any automated methods. More complex methods of image classification included OBIA (19%), with accuracy assessments undertaken manually, and machine learning (15%), again with accuracy assessments undertaken manually. Almost all studies utilising OBIA undertook classification in the eCognition environment (89%; Figure 2), while studies utilising supervised machine learning employed eight different algorithms with little comparability in methodology. No studies compared the results of classification by machine learning with OBIA.

Multinomial logistic regression indicated a statistically significant relationship was present between sensor complexity and the complexity of classification with year as a control variable ($\chi2 = 43.68$, d.f. = 24, P = 0.008). Likelihood Ratio Tests indicating that the effect of sensor complexity on the complexity of analytics was significant ($\chi2 = 30.11$, d.f. = 6, P < 0.001) while the effect of year was not ($\chi2 = 16.34$, d.f. = 18, P = 0.569), indicating that this trend was unlikely to reflect the application of more technically complex platforms by more recent studies (Figure 3).

4. Discussion

The application of UAVs in plant health monitoring is well established for agriculture [9,33–36] and forestry [14,37,38]. However, there is little evidence of translational research applying these approaches to ecological restoration, with UAV use in the monitoring of ecological recovery comprising less than 7% of studies (56 out of 2133 studies) of the UAV monitoring literature. Importantly, we found no evidence of UAV assisted plant condition analysis being proven despite this claim by many in the UAV vegetation assessment industry. This low representation may reflect the nascence of UAVs for ecological monitoring, as > 65% of all literature analysed was published in the three years preceding this study. However, trends observed in the growing body of literature in this field suggest a level of regional bias, poor reporting of project-specific monitoring goals and outcomes, a degree of inconsistency in the application of different sensors to achieving monitoring goals, and low comparability between image processing techniques. We propose that if UAVs are to represent the future of ecological monitoring, research requires a) greater consistency in the application of different platforms and sensors to the monitoring of target landforms, organisms and ecosystems, underpinned by clearly articulated monitoring goals and outcomes; b) optimization of data analysis techniques and the manner in which data are reported, undertaken in cross-disciplinary partnership with fields such as bioinformatics and machine learning; c) the development of sound, reasonable and multi-laterally homogenous regulatory and policy frameworks supporting the application of UAVs to the large-scale and potentially trans-boundary ecological applications of the future; and d) links to plant physiologists to validate UAV-based interpretations of plant health and vegetation condition.

4.1. Regional Bias in UAV Application

Despite the increasingly lower costs of UAVs and their sensor payloads, the use of UAVs in restoration monitoring is still geographically limited. Much of the literature analysed (ca. 58% of studies) originated from Europe and North America, with most remaining studies from China, Australia, and Central and South America (Figure 1). However, given the relatively low skillset required for UAV use [39], UAVs are a viable prospect for monitoring of restoration and conservation projects in developing countries [40]. The coming years are likely to see a far greater global application of UAV monitoring research, particularly as developing countries begin to take advantage of the increasing availability and affordability of UAVs.

4.2. A Need to Realise the Full Potential of UAV Platforms and Sensors in Monitoring Ecological Recovery

Despite the increasing complexity, affordability and reliability of commercially available UAVs, the use of UAVs in ecological recovery monitoring is lagging compared with the rapidly growing portfolio of their novel application in other ecological contexts such as fauna monitoring [40–42]. Where studies have utilised UAVs for rehabilitation or restoration applications, there continues to be a propensity for single-sensor monitoring using commercially available drones fitted with modest-resolution RGB sensors (Figure 1). Fixed wing and multirotor UAVs were used in similar numbers, perhaps due to the different scales of operation. While multirotor UAVs have several advantages over fixed wing platforms, such as reduced vibration and a smaller required area for takeoff and landing, the higher cruise altitude and greater endurance of fixed wing UAVs makes them a superior choice when monitoring large areas that do not require sub-centimetre resolution [13]. Very few articles sourced (29% of studies) employed complex sensors or multiple-sensor assemblies (Figure 1). The strong correlation between the application of these complex sensors and increasingly detailed image analytical techniques (Figure 3), both representing comparatively expensive technologies, suggests that cost may be a primary constraint to their wide application in research.

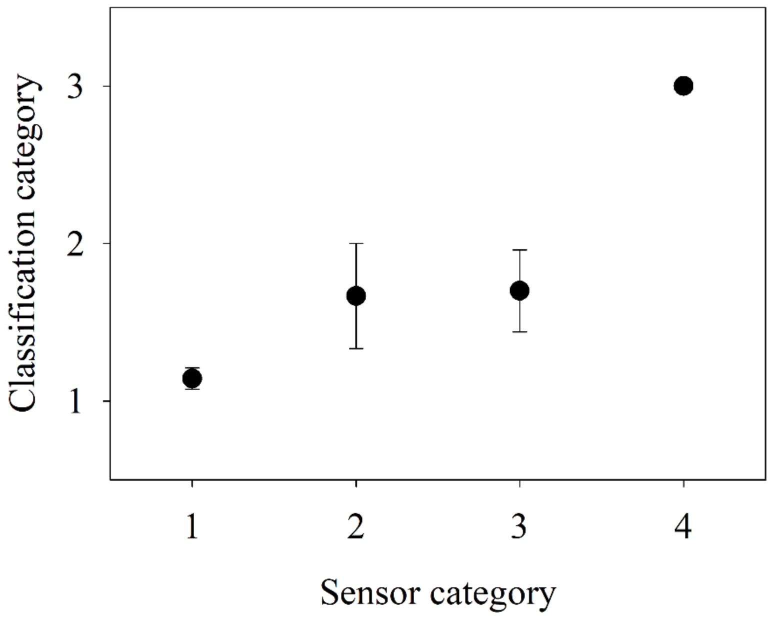

Figure 3. Association between categorised sensor complexity and categorised classification technique complexity in analysed studies of UAV-based monitoring of ecological recovery. Annotated lettering represents the results of pairwise tests among sensor complexity categories. Values followed by the same letters are not significantly different at P = 0.05.

RGB imagery represents the most rudimentary remote sensing option for UAV use, but the near-universal application of RGB sensors in the literature analysed highlights their significant utility (and accessibility) for restoration monitoring. For example, low-altitude high-resolution RGB imagery has been used to accurately estimate tree height, crown diameter and biomass in forested areas [14,43–45], and has been employed to determine the presence or absence of target plant species [46–49]. One particularly attractive feature of RGB imagery is its capacity to be stitched into large orthomosaics using, for example, Structure from Motion (SfM) techniques [50]. Orthomosaics retain the ground sampling distance (GSD) of initial images, and high-resolution imagery provides a high level of detail facilitating visual recognition and classification of plant and animal species or individuals [23]. SfM technology also allows for the creation of digital elevation models (DEMs) from RGB images. DEMs created in this manner can approach the accuracy of more expensive and complex sensors such as LiDAR, and have been employed for measurements of tree height and biomass estimation [51,52]. Studies have used DEMs generated from UAV imagery for the creation of slope maps [53], and for the monitoring of the stability of tailings stockpiles [54]. Given the accuracy of these DEMs and the cost of LiDAR surveys, geomorphological mapping from RGB imagery obtained from UAVs is a viable alternative [3].

We found few examples of fauna monitoring using UAVs in a restoration context, potentially reflecting the global paucity of literature relating to fauna recovery following disturbance [55]. However, RGB-equipped UAVs have been employed to assess distribution and density in fauna species that are challenging to survey on foot, such as orangutan (*Pongo abelii*) [56] and seabird colonies [57,58]. Their application appears most suitable for the covert observation and recording of ecological, behavioural and demographic data from fauna communities in poorly accessible habitats [59–61], and UAV-derived count data can be up to twice as accurate than ground-based counts [62,63]. Although the ontogenetic and species-specific responses of fauna to UAVs remain unknown [64,65], particularly in regard to behavioural responses of fauna following interactions with UAVs [66–68], it seems likely that UAV-use in fauna monitoring will rapidly expand as technology improves and costs decline [42]. For example, many GPS units attached to animals for research are accessed by remote download, and UAVs may facilitate both target location via VHF signal and remote download of GPS data without the need to approach, or disturb, the animal.

Multi- and hyperspectral imagery has been employed to successfully discriminate between horticultural and agricultural crops on the basis of spectral signature [18,69–71], to estimate leaf carotenoid content in vineyards [72], to determine plant water stress in commercial citrus orchards [73], assess the health of pest-afflicted crops [74], and estimate the leaf area index of wheat [7]. However, there are few examples of multi- or hyperspectral sensor application in native species ecological recovery monitoring (only 23% of studies used a multispectral sensor, and only 6% used a hyperspectral sensor), possibly due to the markedly higher cost of these sensors compared with RGB sensors. Although it should be noted that commercial RGB cameras can be modified in such a way that they can detect red-edge and near infra-red light in a similar fashion to multispectral sensors, and that this technique has been used effectively, for example, to map the distribution and health of different species in vegetation surveys of forested areas and peat bogs [18,20]. Combinations of RGB and multispectral imagery may allow for high levels of accuracy in species-level discrimination, which would be of significant utility for the identification and potential classification of small plants (e.g., seedlings), estimation of invasive plant cover, or the targeted mapping of rare or keystone species in restoration projects.

Where multiple sensors have been employed, they often required multiple flights with sensor payloads being exchanged between flights [75–77]. Thermal imagery was used by Iizuka et al. [76] to monitor thermal trends in peat forest, and a LiDAR/hyperspectral fusion has been proven to be more effective in vegetation classification than either sensor alone [77]. UAVs equipped with thermal sensors have been used to locate and monitor fauna species such as deer, kangaroos and koalas [78,79], including large mammals of conservation concern such as white rhinos (*Ceratotherium simum*) [80]. They have also been applied to detecting water stress in citrus orchards [73], olive orchards [6], and barley fields [81], although only at small scales (<32 ha). More advanced sensors such as multispectral and thermal cameras may also have utility in the monitoring of disturbance-related environmental issues important for some rehabilitation and restoration scenarios, such as methane seepage, acid mine drainage and collapse hazards in near-surface underground mining [82]. However, further work is required to translate thermal imagery studies conducted in agricultural or forestry settings to a restoration context.

4.3. Analysis and Reporting of Restoration Monitoring Data

Comparison of the data gathered by UAVs is limited by considerable disparity in the analytical and processing methods used by various studies. While manual classification of imagery is generally undertaken comparably across all studies, it can be slow, time-consuming, and has potential for operator error, offsetting the time benefits of UAV-based monitoring [28]. Thus, automated image classification is a clear direction for the technology to maximise the utility, efficiency and cost-effectiveness inherent in UAV monitoring. Importantly, techniques such as OBIA and machine learning have proven to be of significant utility in UAV monitoring to date [35].

In OBIA, images are split into small "objects" on the basis of spectral similarity and are then classified using a defined set of rules [35]. The advantage of this method is that the objects created can be classified based on contextual information such as an object's shape, size, and texture, which provides additional capacity to the spectral characteristics [83]. These factors make object-based image analysis an extremely useful tool for restoration monitoring, as species-level classification can be undertaken on imagery of sufficiently high resolution. The first OBIA software on the market was eCognition [83], and this continues to be employed almost ubiquitously (89% of studies). eCognition has been employed in a diverse range of applications, for example to identify and count individual plant species of interest within a study area from multispectral UAV imagery [84], to map weed abundance in commercial crops [85,86], and to identify infestations of oak splendour beetles in oak trees [38]. Studies employing eCognition for automated imagery classification in the context of ecological recovery generally undertook relatively simple tasks such as vegetation classification and counting [20,46,47], generating maps of vegetation cover over time [87], and identifying terraces on a plateau [88]. However, some recent studies have broadened the scope of their classification processes and assessed additional information in order to provide a greater level of detail. For example, while many studies identified areas of bare ground, Johansen et al. [89] further classified bare ground based on topographical position. While most studies on species discrimination were content to only identify target species, Baena et al. [18] further separated the keystone species *Prosopis pallida* (Algarrobo) into distinct classes representing the health of the tree supplemented by ground truthing. Even without the additional spectral bands offered by multispectral imagery, object-based image analysis is a powerful classification tool, in some applications, such as the classification of coastal fish nursery grounds by identifying seabed cover type [90]. Notably, this study compared OBIA with non-OBIA and two different methods of machine learning, and found OBIA to be the most accurate. Given the ability of OBIA to layer the output from multiple sensors into one project for classification, the technique will only increase in utility as more and more sensors become practical for UAV-based deployment. OBIA represents a powerful tool for reducing the workload required for repeated monitoring of restoration areas.

Machine learning can be separated into supervised and unsupervised methods [17]. Both have been applied to UAV surveys to good effect. Unsupervised machine learning relies on user input to define how many classes an image should be separated into, and then classifies each pixel in such a way that the mean similarity between each class is minimised [17]. This technique has been applied successfully to UAV imagery to classify vernal pool habitats [17], to classify vegetation in mountain landscapes [91], and to detect deer in forested areas [78]. Supervised classification relies on the input of selected examples as training data, but provides a higher level of accuracy [17]. However, supervised learning requires sufficient training data to capture the variance of the targets, and should avoid spatially dependent samples [92]. For example, one study that assessed the automated identification of cars from UAV imagery required 1.2 million vehicle images as training data, all of a common required size, to minimise false negatives [93]. Negative examples must also be provided. However, despite these requirements, machine learning is a powerful tool for automated classification and has previously been used in UAV-based studies to accurately classify tree crowns in orchards

[6,71], to identify different species of trees and weeds [94,95], to detect algae in river systems [96], to estimate biomass of wheat and barley crops [34], and to identify and map riparian invasive species [84]. Several of the reviewed studies (15%) applied machine learning techniques to ecological recovery projects, reporting accuracies over 90% in creating classified vegetation maps of forests [76,97] and restored quarries [98]. Overall, supervised classification returns superior levels of accuracy compared with unsupervised classification, particularly in cases with minimal spectral differentiation [35].

While UAVs can rapidly and effectively map restoration areas with multiple different sensors, the increasingly large volumes of data gathered render image classification a complex task. Although machine learning programs and other automated methods of image classification can overcome this limitation, there appears to be little consistency in the approaches that have been employed in image classification automation. We found studies utilised an even mix of machine learning and OBIA approaches, with little comparability or uniformity in specific software or methodology used. Only three studies provided a comparison of different methods of automated classification. While eCognition was by far the most common software package employed in automated image classification, it was generally utilised without reference to the methodological approaches of previous studies and few articles considered other potential options for classification. We propose that the apparent lack of clarity surrounding the most appropriate methodological approaches to image classification in ecological monitoring could be addressed through greater collaboration between restoration ecologists and specialised data analysts, remote sensing experts, and computer scientists to ensure that the most accurate and efficient methods are being used. Additionally, consideration should be paid to whether or not UAV-based remote sensing is the most appropriate method of data acquisition for the specific requirements of each project.

It is also noteworthy to recognise the increasing use of UAV-based LiDAR, which is rapidly becoming a practical alternative to UAV-based imagery in situations where penetration of water of plant canopy is desired in order to image underlying topography [99]. Although drone laser scanning technology (e.g., LiDAR) is currently more commonly employed by non-UAV remote sensing platforms (e.g., manned aircraft and satellites), increasing technological development of these sensors will improve accessibility of this technology as a surveying option and thus greatly improve its utility for UAV application into the future [99].

4.4. Regulatory and Community Expectations of Restoration Monitoring

The regulatory and community expectations of restoration monitoring continue to increase as the international community aspire towards better ecological recovery outcomes at larger scales [30,32]. Monitoring must be tied to specific targets and measurable goals and objectives identified at the start of the project, and the establishment of baseline data, as well as the collection of data at appropriate intervals after restoration works, is fundamental for achieving appropriate measures of success [32]. Additionally, sampling units must be of an appropriate size for the attributes measured, and must be replicated sufficiently within the site to allow for meaningful interpretation [32]. These requirements concur with the possibilities offered by UAV-based remote sensing, as highly detailed data can be gathered quickly and easily from small to medium areas (up to ca. 250 hectares with current technology) [100].

The National Standards for the Practice of Ecological Restoration [32] state that restoration science and practice are synergistic, particularly in the field of monitoring. Restoration practice is often determined by legislative requirements, and thus legislative requirements should be supported by scientific knowledge. While UAVs are being increasingly integrated into aviation legislation worldwide, and following local aviation law is undeniably important, legislation covering the use of UAVs in restoration monitoring is also needed to provide guidelines for practitioners to follow. However, the confusion in the use of UAVs ranging from choice of platform, choice of sensor, and choice of classification method is such that enacting legislative requirements would be of very little value. Until there is a strong scientific consensus on best practice, there should be no legislative guidelines introduced. Rather, effort should be focused towards conducting further studies in order to bring about consensus.

5. Conclusions

Monitoring of ecological restoration efforts is a requirement for ensuring goals are met, determining trajectories and averting potential failures. However, despite technological advances in its practice, ecological monitoring generally continues to be conducted with a 'boots on the ground' philosophy. Although UAV-mounted sensors are increasingly being used in novel ways to monitor a wide variety of indicators of ecosystem health, the technology is consistently applied to examine only highly specific aims or questions and fails to consider the wide and increasing potential for capturing associated ecological data. The ongoing miniaturization, affordability and accessibility of UAV-mounted sensors presents an increasing opportunity for their application to broad-scale restoration monitoring. Although the wide application and low cost of RGB sensors mean they are likely to remain a staple component of the UAV-based monitoring toolbox into the future, sensing in the visible spectrum alone may represent a missed opportunity to gather significantly more complex and meaningful data where project goals and finance allow. We propose that the collection of default RGB imagery is self-limiting, and the future of monitoring lies in UAVs equipped with multiple sensors that can provide a complete understanding of restoration efforts in the span of a single flight, supported by robust computer-aided analytical technologies to mitigate time-intensive image classification processes. However, few studies currently utilise more than one or two sensors, and even those that do use three or more require multiple flights with different drones due to weight and power limitations.

The ecological monitoring industry requires more resilient and reliable UAVs capable of multiple sensor payloads, enabling more effective data acquisition. However, future UAV research efforts should also adopt a more consistent and replicable approach to undertaking ecological research, incorporating a broader scope of complementary sensors into UAV-based monitoring projects and undertaking image analysis and classification using comparable methodological approaches. Although we are rapidly approaching an era in which UAVs are likely to represent the most cost-efficient and effective monitoring technology for ecological recovery projects, the full potential of UAVs as ecological monitoring tools is unlikely to be realized without greater trans-disciplinary collaboration between industry, practitioners and academia to create evidence-based frameworks.

Supplementary Materials: The following are available online at www.mdpi.com/xxx/s1, Figure S1: "The PRISMA flow diagram for Buters et al. (2019), detailing the databases searched, the number of non-duplicate articles found, and the final number of relevant articles retrieved, after discarding papers that did not present experimental results.", Table S1: "All analysed unmanned aerial vehicle (UAV) studies providing empirical data on the use of UAVs in the monitoring of ecological recovery by Buters et al. 2019, including year of publication, region of study interest, landscape of study interest, ecological recovery terminology utilised, UAV platform employed, captured imagery classification technique, maximum number of sensors utilised in a single flight, and most advanced sensor type utilised during the study."

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Article

Seed and Seedling Detection Using Unmanned Aerial Vehicles and Automated Image Classification in the Monitoring of Ecological Recovery

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Abstract: Monitoring is a crucial component of ecological recovery projects, yet it can be challenging to achieve at scale and during the formative stages of plant establishment. The monitoring of seeds and seedlings, which represent extremely vulnerable stages in the plant life cycle, is particularly challenging due to their diminutive size and lack of distinctive morphological characteristics. Counting and classifying seedlings to species level can be time-consuming and extremely difficult, and there is a need for technological approaches offering restoration practitioners with fine-resolution, rapid and scalable plant-based monitoring solutions. Unmanned aerial vehicles (UAVs) offer a novel approach to seed and seedling monitoring, as the combination of high-resolution sensors and low flight altitudes allow for the detection and monitoring of small objects, even in challenging terrain and in remote areas. This study utilized low-altitude UAV imagery and an automated object-based image analysis software to detect and count target seeds and seedlings from a matrix of non-target grasses across a variety of substrates reflective of local restoration substrates. Automated classification of target seeds and target seedlings was achieved at accuracies exceeding 90% and 80%, respectively, although the classification accuracy decreased with increasing flight altitude (i.e., decreasing image resolution) and increasing background surface complexity (increasing percentage cover of non-target grasses and substrate surface texture). Results represent the first empirical evidence that small objects such as seeds and seedlings can be classified from complex ecological backgrounds using automated processes from UAV-imagery with high levels of accuracy. We suggest that this novel application of UAV use in ecological monitoring offers restoration practitioners an excellent tool for rapid, reliable and non-destructive early restoration trajectory assessment.

Keywords: ecological restoration; object-based image analysis; rehabilitation; remote sensing; monitoring

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1. Introduction

Ecological restoration is an intentional activity that initiates or accelerates the recovery of an ecosystem with respect to its health, integrity and sustainability [1]. In order to identify whether recovery efforts result in acceptable trajectories toward a desired reference endpoint (e.g., plant communities are establishing towards predetermined targets or goals, usually a resilient, functional and self-sustaining ecosystem), restoration projects undertake ongoing monitoring of a wide range of environmental parameters [2]. However, these diverse parameters often require monitoring at different spatial and temporal scales.

Monitoring needs can be idiosyncratic and complex between different sites, as project requirements can be influenced by factors such as substrate, local vegetation communities and local geomorphology [3]. Examinations of developing plant communities, such as plant establishment from broadcast seeds, species diversity, canopy cover and plant performance, are increasingly becoming commonly desired monitoring outcomes for ecological restoration projects [4,5].

High levels of mortality during the seed germination, seedling emergence and early establishment phases, often exceeding 95%, are considered the most significant bottleneck in ecological restoration [6,7]. The scale of seed-based ecological restoration projects has increased dramatically in recent decades [8], and it is now frequently undertaken in areas where traditional on-foot surveys of seedling emergence and early plant establishment can be challenging (e.g., large post-mining landforms such as waste rock dumps and tailings storage facilities). Additionally, counting and classifying seedlings to species level can be time-consuming and extremely difficult in diverse seedling communities [9–11]. As such, there is an increasingly urgent need for technological approaches offering restoration practitioners with fine-resolution, rapid and scalable plant-based monitoring solutions.

Among the suite of new technologies offering advances in ecological monitoring, unmanned aerial vehicles (UAVs) have significant potential for improving plant-based outcomes. The monitoring of vegetation growth and performance greatly benefits from UAV-based remote sensing [12], mainly due to the scales at which monitoring must be undertaken. UAVs are considered the best option for remote sensing at scales up to 250 ha [13], as they offer greater spatial resolution compared with satellite imagery [14], significantly lower operational costs compared with manned aircraft [15], and can be operated in weather conditions that would prevent both satellites and manned aircraft from gathering meaningful data [16,17]. Furthermore, remote sensing using UAVs offer greater repeatability and lower turnaround times than both satellites and manned aircraft [18]. While alternative sensing platforms such as pole photogrammetry may offer utility in the monitoring of fixed and small-scale areas of interest (e.g., vegetation monitoring quadrats), UAV-based remote sensing techniques have been successfully applied to the classification of adult plants (species of invasive trees and shrubs) in bogs and disturbed forest environments [19–21], as well as to monitor plant health in a variety of agricultural species [22–25]. However, the utility of UAVs has been demonstrated almost exclusively in agricultural settings, and little translational research has been undertaken to apply this technology to plant-based monitoring in ecological restoration [26].

This study tested the utility of a small, commercially-available UAV to monitor seed germination and seedling establishment in restoration. We tested the accuracy of eCognition, an object-based image analysis (OBIA) software package, for seed and seedling classification from captured imagery by comparing two OBIA rulesets we developed with the manual object classification of seeds and seedlings on soil surfaces of different color and texture (reflective of local restoration substrates), at multiple flight altitudes. It was hypothesized that OBIA using UAV imagery would readily and effectively discriminate target seeds and seedlings from background soil with a high level of accuracy, but that this accuracy would decrease, and then the rates of false positive classifications would increase, with in turn, increasing background soil complexity (greater texture and lower contrast), increasing cover of non-target seedlings, and increasing flight altitude.

2. Materials and Methods

2.1. Study Site

The study was conducted at the University of Western Australia Shenton Park Field Station, Perth, Western Australia. Unmanned aerial vehicle (UAV) flights were undertaken over a 400 m² trial area divided into four experimental 100 m² plots (10 m × 10 m) with different surface treatments representing local restoration substrates in Western Australia (Figure 1).

Surface treatments included a '*control*' of undisturbed local sandy soil (smooth, homogenous texture and light background color), representative of typical post-mining substrate in *Banksia* woodland restoration [27]; a '*textured*' treatment of undisturbed local sandy soil with scattered crushed overburden rock (2–20 cm in size, giving increased surface heterogeneity) used to rock armor the slopes of restoration landforms [28]; a '*dark*' treatment of undisturbed local sandy soil capped with a 1 cm layer of tailings generated from the processing of magnetite ore [29]; and a '*high red-ratio*' treatment of undisturbed local sandy soil capped with a 1 cm layer of red clay loam soil from a mine site in the Midwest region of Western Australia [28]. All capping materials were sourced from a major magnetite mining operation located approximately 400 km northeast of Perth, Western Australia. Additionally, to examine whether seed and seedling detection rates were affected by surface topography, half of each treatment plot was manually ripped to a depth of 20 cm, using a backhoe (Figure 1) to mimic standard ripping practices in post-mining restoration [27].

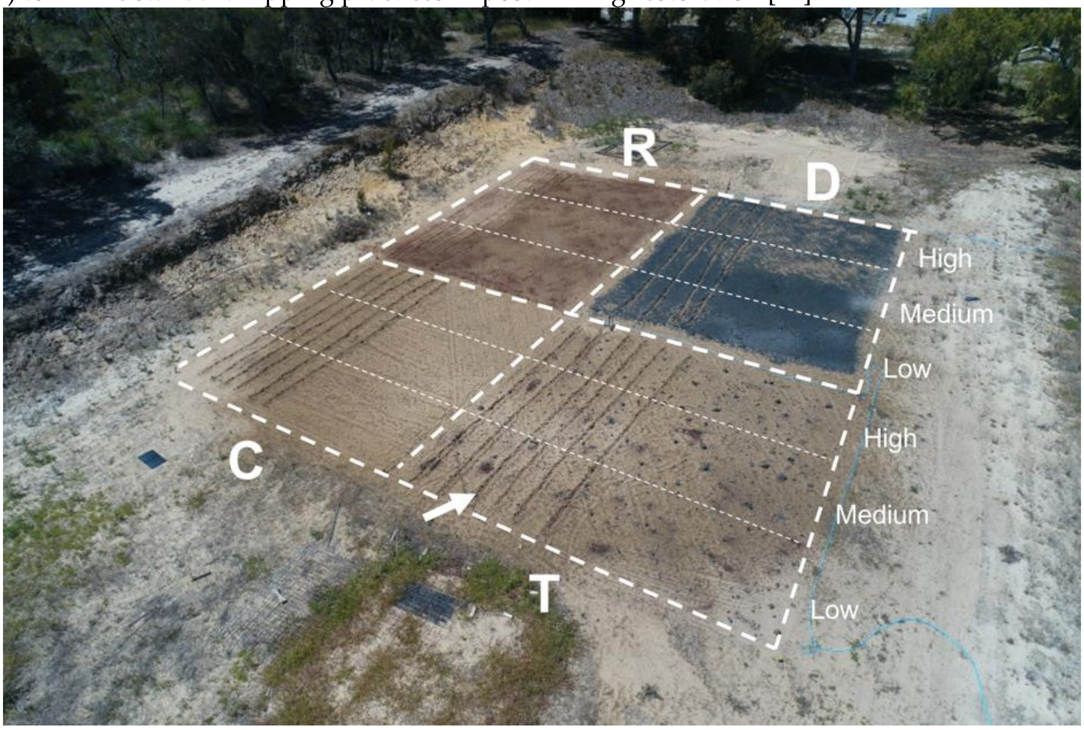

Figure 1. Layout of experimental plots (individual plot area 100 m²) illustrating surface treatments (annotated lettering), ripping sub-treatments (individual rip line indicated by annotated arrow), and broadcast seeding densities. Surface treatments included a 'control' of undisturbed local sandy soil (C), a 'textured' treatment of undisturbed local sandy soil with scattered crushed overburden rock (T), a 'dark' treatment of undisturbed local sandy soil capped with a 1 cm layer of tailings (D), and a 'high red ratio' treatment of undisturbed local sandy soil capped with a 1 cm layer of red clay loam soil (R). Broadcast seeding density treatments included low (15 seeds m^{-2} of target species, 50 seeds m^{-2} of grasses), medium (25 seeds m^{-2} of target species, 250 seeds m^{-2} of grasses) and high (50 seeds m^{-2} of target species, 1000 seeds m^{-2} of grasses). The image was taken from an altitude of 20 m using a DJI Phantom 4 Pro UAV.

Plots were seeded in September 2017, split into three seeding sub-treatments, representing increasing volumes of broadcast seeds (and, correspondingly, increasing seedling density; Figure 1). The seed mix broadcast included seeds of a target species, *Lupinus angustifolia* L. (Fabaceae), in a commercial grass species mix comprising *Festuca* arundinacea Schreb. and *Stenotaphrum secundatum* (Walter) Kuntze (Poaceae). The target species was selected as its seeds were large (ca. 1 cm) and distinctly colored (white), and its distinctive large, dark green palmate leaves produced from a central stem provided a strong comparison with the small linear leaves of non-target grasses.

The seed mix was intended to represent a potential restoration scenario where monitoring was required for a species of restoration interest with large, distinctly colored seeds and distinctive foliage (e.g., *Banksia* species, where seeds have been polymer coated [30]) among a matrix of grassy invasive weeds (a situation common in regional restoration initiatives [27]). The seed mix was broadcast at three densities: Low, comprising 15 *L. angustifolia* seeds and 50 grass seeds per m^{-2}; medium, comprising 25 *L. angustifolia* and 250 grass seeds per m^{-2}; and high, comprising 50 *L. angustifolia* and 1000 grass seeds per m^{-2}. In total, 12,000 *L. angustifolia* seeds were broadcast.

In total, the experimental area comprised a combined 24 sub-treatments (four surface treatments × two ripping sub-treatments × three broadcast seeding densities). Plots were irrigated daily for a 10-week growing season.

2.2. Flights and Image Capture

Manual flights of the study site were conducted daily for the entire 10-week duration of the experiment using a DJI Phantom 4 Pro (Dà-Jiāng Innovations, Shenzhen, China) equipped with a 20 Megapixel red-green-blue (RGB) camera. Each day, flights were conducted at 5, 10 and 15 meter flight altitudes, with 70% sidelap and frontlap. While flight planning software exists that could conduct the flights automatically and with calibrated overlaps, flights were conducted manually, as the low area covered and low flight altitude made such software impractical.

2.3. Image Processing and Classification

Raw images from all UAV flights were stitched together using the Agisoft Photoscan software, to produce RGB-rectified orthomosaics as well as Digital Elevation Models (DEMs) for each flight. The final resolution of orthomosaics was 1.02 mm per pixel for flights undertaken at a 5 m altitude, 2.63 mm per pixel for flights undertaken at the 10 m altitude, and 4.04 mm per pixel for flights undertaken at the 15 m altitude. Manual and automated seed classifications were undertaken on imagery captured the day following broadcast seeding (day 1). Manual image classification was also undertaken daily for all captured imagery to identify the point at which seedlings first became visible in imagery from each flight altitude (e.g., the distinctive palmate leaves of the target species were distinctly identifiable by a manual inspection of orthomosaics). Following the earliest point at which the target seedlings were discernable from the orthomosaics (day 25), total seedling counts were made from daily orthomosaics at each flight altitude until day 68.

For seed classifications, captured orthomosaics of the trial area were split into sub-treatment sections (representing 24 discrete sections), and each sub-treatment section was split into sixteen equal replicates, each representing a 1 m^2 area. Five replicates from each sub-treatment section were randomly selected to undertake classification, giving a total of 120 m^2 (30% of the trial area) that was examined for seeds. Automated classification of the target seeds from captured imagery was undertaken entirely dependent upon the color and shape of the classified image objects using object-based image analysis (OBIA). Following automated classification, manual classification was undertaken by a visual inspection of the orthomosaics, with all target seeds manually counted to determine the number of missed automated classifications and misclassified seed objects.

Seed Classification Workflow: *Image Capture → Orthomosaic Generation → Orthomosaic splitting (24 subtreatments) → Subtreatment splitting (16 sections) → Random selection of sections → Sections loaded into eCognition → Multiresolution segmentation → Automated seed object classification → Manual validation of classification*

For seedling classification, each sub-treatment section was split into three equal replicates for manual and automated examination, each representing a ca. 5.3 m^2 area. Automated classification of target seedlings from captured imagery was undertaken using two different OBIA methods: "*single-date*" (utilizing orthomosaics and DEMs generated from RGB imagery from a single point in time, with no post-processing alignment required) and "*layered*" approaches (utilizing layered ortho-mosaics and DEMs from two different flight times (day 25 and day 68), which requires post-processing alignment).

Single-date classification was undertaken on captured imagery from day 68, after a period of seven consecutive days in which no new seedlings were scored in any treatment at any flight altitude. Seedlings were classified after an initial multiresolution segmentation, with a process in which all objects with a green ratio (defined here as green light intensity divided by total light intensity) above a set threshold, determined by trial and error, were assigned to the 'target' class, followed by additional refining of the rule set using Hue-Saturation-Intensity (HSI; for definition see Supplementary S1) transformations, Triangular Greenness Index (TGI, defined here as TGI = $R_{GREEN} - 0.39 * R_{RED} - 0.61 * R_{BLUE}$ [31]), area (of the object, in cm^2), compactness (for definition see Supplementary S1), height (represented by the mean difference to neighbor objects in the DEM; see Supplementary S1), perimeter/width and length/width. For each replicate image, the total leaf cover of non-target grass seedlings (including false positive seedling classifications) was used to classify target seedlings for all objects not already classified using green ratio and TGI. *Layered* classification was undertaken on imagery captured on day 25 as well as imagery captured in the final flight (day 68). Orthomosaics were aligned in QGIS [32] with the 'georeferencer' plugin, and were split into discrete sub-treatment sections using the 'gridsplitter' plugin. Sections were then layered in eCognition, with target seedlings initially classified using green ratio from day 25 sections, and then this classification was confirmed by a continued presence of the target object in the day 68 sections determined again by green ratio. Manual classification was undertaken by a visual inspection of the orthomosaics following automated classification in all cases (discretely for each flight altitude, by the same observer), with all target seedlings manually counted to determine the number of missed automated classifications and misclassified seedling objects.

Single-date Seedling Classification Workflow: *Image Capture → Orthomosaic Generation → Orthomosaic splitting (24 subtreatments) → Subtreatment splitting (three sections) → Sections loaded into eCognition → Multiresolution segmentation → Automated seedling object classification → Manual validation of classification*

Layered Seedling Classification Workflow: *Image Capture → Orthomosaic Generation → Alignment of orthomosaics from different dates → Orthomosaic splitting (24 subtreatments) → Subtreatment splitting (3 sections) → Sections loaded into eCognition → Multiresolution segmentation → Automated seedling object classification → Manual validation of classification*

Full rule sets used in classification, and example outputs, are presented in Supplementary S1.

2.4. Statistical Analyses:

Dependent variables for seed classification analyses included *percentage of correct automated seed classifications* (PCA_{seeds}), calculated as the number of correct automated seed classifications in each replicate as a percentage of the total number of seeds manually classified in each replicate, and *number of false positive automated seed classifications* (FPA_{seeds}), calculated as the number of incorrect automated seed classifications in each replicate (e.g., objects classified as seeds by automated classification that instead represented non-target seed objects). Dependent variables for seedling classification analyses included *percentage seedling establishment*, calculated as the number of target seedlings classified manually in each replicate from imagery captured at the 5 m flight altitude at day 68 as a percentage of seeds broadcast, *percentage of correct automated seedling classifications* ($PCA_{seedlings}$), calculated as the number of correct automated seedling classifications (using OBIA) in each replicate as a percentage of the total number of seedlings manually classified in each replicate (discretely for *single-date* versus *layered* classification approaches), and *number of false positive automated seedling classifications* ($FPA_{seedlings}$), calculated as the number of incorrect automated seedling classifications (using OBIA) in each replicate (e.g., objects classified as seedlings by automated classification that instead represented non-target seedling objects; independently for *single-date* versus *layered* classification approaches). Additionally, the *cover of non-target grasses*, calculated as the percentage of each classified image representing leaf cover of non-target grass seedlings (excluding false positive seedling classifications) at day 68 (using OBIA) was determined from captured imagery at each flight altitude.

All dependent variables were square root transformed prior to analyses to meet assumptions of normality (assessed by Shapiro-Wilk tests of normality) and homogeneity of variances (as assessed by Levene's test for equality of variances).

One-way Analyses of Variance (ANOVA) were conducted to determine the effect of flight altitude on PCA_{seeds}, $PCA_{seedlings}$, FPA_{seeds} and $FPA_{seedlings}$ (independently for *single-date* versus *layered* data), and the effect of classification technique (*single-date* versus *layered*) on $PCA_{seedlings}$ and $FPA_{seedlings}$. A two-way ANOVA was conducted to determine the effect of treatment and ripping on *percentage seedling establishment* using data captured at the 5 m flight altitude. Three-way ANOVA were conducted to determine the effects of surface treatment, ripping and seeding density (i.e., the number of seeds in captured imagery) on PCA_{seeds} and FPA_{seeds} independently for 5 m and 10 m flight altitude data. Three-way ANOVA were also conducted to determine the effects of surface treatment, ripping and seeding density (i.e., the seeding density of non-target seedlings in captured imagery) on $PCA_{seedlings}$ for all flight altitude data combined, and on $FPA_{seedlings}$ independently for 5 m, 10 m and 15 m flight altitude data (*single-date* classification method), and on $PCA_{seedlings}$ and $FPA_{seedlings}$ independently for 5 m, 10 m and 15 m flight altitude data (*layered* method). All simple pairwise comparisons were run with a Bonferroni adjustment applied. A two-way ANOVA was conducted to determine the effect of treatment and ripping on *cover of non-target grasses,* using data captured at the 5 m flight altitude. Linear regression models were fitted to determine the effect of increasing *cover of non-target grasses* on $PCA_{seedlings}$ and $FPA_{seedlings}$ independently for 5 m, 10 m and 15 m flight altitude data. Data are presented as mean ± standard error, unless otherwise stated.

3. Results

3.1. Classification of Target Seed Objects

A total of 2291 *L. angustifolia* seeds were manually counted from the 120 m² of imagery examined from flights at a 5 m altitude (representing ca. 64% of seeds broadcast), and 2274 seeds from imagery captured at 10 m (representing ca. 63% of seeds broadcast). The resolution of imagery captured at 15 m was insufficient to manually or automatically classify seeds with a high level of confidence (Figure 2). The automated classification of seed objects from imagery captured by flights at 5 m was highly accurate, with a global PCA_{seeds} mean of 88 ± 1.0%, and was statistically significantly higher (F = 44.280, P <0.001) than from imagery captured by flights at 10 m, with a global PCA_{seeds} mean 74 ± 1.7% (95% CI, 70.3% to 77.1%). Similarly, FPA_{seeds} was significantly greater (F = 25.765, P <0.001) from imagery captured by flights at 10 m, global mean 19 ± 2.5 objects, compared to imagery captured by flights at 5 m, global mean 7 ± 1.4 objects.

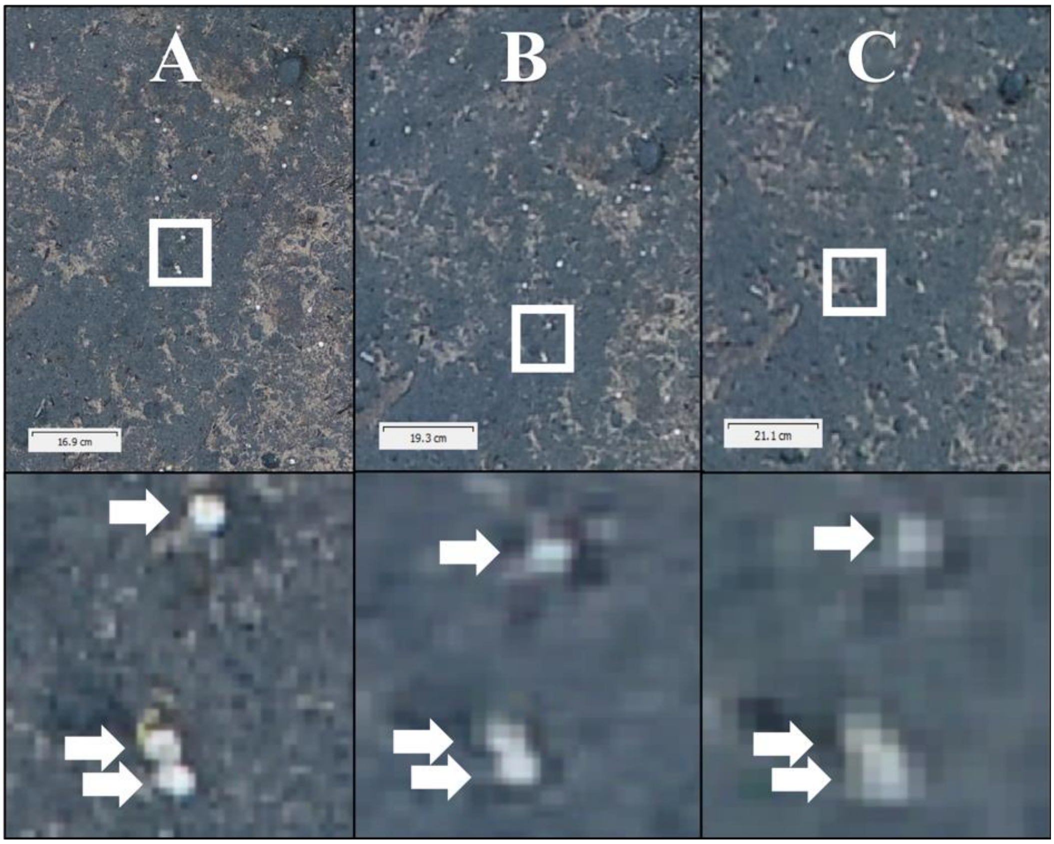

Figure 2. Variable image resolution used for the classification of target *L. angustifolia* seeds on the *Dark* substrate from captured imagery obtained at 5 m (A), 10 m (B) and 15 m (C) flight altitudes using a DJI Phantom 4 Pro unmanned aerial vehicle (UAV). The same region in each image is indicated by a white box, with the same three seeds indicated by annotated arrows.

There was no statistically significant three-way interaction between surface treatment, ripping and seeding density on PCA_{seeds} from imagery captured at the 5 m flight altitude, $F(6, 60) = 0.079$, $P = 0.380$, nor any simple two-way interactions ($P > 0.05$). There was a statistically significant simple main effect of both surface treatment, $F(3, 30) = 6.134$, $P = 0.001$, and ripping, $F(1, 60) = 19.693$, $P < 0.001$, on PCA_{seeds}. PCA_{seeds} was generally higher for unripped sub-treatments compared to ripped sub-treatments at the 5 m flight altitude (Figure 3), with the highest values recorded for *dark* (93 ± 2.8%) and the lowest for *high red-ratio* (86 ± 3.8%). There was no statistically significant three-way interaction between surface treatment, ripping and seeding density on FPA_{seeds} from imagery captured at this 5 m flight altitude, $F(6, 60) = 0.497$, $P = 0.809$, but there was a statistically significant simple two-way interaction between surface treatment and ripping, $F(3, 60) = 3.625$, $P = 0.028$. While FPA_{seeds} from imagery at 5 m was zero for *control, textured* and *high red-ratio* treatments regardless of ripping, there were 21 ± 9.4 and 33 ± 6.9 falsely classified objects from unripped and ripped *dark* sub-treatments, respectively.

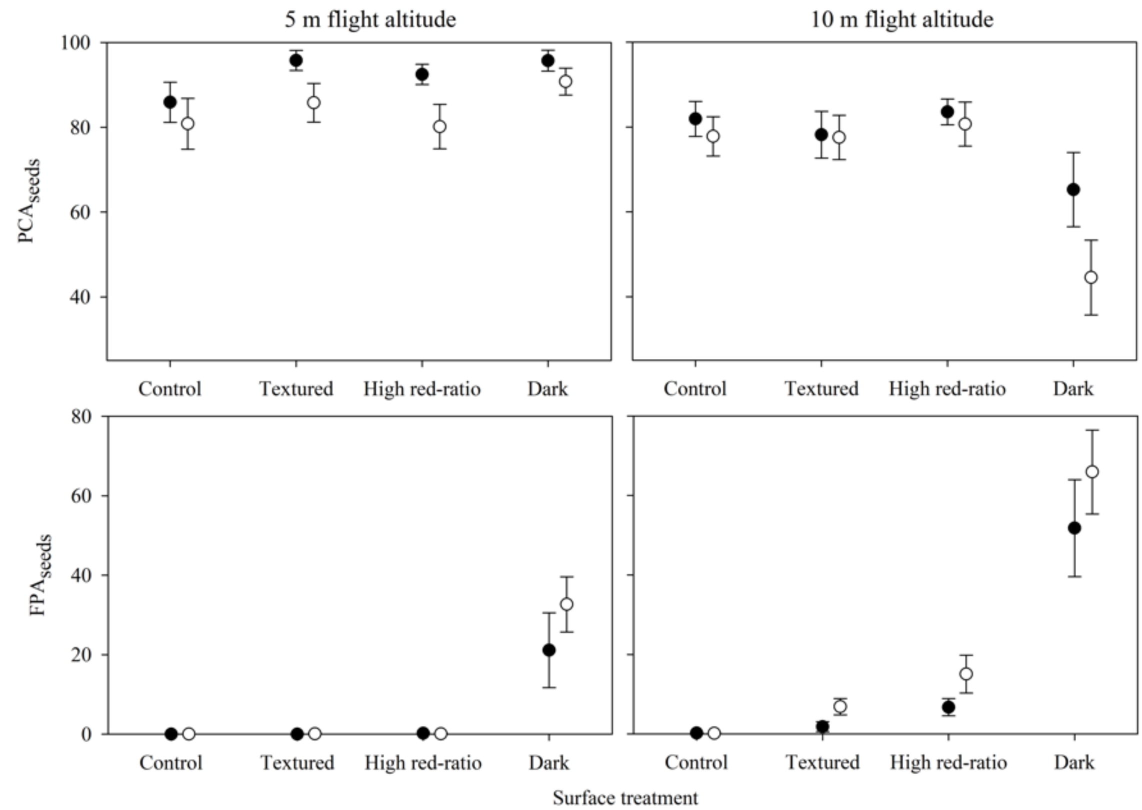

Figure 3. *Percentage of correct automated seed classifications* (*PCA_{seeds}*) and *number of false positive automated seed classifications* (*FPA_{seeds}*) among different surface treatments for unripped (filled symbols) and ripped (open symbols) sub-treatments from the imagery obtained at 5 m and 10 m flight altitudes using a DJI Phantom 4 Pro UAV. Control: Undisturbed local sandy soil. Textured: Undisturbed local sandy soil with scattered crushed overburden rock. Dark: Undisturbed local sandy soil capped with a 1 cm layer of mine tailings. High red-ratio: Undisturbed local sandy soil capped with a 1 cm layer of red clay loam soil. Data are presented as mean ± 1 s.e.

There was no statistically significant three-way interaction between surface treatment, ripping and seeding density on *PCA_{seeds}* from imagery captured at the 10 m flight altitude, $F(6, 60) = 0.853$, $P = 0.532$, but there was a statistically significant simple two-way interaction between surface treatment and ripping, $F(3, 60) = 3.963$, $P = 0.010$. *PCA_{seeds}* from imagery captured at our 10 m flight altitude was slightly lower (1–3%) in ripped sub-treatments than unripped sub-treatments for *control, textured* and *high red-ratio*, but remained >75% (Figure 3), while *PCA_{seeds}* fell to 65 ± 8.8% and 45 ± 8.8% for unripped and ripped *dark* sub-treatments, respectively. There was no statistically significant three-way interaction between the surface treatment, ripping and seeding density on *FPA_{seeds}* from imagery captured at this 10 m flight altitude, $F(6, 60) = 1.225$, $P = 0.300$, nor any simple two-way interactions ($P > 0.05$). There was a statistically significant simple main effect of both surface treatment, $F(3, 30) = 162.398$, $P < 0.001$, and ripping, $F(1, 60) = 15.131$, $P < 0.001$, on *FPA_{seeds}*. While *FPA_{seeds}* from imagery at 10 m was zero for *control*, it increased from 2 ± 1.3 and 7 ± 2.0 in unripped and ripped *textured* sub-treatments, respectively, to 7 ± 2.2 and 15 ± 4.8 in unripped and ripped *high red-ratio* sub-treatments, respectively, and to 52 ± 12.2 and 66 ± 10.6 in unripped and ripped *dark* sub-treatments, respectively (Figure 3).

3.2. Classification of Target Seedling Objects

A total of 242 *L. angustifolia* seedlings were manually classified from imagery captured at a 5 m flight altitude, representing an overall seedling establishment rate of ca. 2% of broadcast seeds. A total of 217 target seedlings were manually classified from imagery captured at a 10 m altitude, and 184 target seedlings from imagery captured at the 15 m altitude (Figure 4).

A statistically significant two-way interaction was present between surface treatment and ripping on *percentage seedling establishment*, $F(3, 36) = 6.652$, $P = 0.001$. Higher rates of *percentage seedling establishment* (increases of ca. 1.5–3%) were reported from ripped compared with unripped sub-treatments in all surface treatments (particularly in ripped *dark sub*-treatments where *percentage seedling establishment* reached 4.2 ± 0.2%), except in *high red-ratio* where values for unripped sub-treatments increased to levels comparable with ripped sub-treatments.

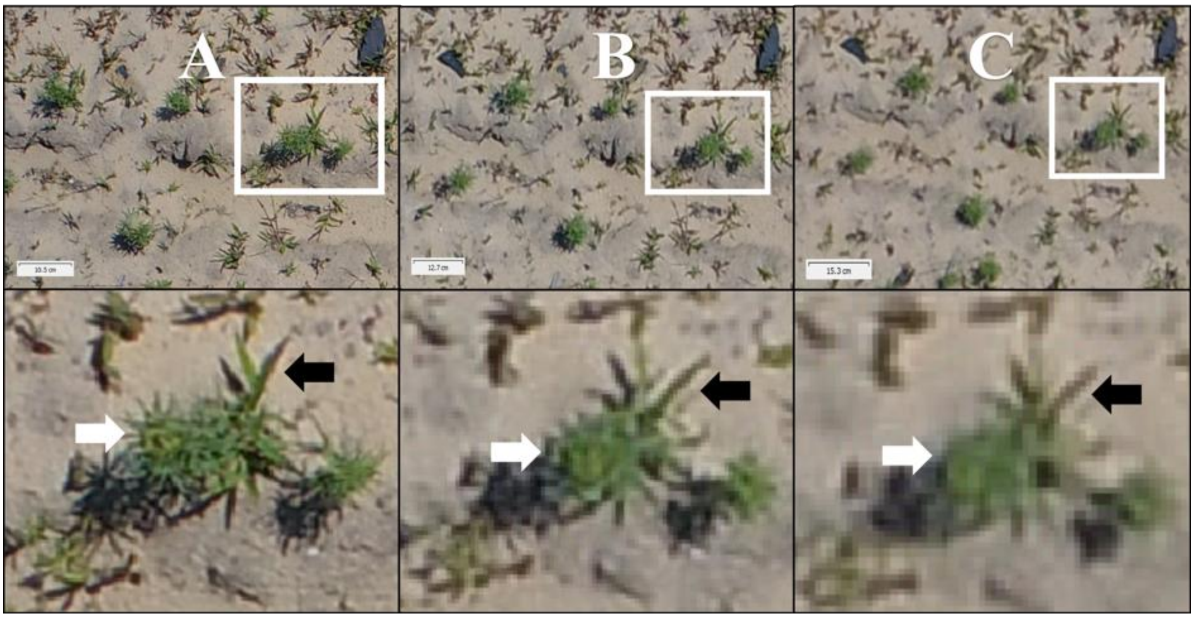

Figure 4. Variable image resolution used for classification of target *L. angustifolia* seedlings and non-target grasses from captured imagery obtained at the 5 m (**A**), 10 m (**B**) and 15 m (**C**) flight altitudes using a DJI Phantom 4 Pro UAV. The same region in each image is indicated by a white box, with the same *L. angustifolia* seedling (white arrows) and non-target grass seedling (black arrows) indicated.

The automated classification of seedlings was significantly more accurate (ca. 10–20% averaged across all sub-treatments and flight altitudes), using *single-date* classification compared with *layered* classification, $F(1, 404) = 32.430$, $P < 0.001$ (Figure 5). However, *layered* classification resulted in an average three-fold reduction in false positive classifications, $F(1, 430) = 55.708$, $P < 0.001$ (Figure 5).

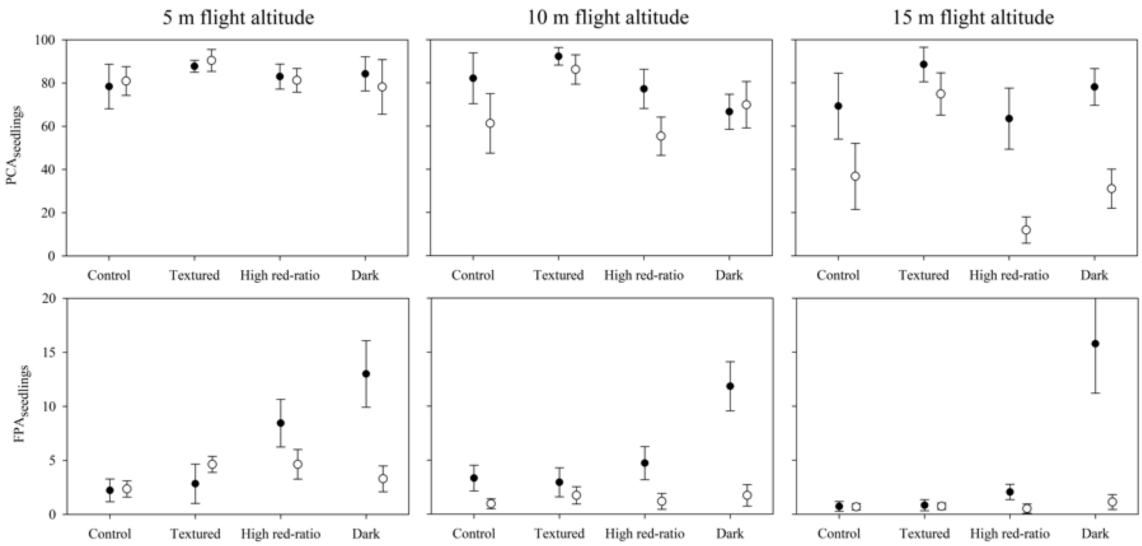

Figure 5. Comparison of single-date (filled symbols) versus layered (open symbols) classification approaches for percentage of correct automated seedling classifications ($PCA_{seedlings}$) and number of false positive automated seedling classifications ($FPA_{seedlings}$) among different surface treatments from imagery obtained at the 5 m and 10 m flight altitudes using a DJI Phantom 4 Pro UAV. Control: Undisturbed local sandy soil. Textured: Undisturbed local sandy soil with scattered crushed overburden rock. Dark: Undisturbed local sandy soil capped with a 1 cm layer of mine tailings. High red-ratio: Undisturbed local sandy soil capped with a 1 cm layer of red clay loam soil. Data are presented as mean ± 1 s.e.

Automated classification of seedling objects using *single-date* classification from imagery captured by flights at 5 m was highly accurate. The global $PCA_{seedlings}$ mean was 83 ± 2.3%, and although the classification accuracy was reduced from imagery captured by flights at 10 m (79 ± 2.5%) or 15 m (75 ± 3.2%) the effect of flight altitude on $PCA_{seedlings}$ was not statistically significant, $F(2, 72) = 2.049$, $P = 0.132$. The automated classification of seedling objects using *layered* classification from imagery captured by flights at 5 m was comparably accurate (global $PCA_{seedlings}$ mean of 82 ± 2.1%), but was significantly reduced from imagery captured by flights at 10 m (67 ± 3.2%) and 15 m (38 ± 4.2%), $F(2, 205) = 48.424$, $P < 0.001$.

The effect of flight altitude on $FPA_{seedlings}$ using *single-date* classification was statistically significant ($F(2, 213) = 4.030$, $P = 0.019$), with slightly fewer objects misclassified from imagery captured at 15 m (4 ± 0.3 objects) compared with imagery captured at 10 m (6 ± 0.7 objects) or 5 m (7 ± 0.8 objects). This trend was more strongly evident for $FPA_{seedlings}$ using *layered* classification ($F(2, 213) = 39.390$, $P < 0.001$), with fewer objects misclassified from imagery captured at 15 m (0.8 ± 0.14 objects) and from imagery captured at 10 m (global mean 1.4 ± 0.20 objects) than from imagery captured at 5 m (global mean 3.7 ± 0.36 objects).

There was no statistically significant three-way interaction between surface treatment, ripping and seeding density on $PCA_{seedlings}$ using *single-date* classification, $F(6, 105) = 0.985$, $P = 0.437$, but there were statistically significant simple two-way interactions between surface treatment and ripping, $F(3, 105) = 3.581$, $P = 0.015$, and surface treatment and seeding density, $(3, 67) = 8.601$, $P = 0.005$. $PCA_{seedlings}$ using *single-date* classification was higher in unripped compared to ripped *textured* sub-treatments (97 ± 1.5% and 82 ± 2.2%, respectively), but was otherwise comparable between unripped and ripped sub-treatments for all other treatments. $PCA_{seedlings}$ using *single-date* classification broadly increased with an increasing seeding density, although it remained >70% in all sub-treatments except for low seeding density *control* (58 ± 4.0%). There was no statistically significant three-way interaction between the surface treatment, ripping and seeding density on $PCA_{seedlings}$ using *layered* classification, $F(6, 105) = 1.193$, $P = 0.327$, nor any statistically significant simple two-way interactions or simple main effects ($P > 0.05$ in all cases).

There was no statistically significant three-way interaction between surface treatment, ripping and seeding density on $FPA_{seedlings}$ using *single-date* classification, $F(6, 105) = 0.767$, $P = 0.599$, but there was a statistically significant simple two-way interaction between surface treatment and seeding density, $F(6, 105) = 3.766$, $P = 0.004$. This interaction effect stemmed predominantly from a 3–4-fold increase in the number of misclassified objects from *dark* treatment at medium and high seeding density (ca. 20 and 15 objects per image, respectively), compared with low seeding density (ca. 5 objects per image). There was no statistically significant three-way interaction between surface treatment, ripping and seeding density on $FPA_{seedlings}$ using *layered* classification, $F(6, 105) = 1.061$, $P = 0.399$, but there were statistically significant simple two-way interactions between surface treatment and ripping, $F(3, 105) = 7.803$, $P < 0.001$, and ripping and seeding density, $(3, 67) = 6.044$, $P = 0.005$. $FPA_{seedlings}$ using *layered* classification remained unchanged at ca. three objects per image for unripped sub-treatments, regardless of seeding density, but increased with seeding density from ca. two objects per image at low seeding density to ca. six objects per image at high seeding density. While $FPA_{seedlings}$ using *layered* classification generally differed little among ripped sub-treatments by surface treatment, it increased markedly from ca. two objects per image to ca. eight objects per image in unripped and ripped *high red-ratio*, respectively.

After the 10-week growing period, the mean *cover of non-target grasses* determined from imagery captured at 5 m was 7 ± 1.1% in low seeding density treatments, 10 ± 0.8 in medium seeding density treatments, and 12 ± 0.8 in high seeding density treatments.

The automated detection of *cover of non-target grasses* was significantly affected by flight altitude, $F(2, 72) = 20.968$, $P < 0.001$, with a lower detection accuracy from imagery captured at 10 m (mean difference 4 ± 0.74%, 95% CI 2.2–5.8, $P < 0.001$) and 15 m (mean difference 5 ± 0.74%, 95% CI 2.8–6.3, $P < 0.001$) flight altitudes compared with imagery captured at 5 m.

Linear regression established that $PCA_{seedlings}$ using *single-date* classification at the 5 m flight altitude was not significantly predicted by *cover of non-target grasses*, $F(1, 64) = 0.016$, $P = 0.899$, and *cover of non-target grasses* accounted for only 1.5% of the explained variability in $PCA_{seedlings}$. $PCA_{seedlings}$ was also not significantly predicted by *cover of non-target grasses* at 10 m, $F(1, 66) = 0.269$, $P = 0.606$ (accounting for only 1.1% of the explained variability), or at the 15 m flight altitude, $F(1, 62) = 0.355$, $P = 0.553$ (accounting for only 1.0% of the explained variability). *Cover of non-target grasses* similarly had no statistical effect on $PCA_{seedlings}$ using *layered* classification at either 5 m flight altitude ($F(1, 69) = 0.571$, $P = 0.453$, accounting for only 0.03% of the explained variability), 10 m flight altitude ($F(1, 68) = 2.395$, $P = 0.126$, accounting for only 0.1% of the explained variability) or 15 m flight altitude ($F(1, 65) = 3.865$, $P = 0.054$, accounting for only 0.2% of the explained variability).

Increasing *cover of non-target grasses* accounted for 28.8% of the explained variability in $FPA_{seedlings}$ at the 5 m flight altitude using *single-date* classification, and could statistically significantly predict $FPA_{seedlings}$, $F(1, 70) = 29.773$, $P < 0.001$. The strength of this prediction increased with the increasing flight altitude, with c*over of non-target grasses* accounting for 46.4% of the explained variability in $FPA_{seedlings}$ at 10 m, $F(1, 70) = 60.623$, $P < 0.001$, and for 56.0% of the explained variability in $FPA_{seedlings}$ at 15 m altitude, $F(1, 70) = 91.411$, $P < 0.001$. *Cover of non-target grasses* had no statistical effect on $FPA_{seedlings}$ using *layered* classification at either 5 m flight altitude ($F(1, 70) = 0.102$, $P = 0.750$, accounting for only 0.01% of the explained variability), 10 m flight altitude ($F(1, 70) = 0.264$, $P = 0.609$, accounting for only 0.01% of the explained variability) or 15 m flight altitude ($F(1, 70) = 0.335$, $P = 0.565$, accounting for only 0.01% of the explained variability).

4. Discussion

Our data suggest that performing OBIA with the eCognition software is a suitable method for classifying and counting both seeds and seedlings. Results from this study provide empirical support for the utility of even low-cost commercially-available UAVs in monitoring ecological recovery, and suggest that UAV-based monitoring of broadcast seeding and seedling establishment can be a viable and effective tool in rehabilitation and ecological restoration. The automated classification of target seeds from the study area was achieved with a high level of accuracy from imagery captured at both 5 m and 10 m flight altitudes. Although this accuracy was reduced at higher altitudes, it should be noted that we employed the 20 megapixel RBG sensor integrated into the commercially-available UAV platform. UAV-mounted sensors will likely become increasingly higher-resolution and affordable with technological improvement [26,33], and undertaking sensing at denser resolution would maintain the accuracy of automated target object classification from imagery obtained at far greater flight altitudes. Not all seeds that were broadcast were classified from captured imagery (64% and 63% of broadcast seeds were classified from imagery captured at 5m and 10 m, respectively), probably due to seed burial during heavy rain the morning following seeding (11.8 mm; www.bom.gov.au, 'Swanbourne' weather station 9215) and seed predation by birds. Numerous granivorous birds were observed in the trial area, including Galah (*Eolophus roseicapilla*) and Western Corella (*Cacatua pastinator*), and Australian Magpie

(*Cracticus tibicen*) were even captured on UAV imagery in the trial area during sequential flights at 5 m and 15 m altitudes.

OBIA on imagery obtained at 5 m and 10 m flight altitudes provided a high level of accuracy in the automated classification of target seeds (nearly 90% at 5 m, and nearly 75% at 10 m; Figure 3) and target seedlings (ca. 80% at both altitudes; Figure 5) on soil surfaces representing local restoration substrates. The resolution of imagery from flights at the 15 m altitude (4.04 mm per pixel) was too poor for the classification of target seeds (either manually or through automated processes; Figure 2), but remained sufficient for the classification of seedlings (Figure 4), albeit at lower accuracy than 5 and 10 m (ca. 75%). The accuracy of OBIA at classifying seeds was reduced by ca. 14% as the resolution of the imagery decreased from 5 m to 10 m.

Accuracy was slightly lower in *dark* treatments and in ripped sub-treatments suggesting a significant effect of substrate complexity (e.g., surface texture and color) on classification success.

Rates of false positive classifications were generally very low among treatments (0–10 items m^{-2}), but automated classification of both seeds and seedlings became less successful as the complexity of the non-target background image increased. Rates of false positive classifications for both seeds and seedlings were generally zero or very low for most sub-treatments (<10 objects m^{-2}), but increased markedly as the image resolution decreased for seeds (Figure 3), and as the cover of non-target grasses increased for seedlings. Rated of false positive classifications were highest in *dark* treatments and in ripped sub-treatments for both seeds (up to ca. 66 objects m^{-2}) and seedlings (up to ca. 20 objects m^{-2}). These high rates likely reflect higher substrate heterogeneity compared with *control* and *textured* treatments; as surface cover treatments represented only a one centimeter capping over natural sands, rain over the study period resulted in patchiness in *dark* and *high red-ratio* treatments and, thus, less homogeneity compared with other treatments in terms of surface color. This color variability resulted in the manual classification of seeds and seedlings from *dark* and *high red-ratio* treatments being more challenging than for *control* and *textured* treatments, and a greater number of misclassifications during automated object classification. Future studies should field-test the efficacy of automated seed and seedling classification from imagery captured over real-world restoration trials, as restoration substrates commonly exhibit high surface heterogeneity resulting from land-forming activities, ripping and the inclusion of rocky material and woody debris [29,34].

We present two methods of classifying seedlings from aerial imagery, *single-date* and *layered* classification. While these approaches both yielded similar accuracies for target seedling classification from flights undertaken at a 5 m altitude, the accuracy of *layered* classification declined much more rapidly with the increasing altitude. This suggests that *layered* classification was significantly more reliant upon high spatial resolution than *single-date* classification, and may become more viable as sensor resolution improves into the future. However, *layered* classification also exhibited a markedly lower false positive detection rate than *single-date* classification, as well as a lack of correlation between the accuracy of target seedling classification and the percentage target area covered by background grasses. This suggests that *layered* classification may represent a more appropriate method to employ in areas with a high percentage of non-target objects (e.g., high weed coverage), due to the lower prevalence of false positives. Additionally, we employed *layered* classification solely to classify healthy seedlings—no stress conditions were introduced over the course of the experiment. Future studies could utilize this classification technique to track plant performance in target seedlings over time. This could, for example, allow for the detection of plant performance declines in seedlings, or track the recovery of communities following disturbance events such as droughts or wildfire.

Although our study is not the first to present methods for the automated classification of plants to species level from UAV imagery, previous studies have focused on large, established plants rather than seedlings in early developmental stages [19,20,35]. As seed germination and seedling emergence and early establishment are a critical stage of any restoration project [6,7], developing methods of monitoring this bottleneck phase are critical. Assessments of seedling numbers and species diversity from seed broadcasting efforts are traditionally determined by foot surveys, and UAV-based monitoring represents a significantly faster and lower risk method of undertaking these assessments. While this study focused on a single target species, it represents a successful proof of concept in demonstrating that seedlings of a target species can be classified despite the presence of non-target grasses. Future studies should investigate the viability of this approach when classifying multiple different target species within a more complex background area. Additionally, the ability to provide simultaneous assessments of factors such as weed coverage from captured imagery provides restoration practitioners with a useful tool to achieve multiple restoration monitoring goals in a single pass. However, significantly more research into areas such as developing and testing multi-sensor pods and more robust, reliable UAV platforms is required to realize the full potential of UAV use in ecological monitoring [26].

The techniques described in this study for classifying individual seeds broadcast in restoration areas have significant applicability to large-scale ecological monitoring, as results clearly demonstrate that large and distinctive seeds can be easily be distinguished from background substrates. Restoration projects commonly identify keystone taxa, including rare, threatened or structurally or functionally important species [27], and the seeds of these species are sometimes polymer coated (often in distinctive colors) in order to improve seed delivery or reduce seed predation [29,30]. The monitoring of seeds post broadcasting could be important for adaptive management in ecological restoration [2]; for example, repeated surveys of seeded areas could allow for accurate estimates of seed predation rates, and for examining differences in seedling establishment success among different microhabitats. Microsite availability and diversity can more strongly influence plant species richness at fine scales than seed availability [36], particularly in restoration [34,37], and numerous studies provide evidence that species establishment from seeds is strongly linked to microsite suitability [38-40]. UAV-captured imagery can generate a diverse range of ecological data to complement the classification of target objects (e.g., micro-topographic data from DEMs; Figure 6), and quantifying and identifying different microhabitats from aerial imagery prior to broadcast seeding might also allow for more targeted seed delivery. For species with highly specific seed germination biology requirements, which are often rare and threatened species for which seeds are often in limited supply or highly valued [41], ensuring seeds are delivered to the right microsite could maximize establishment, improving restoration outcomes and reducing costs. It should be possible through UAV-based monitoring to follow target geo-referenced individuals through life cycle phases from seed germination and early development to establishment and reproduction, which, when coupled with sensors capturing data relating to plant health and performance, such as hyperspectral and thermal sensors [22,24,26,42], could offer an unprecedented level of resolution in ecological monitoring, particularly for target species of particular interest or value. Given that the agricultural sector has already begun exploring options for UAV-based seed delivery and weed-control methods [43,44], it seems likely that the coming decades will see the rise of increasingly hands-free and increasingly replicable, accurate and reliable approaches to undertaking and monitoring ecological restoration. Further improvement to computer-aided classification techniques, such as more accessible means of utilizing machine learning software, would also improve classification accuracy. This study focused on the classification of large, white seeds (representing a relatively favorable target object in terms of color and contrast), and future work should test the approaches outlined here with smaller and less distinctive seeds. This is likely to require the application of higher resolution sensors, or sensors reliant upon sensing light outside of the visible spectrum. However, it is possible that the application of pelleting and polymer coating technologies sometimes employed in ecological restoration may assist in improving the identification of many smaller and less distinctive seeds if aerial identification of these seeds was desirable.

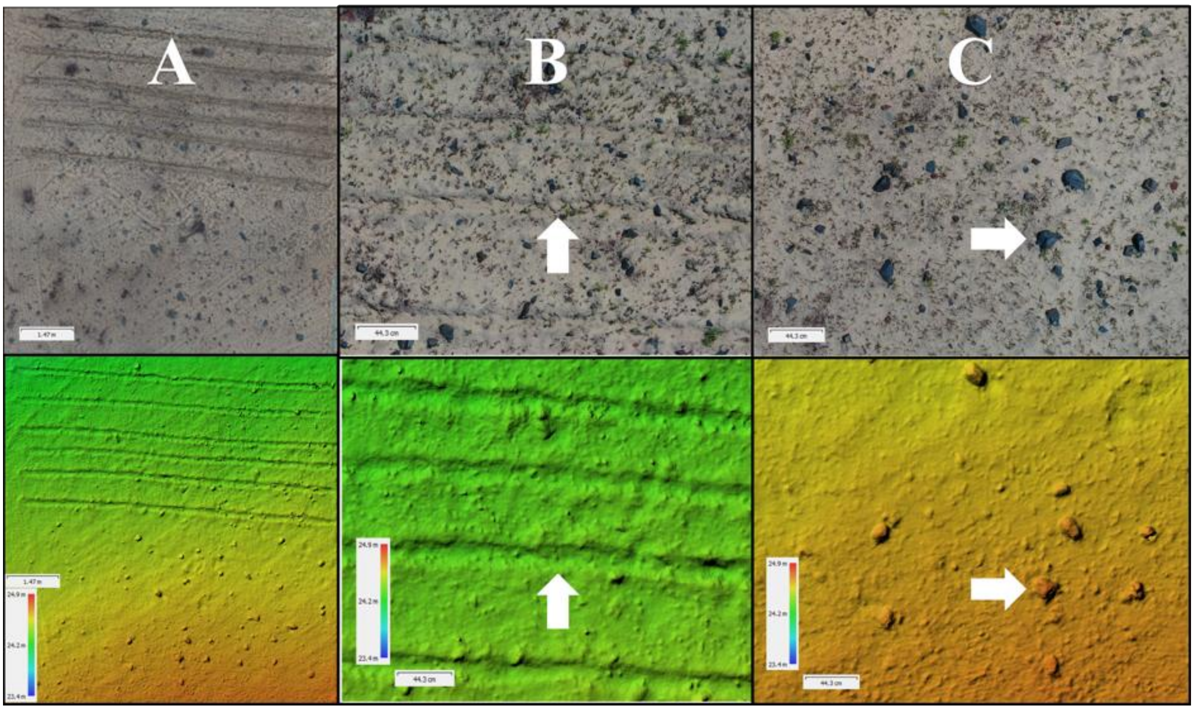

Figure 6. Digital elevation models (DEMs) can offer fine-resolution surface micro-topography data complementing the orthomosaics of target regions (**A**), with surface textures providing microhabitats for seedling establishment such as ripping contours ((**B**), arrows indicating an individual rip line) and rocks ((**C**), arrows indicating an individual rock) shown in relief that may not be easily visible from red-green-blue (RGB) imagery alone. DEMs generated in Agisoft Photoscan Professional from RGB imagery of *textured* treatment captured at 5 m flight altitude using a DJI Phantom 4 Pro UAV.

5. Conclusions

This study provides the first empirical evidence to support the utility of small, consumer grade UAVs as effective tools to monitor seed germination and seedling establishment in ecological restoration. Data show that undertaking OBIA on imagery captured using even low-cost commercially-available UAVs can achieve a high level of accuracy in classifying small target objects such as seeds and seedlings. Additionally, complementary ecological and site data (e.g., microsite topography and landform relief, cover of associated non-target species) can be easily gathered from the same imagery. The use of UAVs and the automated classification of captured sensor data holds great prospects to support ecological monitoring [26], and the application of UAVs to monitoring ecological restoration will increase the reliability, replicability, scale, diversity, speed and accuracy of data collection. This is likely to yield significant long-term cost-savings for restoration practitioners and industry, and has the potential to dramatically improve the quality and success of ecological monitoring efforts. The high spatial resolution required for fine-scale classification and the classification of small objects requires the current generation of UAVs equipped with low-resolution sensor payloads to operate at low altitudes. While the spatial area that can be covered by individual flights is constrained by the interplay between battery life and image footprint width at variable flight altitudes, rapid technological advances in sensor and platform design are likely to improve sensor resolution and improve battery life to increase the UAV operational area [26]. Future studies should explore further opportunities for capturing ecological data using multiple sensors in single flights (such as thermal, multispectral, or hyperspectral sensors), which will improve the amount of information gathered in shorter periods, and bring us closer to a one-pass solution for ecological monitoring.

Supplementary Materials: The following are available online at www.mdpi.com/xxx/s1, Supplementary S1: Full eCognition rule sets for automated seed and seedling identification from Buters et al. 2019.

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Article

Multi-Sensor UAV Tracking of Individual Seedlings and Seedling Communities at Millimetre Accuracy

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Abstract: The increasing spatial and temporal scales of ecological recovery projects demand more rapid and accurate methods of predicting restoration trajectory. Unmanned aerial vehicles (UAVs) offer greatly improved rapidity and efficiency compared to traditional biodiversity monitoring surveys and are increasingly employed in the monitoring of ecological restoration. However, the applicability of UAV-based remote sensing in the identification of small features of interest from captured imagery (e.g., small individual plants, < 100 cm^2) remains untested and the potential of UAVs to track the performance of individual plants or the development of seedlings remains unexplored. This study utilised low-altitude UAV imagery from multi-sensor flights (Red-Green-Blue and multispectral sensors) and an automated object-based image analysis software to detect target seedlings from among a matrix of non-target grasses in order to track the performance of individual target seedlings and the seedling community over a 14-week period. Object-based Image Analysis (OBIA) classification effectively and accurately discriminated among target and non-target seedling objects and these groups exhibited distinct spectral signatures (six different visible-spectrum and multispectral indices) that responded differently over a 24-day drying period. OBIA classification from captured imagery also allowed for the accurate tracking of individual target seedling objects through time, clearly illustrating the capacity of UAV-based monitoring to undertake plant performance monitoring of individual plants at very fine spatial scales.

Keywords: drones; multispectral; ecological restoration; rehabilitation; Object-based image analysis

1. Introduction

Ecological restoration and other recovery activities directed at returning ecological functioning to degraded ecosystems, is being undertaken at increasing scale around the world [1–3]. It is being increasingly recognised that humans must achieve a net gain in the extent and function of indigenous ecosystems in coming decades if ambitious global targets relating to sustainable development and biodiversity preservation are to be met [1,4,5]. However, ecological restoration is a complex process and achieving desired restoration trajectories requires significant planning, careful and targeted on-ground activities and detailed subsequent monitoring and adaptive management over long time periods [4,6,7]. The monitoring of ecological recovery projects such as ecological restoration is particularly important, both to ensure that predetermined goals are being met and to inform adaptive management in situations where trajectories are unsatisfactory [4,8,9]. Many studies have utilised keystone plant species (e.g., species of notable abundance or importance to ecological functioning) as indicators to project restoration trajectory [9,10]. However, the demand for more rapid and accurate methods of predicting restoration trajectory continues to grow with the increasing spatial and temporal scales of ecological recovery projects [1–3,11]. Unmanned Aerial Vehicles (UAVs) have increasingly been applied to meet this demand, as they offer greater cost-efficiency and ease of use, increased spatial and temporal resolution and improved rapidity and safety compared to traditional biodiversity monitoring surveys [12,13].

One area of particularly strong uptake in UAV-based monitoring in in the monitoring of post-mining rehabilitation and ecological restoration [14]. Many mining operations are located in relatively remote regions and post-mining landforms are often steep, unstable or hazardous to traverse on foot (e.g., waste rock landforms and tailings storage facilities). UAVs offer an effective monitoring solution for these landforms, on which rehabilitation or ecological restoration are often regulatory requirements [15,16], as their operation avoids human exposure to hazardous conditions, can access areas not able to be monitored on foot and does not risk degrading or trampling sensitive or regenerating communities [17]. Additionally, UAVs do not pose a pathogen transmission risk in areas where soil- or water-borne pathogens such as dieback (*Phytopthora* spp.; [18]) represent a serious threat to ecological recovery and to the integrity of natural communities. The ability of UAVs to fly at extremely low altitudes, coupled with lower operating costs and minimal infrastructure requirements, yields more accessible and cost-efficient data capture at far greater spatial resolution than can be achieved by manned aircraft or satellites [19].

Higher spatial resolution greatly improves the accuracy of automatic image classification techniques such as Object-Based Image Analysis (OBIA; a technique that splits each image into spectrally similar 'objects,' which allows for classification not just on colour but other factors like shape, size and relationship to surrounding objects [20]) and supervised machine learning (a process that "teaches" a program what set classes are meant to look like by providing training examples; based off of the given examples, the program then classifies the remaining images by their similarity to the specified classes [21]). Very high spatial resolution is a prerequisite where monitoring goals require the identification of small features of interest (e.g., individual plants) [20,22]. The mining industry are increasingly seeking monitoring tools that provide accurate and reliable assessments of restorative trajectories [14,23] and studies have identified examinations of growth, phenology and physiological performance in restored communities at the level of individual plants as a crucial desired component of this toolbox [16,24,25].

As UAVs can carry a variety of sensors and multiple sensors can be mounted on a single platform to capture data simultaneously in a single flight [26], they represent an increasingly appropriate tool for monitoring plant performance. UAVs are widely employed in the monitoring of plant health and performance in the agricultural sector [27], with recent studies demonstrating their effective application to the estimation of leaf carotenoid content in vineyards [28], the identification of nitrogen stress in maize [29], the monitoring of crop pests [30], in assessments of leaf area index in wheat [31], in the diagnosis of various ailments such as sheath blight in rice [32] and in assessments of water stress in barley fields [33] and olive orchards [34]. Many of these applications employed multispectral sensors, due primarily to their ability to provide early warning of plant stressors at a relatively affordable price point compared to other, more advanced sensors such as hyperspectral or thermal cameras [35]. Multispectral sensors can detect near-infrared (NIR) light, which affords the ability to monitor various multispectral indices (e.g., Normalised Difference Vegetation Index, NDVI) that can show changes in plant health before any visible signs appear [36]. The use of multispectral imagery to improve classification accuracy has been previously demonstrated at larger scales in dry forest and peat bogs [20,37], to classify trees and shrubs on the basis of plant health [20] and to provide early warning of plant stress in plantation trees [38]. Indeed, the addition of multispectral cameras to automated image classification processes broadly may represent an opportunity to markedly increase the accuracy of target plant object classification. More advanced sensors such as thermal and hyperspectral sensors are likely to significantly improve this capability, as multispectral sensors do not provide adequate data to determine the reason for observed plant performance declines due to limited spectral resolution [39]. Determining the causes of observed declines is important to action correct management and at least in agricultural crop species requires the spectral resolution offered by hyperspectral sensors and classification accuracies nearing 100% have been achieved when using hyperspectral sensors to differentiate between three different fungal diseases in sugar beets [40] and in detecting head blight in wheat [41]. Additionally, thermal sensors offer a highly accurate tool for assessing water stress in vegetation [33,42–44] and have also been applied variously in the monitoring of mammalian fauna [45–47]. The use of thermal and other UAV-mounted sensors in fauna monitoring is likely to increase to meet the growing demands for more rapid and reliable assessments of fauna biodiversity and behaviour [11]. However, the use of more advanced sensors remains predominantly constrained by cost [14], as thermal and hyperspectral sensors are currently between three to twelve times more expensive than multispectral sensors [35] and translational research from agriculture to the monitoring of ecological restoration projects has to date been broadly restricted to the use of multispectral sensors (e.g., the identification and mapping of woody vegetation cover in post-mining revegetation [48]). Translational research has not yet tested the potential of UAVs to monitor heterogenous natural vegetation communities at very fine spatial scales and is yet to demonstrate that plant health and performance can be monitored at the scale of individual plants or that establishment and development can be reliably tracked at the seedling stage when plants are most vulnerable to environmental stressors [49].

This study aimed to utilise the fine-scale spatial and temporal resolution offered by UAV-mounted sensors to identify target seedlings and track and assess their growth and development from a simple background community on representative restoration substrates. Target seedlings can be identified with a high level of accuracy from RGB imagery using OBIA [50] and multispectral imagery works well in automated image classification approaches as the extra information provided allows users to split classification classes not only by species but by level of plant health based off of multispectral indices [20]. We utilised a UAV equipped with both RGB and multispectral cameras to monitor a juvenile plant community over a period of drying to track individual target seedling objects (*Lupinus angustifolia*) through time and simultaneously gathered data to assess surrogate measures of their ecophysiological performance using a variety of visible and multispectral indices over the course of the drying period to contrast the performance of target seedlings with co-occurring non-target seedlings (co-occurring grasses). Given the often remote and poorly accessible nature of post-mining landforms, we examined whether naturally occurring reference points (local points with defining features that can be located repeatable throughout the data series, that is, small landscape features such as rocks and soil surface microtopography) could be used as common reference points to align orthomosaics produced from images captured by different sensors or on different days. Our objective was to achieve high level of accuracy in the identification, counting and tracking of individual target seedling objects through relative referencing between image captures to facilitate monitoring of a desired area of interest, not to achieve a high level of precision in determining the absolute spatial positioning of target individuals. We hypothesised that target and non-target seedling objects would exhibit different spectral signatures (e.g., different values for visible and multispectral vegetation indices, as surrogates for ecophysiological performance) and that these signatures would respond differently over the drying period facilitating the discrete monitoring of both groups. Additionally, we hypothesised that pre-drying plant performance (determined from values from visible and multispectral indices) would be a significant predictor of the speed of seedling mortality during the drying period.

2. Materials and Methods

2.1. Study Site

The study was conducted at the University of Western Australia Shenton Park Field Station, Perth, Western Australia (31° 56′ 55S, 115° 47′ 39E). UAV flights were undertaken over a 400 m^2 trial area divided into four experimental 100 m^2 plots (10 m × 10 m) with different surface treatments representing generalised restoration substrates in Western Australia (Figure 1). Surface treatments included a '*control*' of undisturbed local sandy soil (smooth, homogenous texture and light background colour) representative of typical post-mining substrate in *Banksia* woodland restoration [16]; a '*textured*' treatment of undisturbed local sandy soil with scattered crushed overburden rock (2–20 cm in size, giving increased surface heterogeneity) used to rock armour the slopes of restoration landforms [51]; a '*dark*' treatment of undisturbed local sandy soil capped with a 1 cm layer of tailings generated from the processing of magnetite ore [52]; and a '*high red-ratio*' treatment of undisturbed local sandy soil capped with a 1 cm layer of red clay loam soil from a mine site in the Midwest region of Western Australia [51]. All capping materials were sourced from a major magnetite mining operation located approximately 400 km northeast of Perth, Western Australia. Additionally, to examine whether seed and seedling detection rates were affected by surface topography, half of each treatment plot was manually ripped to a depth of 20 cm using a backhoe (Figure 1) to mimic standard ripping practices in post-mining restoration [16].

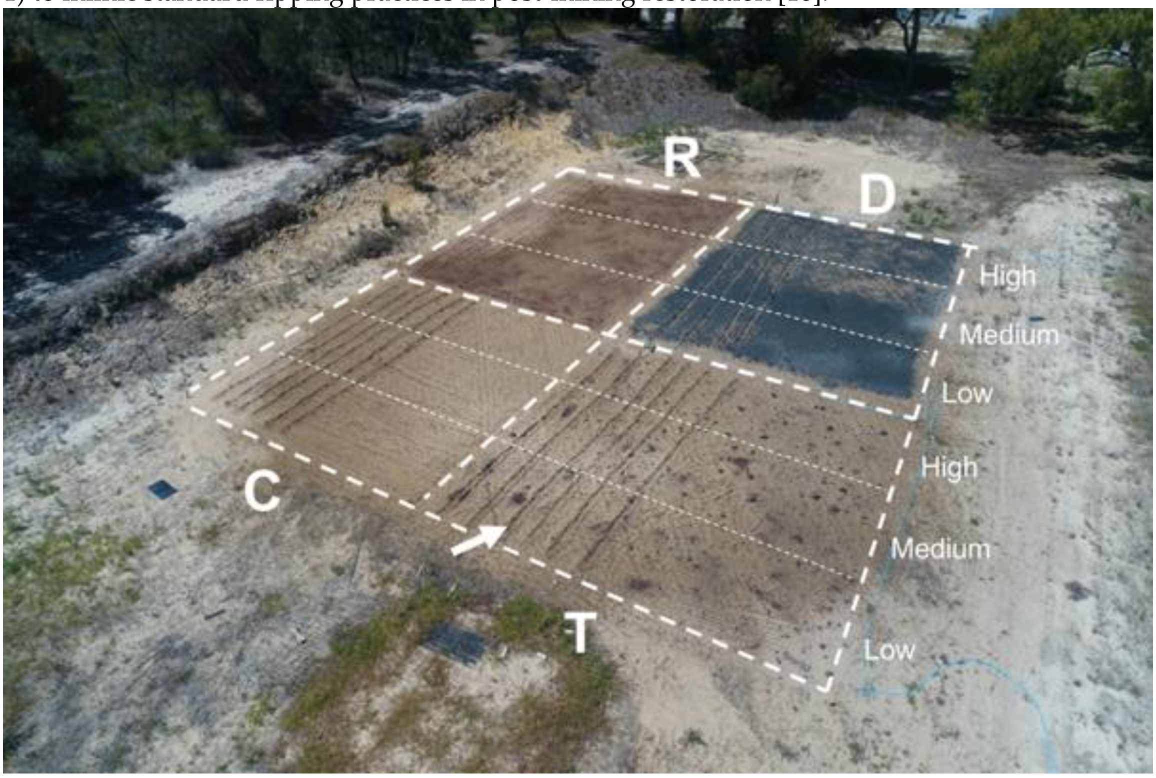

Figure 1. Layout of experimental plots (individual plot area 100 m^2) illustrating surface treatments (annotated lettering), ripping sub-treatments (individual rip line indicated by annotated arrow) and broadcast seeding densities. Surface treatments included a 'control' of undisturbed local sandy soil (C), a 'textured' treatment of undisturbed local sandy soil with scattered crushed overburden rock (T), a 'dark' treatment of undisturbed local sandy soil capped with a 1 cm layer of tailings (D) and a 'high red ratio' treatment of undisturbed local sandy soil capped with a 1 cm layer of red clay loam soil (R). Broadcast seeding density

treatments included low (15 seeds m^{-2} of target species, 50 seeds m^{-2} of grasses), medium (25 seeds m^{-2} of target species, 250 seeds m^{-2} of grasses) and high (50 seeds m^{-2} of target species, 1000 seeds m^{-2} of grasses). The image was taken from an altitude of 20 m using a DJI Phantom 4 Pro unmanned aerial vehicle (UAV).

Plots were seeded in September 2017, split into three seeding sub-treatments representing increasing volumes of broadcast seeds (and, correspondingly, increasing seedling density; Figure 1). The seed mix broadcast included seeds of a target species, *Lupinus angustifolia* L. (Fabaceae), in a commercial grass species mix comprising *Festuca arundinacea* Schreb. and *Stenotaphrum secundatum* (Walter) Kuntze (Poaceae). The target species was selected as its seeds were large (ca. 1 cm) and distinctly coloured (white) and its distinctive large, dark green palmate leaves produced from a central stem provided a strong comparison with the small linear leaves of non-target grasses. The seed mix was intended to represent a potential post mining restoration scenario where monitoring was required for a species of restoration interest with large, distinctly coloured seeds and distinctive foliage (e.g., *Banksia* species, where seeds have been polymer coated [53]) among a matrix of grassy invasive weeds (a situation common in regional restoration initiatives [16]). The seed mix was broadcast at three densities: low, comprising 15 *L. angustifolia* seeds and 50 grass seeds per m^{-2}; medium, comprising 25 *L. angustifolia* and 250 grass seeds per m^{-2}; and high, comprising 50 *L. angustifolia* and 1000 grass seeds per m^{-2}. In total, 12000 *L. angustifolia* seeds were broadcast. Plots were irrigated daily for a 10-week growing season before the irrigation was turned off and soils were allowed to dry for a further four weeks. Daily weather information, as well as daily climatic variable data used in analyses, were retrieved from the Bureau of Meteorology's Swanbourne station, ID 009215 (located ca. 2 km from the study site).

2.2. Flights and Image Capture

Manual flights of the study site were conducted daily (5 metre flight altitude) for 14 weeks using a DJI Phantom 4 Pro (Dà-Jiāng Innovations, Shenzhen, China) equipped with a 20 Megapixel RGB camera and a Parrot Sequoia multispectral camera (only for flights in weeks 9 through 14). Flights were conducted manually due to the small area surveyed and low flight altitudes. All flights were conducted with front- and sidelap of 70% and at low speeds (approximately 3 kph) to avoid image blur. Flights were conducted at the same time each day (early morning, approximately 8.30 am once shadows from adjacent trees no longer fell over the trial area), with the exception of the last week of the experiment when increasing aggression from local avifauna required flights to be conducted at random times throughout the day in an attempt to avoid interactions (see Section 4.5).

2.3. Image Analysis

JPG images from all UAV flights were merged together using the Agisoft Photoscan software, to produce RGB (from the phantom 4 pro integrated camera) and multispectral (from the sequoia) rectified orthomosaics as well as Digital Elevation Models (DEMs) for each flight. The final resolution of RGB orthomosaics was 1.02 mm per pixel and of multispectral orthomosaics was 4.64 mm per pixel. Images were locally geo-referenced and naturally occurring reference points (local points with defining features that can be located repeatable throughout the data series, that is, small landscape features such as rocks and soil surface microtopography) were used as common reference points to align orthomosaics produced from images captured by different sensors or on different days. Orthomosaic alignment thus aimed to facilitate 'identification accuracy' of target seedling objects and the discrete tracking of individual target seedling objects through time, rather than to attain high levels of absolute 'spatial accuracy' in terms of global positioning.

2.4. Monitoring of Plant Response to Water Stress

Ten weeks after seeding, the irrigation to the trial plot was switched off and the soil was left to dry for four weeks. During this period, the plot was monitored with the Phantom 4 Pro and the Parrot Sequoia multispectral camera in order to track response of target and non-target seedlings to water stress. Due to operational difficulties with the multispectral camera, inclement weather and interaction with an aggressive native raptor, successful flights were conducted on 12 out of the 24 days during the drying period. Following all flights, orthomosaics and DEMs were generated from both the Phantom 4 Pro's RGB imagery and the Parrot Sequoia multispectral imagery. Orthomosaics and DEMs were created in Agisoft Photoscan, with the Sequoia imagery requiring initial reflectance calibration from its reflectance target (conducted in Photoscan). Orthomosaics were split into four discrete images representing the four different surface covers and QGIS was used to manually align RGB and multispectral orthomosaics for analysis. However, a lack of identifiable feature objects resulted in insufficient alignment for *dark*, *red* and *control* treatments, a challenge that was made more problematic by the reduced spatial resolution of the multispectral sensor. Only the *textured* treatment could be aligned with an acceptable level of accuracy, with scattered waste rock providing sufficiently distinct features for georeferencing. While target seedling objects were generally well-aligned, occasional misalignment by a few centimetres occasionally resulted in image objects derived from RGB imagery having minimal overlap with image objects derived from multispectral imagery (Figure 2). Following alignment of RGB and multispectral orthomosaics in QGIS, the orthomosaics were imported into eCognition.

Automated identification of target seedlings from captured imagery was initially undertaken using OBIA (utilising orthomosaics and DEMs generated from RGB imagery from a single point in time, with no post-processing alignment required) captured imagery from day 68, after a period of seven consecutive days in which no new seedlings were scored in any treatment. Seedlings were identified by an initial multiresolution segmentation, in which all objects with a green ratio above a set threshold were assigned to the 'target' class, followed by additional refining of the rule set using Hue-Saturation-Intensity (HIS [54]) transformations, Triangular Greenness Index (TGI), area (of the object, in cm^2), compactness [54], height (represented by the mean difference to neighbour objects in the DEM; see Reference [50]), perimeter/width and length/width. Full rule sets used in analyses and example outputs, are presented in Reference [50]. For each image, the total leaf cover of non-target grass seedlings (including false positive seedling identifications) was determined for all objects not already classified using green ratio and TGI. Following target seedling identification, a report was generated from each orthomosaic showing key colour indices of each image object, tracking features including green ratio, TGI, Visible Atmospheric Resistant Index (VARI), Normalised Difference Vegetation Index (NDVI), Soil-Adjusted Vegetation Index (SAVI) and area (of the object, in cm^2). Equations and rationale for all visible and multispectral indices are provided in Table 1.

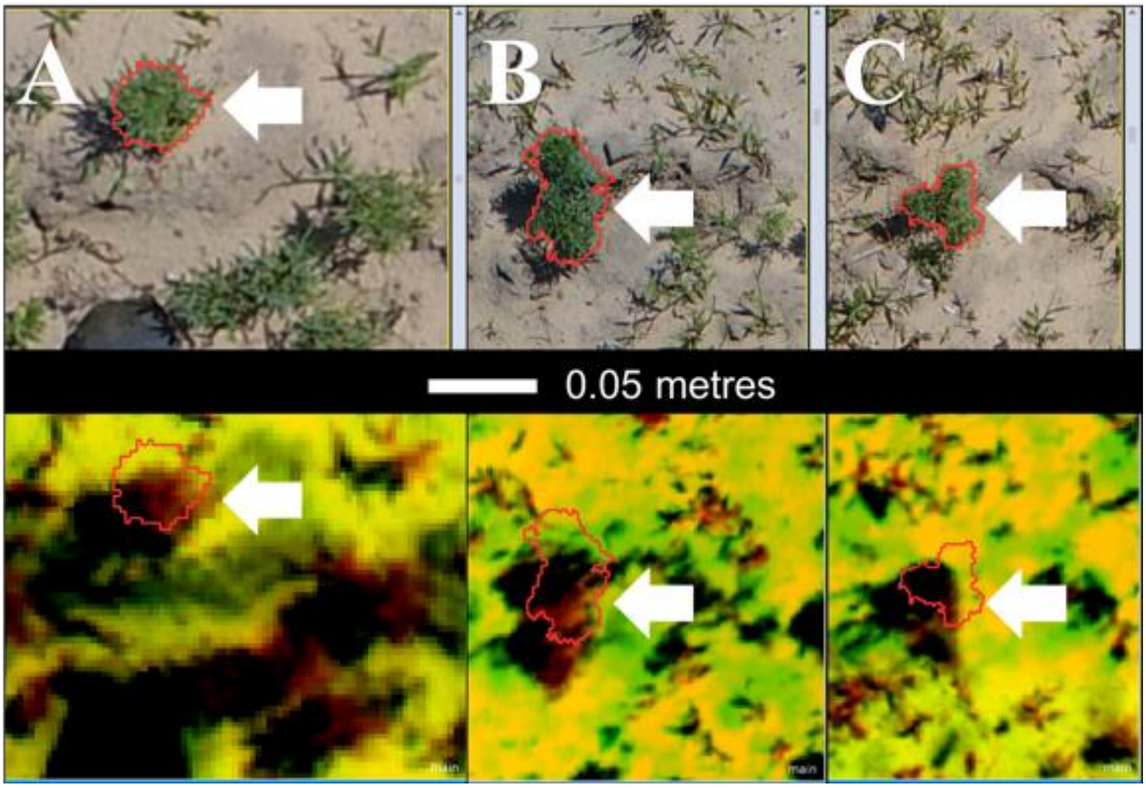

Figure 2. Three examples of target seedling objects (*Lupinus angustifolia*, indicated by white arrows) that exhibited poor overlap between RGB (top) and multispectral (bottom; contrast adjusted for visibility) orthoimages captured by a DJI Phantom 4 UAV at a flight altitude of 5 m. Red polygons indicate the outline of the identified target seedling objects, in terms of their true location and highlight the misalignment between different captured imagery.

Table 1. Equations, sensor used and rationale for each of the five visible and multispectral vegetation indices utilised in analyses.

Index	Equation	Sensor Used	Rationale for Inclusion	Reference
Green ratio	Green/(Green + red + blue)	RGB	Used for initial target seedling identification	
TGI	Green − 0.39 × Red − 0.61 × Blue	RGB	Provides an estimate of chlorophyll content	[55]
VARI	(Green − Red)/(Green + Red − Blue)	RGB	Reduces atmospheric effects	[56]
NDVI	(NIR − Red)/(NIR + Red)	Multispectral	Most widely employed vegetation index in the literature	[57]
SAVI	((1 + L)(NIR − Red))/(NIR + Red + L)	Multispectral	Variant of NDVI intended to be less influenced by soil induced variation	[58]

2.5. Tracking of Specific Individuals

From RGB imagery captured on day 68, 25 individual target seedling objects were randomly selected for individual plant performance monitoring. Orthomosaics from day 68 to day 92 were trimmed to only the *Textured* treatment and the QGIS georeferencer plugin was used to align all future orthomosaics to the coordinates of the day 68 orthomosaic. Target seedlings were identified initially based off of their green ratio, with generous thresholds (see Supplementary 1 for ruleset) used to allow for continued identification even as green ratio of plants changed in response to drying conditions. Following identification via green ratio, each of the chosen 25 target seedlings were identified from their GPS co-ordinates. Following the identification of a target seedling from imagery of the first day of drying, the north-, east-, south- and west- most points were identified and GPS coordinates of each recorded. In future imagery, seedlings were identified as being the seedling of interest if the GPS position of the object's centroid fell within the previously identified extremities. Full rulesets are presented in Supplementary 1. A report was generated from each orthomosaic showing key colour indices of each individual target seedling object, tracking features including area, green ratio, TGI and VARI. Only visible spectrum indices were calculated for the 25 tracked individual target seeding objects, due to georeferencing and overlap errors with multispectral imagery.

2.6. Statistics

To determine whether the spectral signature of the monitored plant community varied in response to climatic conditions, linear regression models were fitted to determine the effect of daily climatic variables (*rainfall, solar exposure, maximum temperature and minimum temperature*) on each of the visible spectrum (TGI, VARI and Green Ratio) and multispectral (NDVI and SAVI) indices utilised. All data were square root transformed prior to multiple linear regression analyses to meet assumptions of normality (assessed by Shapiro-Wilk tests of normality) and homogeneity of variances (as assessed by Levene's test for equality of variances).

To determine whether classified target and non-target seedling object groups exhibited discrete spectral signatures, two-way mixed ANOVA were employed to determine whether mean object area and visible spectrum and multispectral indices varied significantly among the two groups over the experimental period using Greenhouse-Geisser estimates of epsilon (ε) as assumptions of sphericity were not met (SPSS Statistics 25, IBM, United States). Simple main effects among classified object groups at each time point were determined using Tukey post hoc tests.

Two-tailed Pearson correlations were run to determine which visible spectrum or multispectral index provided the strongest association with declines in the target seedling object community (mean object area and number of surviving individuals).

For analyses, the 25 tracked target seedling objects were binned into four size categories on the basis of object area at day 68: small (< 50 cm^2), medium (50–100 cm^2), large (100–150 cm^2) and very large (> 150 cm^2). To determine whether different sized target seedling objects exhibited different spectral signatures in response to drying conditions, two-way mixed ANOVA were employed to determine whether mean object area and visible spectrum indices varied significantly among the four groups over the experimental period using Greenhouse-Geisser estimates of ε as assumptions of sphericity were not met (SPSS Statistics 25, IBM, United States). Simple main effects among classified object groups at each time point were determined using Tukey post hoc tests.

Data are presented as mean ± standard error, unless otherwise stated.

3. Results

3.1. Response of Spectral Signature to Climatic Conditions

Mean object area and both visible spectrum and multispectral indices were significantly predicted by daily climatic variables (Table 2), with strongest effect sizes of regression models evident for multispectral indices (NDVI and SAVI) followed by green-dependent visible spectrum indices (TGI and green ratio). Regression coefficients and standard errors for all multiple regression analyses are presented in Table 3. Object area, TGI, VARI and green ratio were negatively associated with daily temperatures and solar exposure and positively associated with rainfall, while NDVI and SAVI were positively associated with all climatic variables.

Table 2. Overall model effects for multiple regression models examining the effect of climatic variables (*rainfall, solar exposure, maximum temperature and minimum temperature*) on object area and visible spectrum and multispectral indices utilised in this study.

Factor	F (df, *n*)	*P*	Adj. R^2	Variables Statistically Significantly Adding to the Prediction ($P < 0.05$)
Object area	42.302 (4, 109,123)	< 0.001	0.002	*Rainfall* *Solar exposure* *Maximum temperature* *Minimum temperature*
NDVI	5628.883 (4, 109,099)	< 0.001	0.17	*Rainfall* *Solar exposure* *Maximum temperature* *Minimum temperature*
SAVI	5643.055 (4, 109,123)	< 0.001	0.17	*Rainfall* *Solar exposure* *Maximum temperature* *Minimum temperature*
TGI	4770.678 (4, 109,123)	< 0.001	0.15	*Rainfall* *Solar exposure* *Maximum temperature* *Minimum temperature*
VARI	95.538 (4, 109,123)	< 0.001	0.003	*Rainfall* *Minimum temperature*
Green Ratio	1112.903 (4, 109,123)	< 0.001	0.04	*Rainfall* *Solar exposure* *Maximum temperature* *Minimum temperature*

Table 3. Summary of multiple regression analyses examining the effect of daily climatic variables (*rainfall, solar exposure, maximum temperature and minimum temperature*) on object area and visible spectrum and multispectral indices utilised in this study.

Factor	Variable	*B*	SE_B	β
Object area	Intercept	19.961	1.485	
	*Rainfall**	0.016	0.014	0.004
	*Solar Exposure**	−0.391	0.039	0.044
	*Minimum temperature**	−0.157	0.015	−0.038
	*Maximum temperature**	−0.023	0.008	−0.011
NDVI	Intercept	−1.776	0.016	
	*Rainfall**	0.002	< 0.001	0.037
	*Solar Exposure**	0.058	< 0.001	0.542
	*Minimum temperature**	0.006	< 0.001	0.129
	*Maximum temperature**	0.006	< 0.001	0.232
SAVI	Intercept	−2.662	0.024	
	*Rainfall**	0.002	< 0.001	0.037
	*Solar Exposure**	0.086	0.001	0.542
	*Minimum temperature**	0.009	< 0.001	0.129
	*Maximum temperature**	0.009	< 0.001	0.232
TGI	Intercept	1.258	0.654	
	*Rainfall**	0.499	0.006	0.277
	*Solar Exposure**	−0.658	0.017	−0.153
	*Minimum temperature**	−0.108	0.007	−0.055
	*Maximum temperature**	−0.089	0.004	−0.089
VARI	Intercept	−0.001	0.021	
	*Rainfall**	0.001	< 0.001	0.019
	Solar Exposure	−0.001	0.001	−0.006
	*Minimum temperature**	−0.003	< 0.001	−0.047
	Maximum temperature	0	< 0.001	−0.008
Green Ratio	Intercept	0.353	0.001	
	*Rainfall**	0.001	< 0.001	0.149
	*Solar Exposure**	−0.001	< 0.001	−0.061
	*Minimum temperature**	0	< 0.001	−0.052
	*Maximum temperature**	−0.001	< 0.001	−0.011

Note: * $P < 0.05$. *B* = unstandardized regression coefficient; SEB = Standard error of the coefficient; β = standardised coefficient.

3.2. Plant Performance Monitoring for Target and Non-Target Seedling Communities

OBIA classification identified 156 target seedling objects with a total area of 0.8 m^2 and 10,419 non-target seedling objects with a total area of 5.2 m^2 from day 68 imagery. The number and total area of both target and non-target seedling objects declined rapidly over the 24-day drying period. By day 92, only 14 target seedling objects with a total area of 0.04 m^2 (reductions of 91 and 94%, respectively) and 16,585 non-target seedling objects with a total area of 1.7 m^2 (reductions of 47 and 68%, respectively) could be identified.

Classified target seedling objects exhibited distinct spectral signatures from that of non-target seedling objects and these signatures responded differently to the 24-day drying period (Figure 3). There was a statistically significant interaction between all measured indices and time among groups (Table 4), with target objects ca. 10-fold larger than non-target objects and exhibiting higher values for Green Ratio, Normalised Difference Vegetation Index (NDVI), Soil-adjusted Vegetation Index (SAVI), Triangular Green Index (TGI) and Visible Atmospherically-resistant Index (VARI) over the experimental period. Mean values for all measured spectral indices declined for both target and non-target seedling objects over the 24-day drying period. The area, green ratio and TGI of target seedling objects remained significantly higher than that of non-target objects, while values of NDVI, SAVI and VARI for the two groups gradually trended to similarity (Figure 3).

Table 4. Two-way mixed ANOVA outputs for significant interactions between object area and visible spectrum and multispectral indices and time among target and non-target classified seedling objects over the 24-day experimental drought period. Partial η^2 reports the effect size of each interaction, while ε reports Greenhouse-Geisser estimates of sphericity.

Index	*F*	*P*	partial η^2	*E*
Area	1054.134 (8.092, 108,141.318)	< 0.001	0.073	0.736
Green Ratio	68.479 (2.161, 28,878.153)	< 0.001	0.005	0.196
NDVI	167.570 (6.414, 85,717.123)	< 0.001	0.012	0.583
SAVI	167.137 (6.420, 85,800.860)	< 0.001	0.012	0.584
TGI	220.636 (4.965, 66,357.318)	< 0.001	0.016	0.451
VARI	24.311 (6.135, 81,994.764)	< 0.001	0.002	0.558

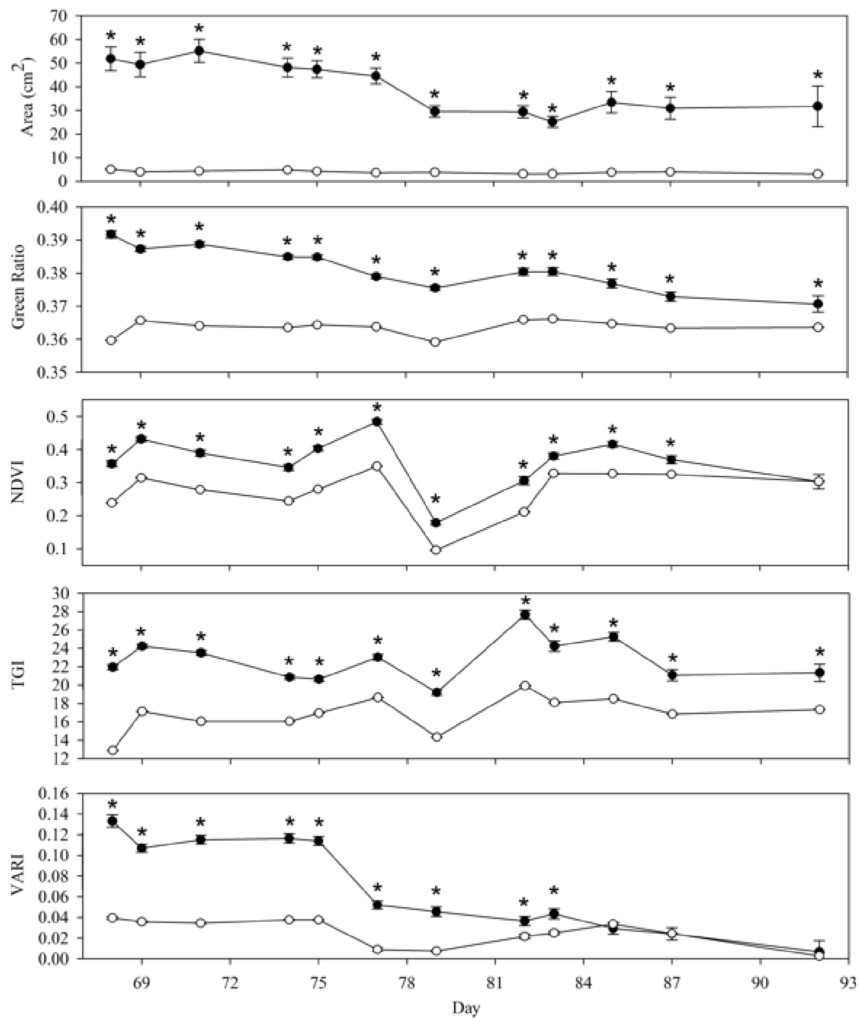

Figure 3. Average values of object area and five visible spectrum and multispectral indices for target (filled symbols) and non-target (open symbols) seedling objects over a 24-day drought period. Error bars indicate 1 s.e. of the mean. Asterisks indicate statistically significant differences among groups at each time point ($P < 0.05$). Data for SAVI showed a near-identical trend to NDVI and are not shown.

VARI and Green Ratio were moderately positively correlated with both mean object area and the number of target individuals over the monitoring period (Table 5), while NDVI and SAVI correlated moderately with mean object area but poorly with the number of target individuals. TGI exhibited no correlation with either variable.

Table 5. Pearson correlations between visible spectrum and multispectral indices for classified target seedling objects (*Lupinus angustifolius*) and the mean object area and number of surviving individuals. $n = 1145$.

	Object Area		Number of Individuals	
Index	**Pearson Correlation**	**Significance**	**Pearson Correlation**	**Significance**
Green Ratio	0.435	< 0.001	0.412	< 0.001
NDVI	0.408	< 0.001	0.120	< 0.001
SAVI	0.408	< 0.001	0.120	< 0.001
TGI	−0.046	0.12	−0.098	0.001
VARI	0.470	< 0.001	0.517	< 0.001

3.3. Monitoring Individual Target Seedling Objects Through Time

A total of 16 naturally occurring reference points (all small rocks) were used to align orthomosaics, ranging in size from 4.9–161.1 cm2 (average 44 ± 11.2). Following orthomosaic alignment, OBIA classification allowed for the tracking of the designated 25 target seedling objects (individuals) over the 24-day drying period with a high level of accuracy and precision. The centroids of target seedling objects exhibited global average X- and Y-axis displacement from their initial recorded position of 10 ± 0.9 mm and 14 ± 1.1 mm, respectively, over the 24-day monitoring period, with the greatest centroid displacement being 66 mm (Table 6). No statistically significant correlation was evident between the area of target individuals and either X- ($R^2 = 0.017$, $P = 0.937$) or Y- ($R^2 = 0.095$, $P = 0.651$) centroid displacement.

Table 6. Average X- and Y- displacement of the centroid of 25 temporally tracked target seedling objects (*Lupinus angustifolius*) from time-zero centroid position across 24 days of captured imagery, with the range of centroid displacement observed for each individual indicated in parentheses.

Individual	**Area at Day 68**	**X-Displacement (mm)**	**Y-Displacement (mm)**
1	170	15 ± 3.3	19 ± 5.1
		(4–37)	(2–45)
2	551	3 ± 2.1	14 ± 5.6
		(0–9)	(0–25)
3	144	0 ± 0	0 ± 0
		(0–0)	(0–0)
4	135	12 ± 6.2	15 ± 7.6
		(0–28)	(1–40)
5	109	4 ± 1.1	3 ± 1.0
		(0–10)	(0–10)
6	43	12 ± 4.5	30 ± 8.2
		(0–23)	(9–57)
7	186	11 ± 3.5	16 ± 7.8
		(2–21)	(0–46)
8	63	8 ± 2.1	10 ± 3.5
		(2–21)	(1–34)
9	218	8 ± 5.6	19 ± 14.4
		(2–13)	(4–33)

10	79	7 ± 1.8 (3–19)	13 ± 3.0 (2–26)
11	125	9 ± 4.2 (1–23)	28 ± 5.7 (16–50)
12	982	15 ± 11.2 (0–47)	18 ± 12.1 (0–51)
13	73	10 ± 2.8 (3–18)	12 ± 3.0 (1–18)
14	92	7 ± 1.9 (2–10)	20 ± 4.8 (10–37)
15	127	34 ± 6.5 (22–44)	17 ± 4.6 (9–25)
16	42	14 ± 3.4 (2–30)	11 ± 3.7 (1–34)
17	70	12 ± 5.9 (1–50)	9 ± 2.3 (3–20)
18	107	11 ± 2.7 (0–25)	17 ± 4.9 (1–45)
19	49	8 ± 2.7 (3–17)	28 ± 6.3 (6–44)
20	119	4 ± 1.4 (0–7)	10 ± 2.5 (3–15)
21	111	3 ± 1.3 (0–6)	5 ± 2.9 (0–17)
22	36	8 ± 8.0 (0–32)	12 ± 11.0 (0–45)
23	33	5 ± 2.7 (0–15)	8 ± 3.0 (1–18)
24	46	10 ± 2.8 (0–26)	15 ± 2.9 (4–28)
25	52	23 ± 9.3 (0–66)	10 ± 2.7 (2–25)

At the beginning of the drying period (day 68) the 25 target seedling objects ranged in Area from 33–982 cm^2 (mean 150 ± 40.3 cm^2; Table 6). Object area and all visible spectrum indices declined rapidly in monitored target individuals over the 24-day drying period (Figure 4), even following a 17 mm rainfall event over days 80–83 (Supplementary 2). The number of days until mortality ranged from 7–24 (mean 16 ± 1.3) across all classes and decreased along a size gradient from Very Large (19 ± 2.8 days; $n = 5$) and Large (18 ± 2.3 days; $n = 6$) individuals to Medium (13 ± 2.2 days; $n = 8$) and Small (12 ± 2.2 days; $n = 6$) individuals.

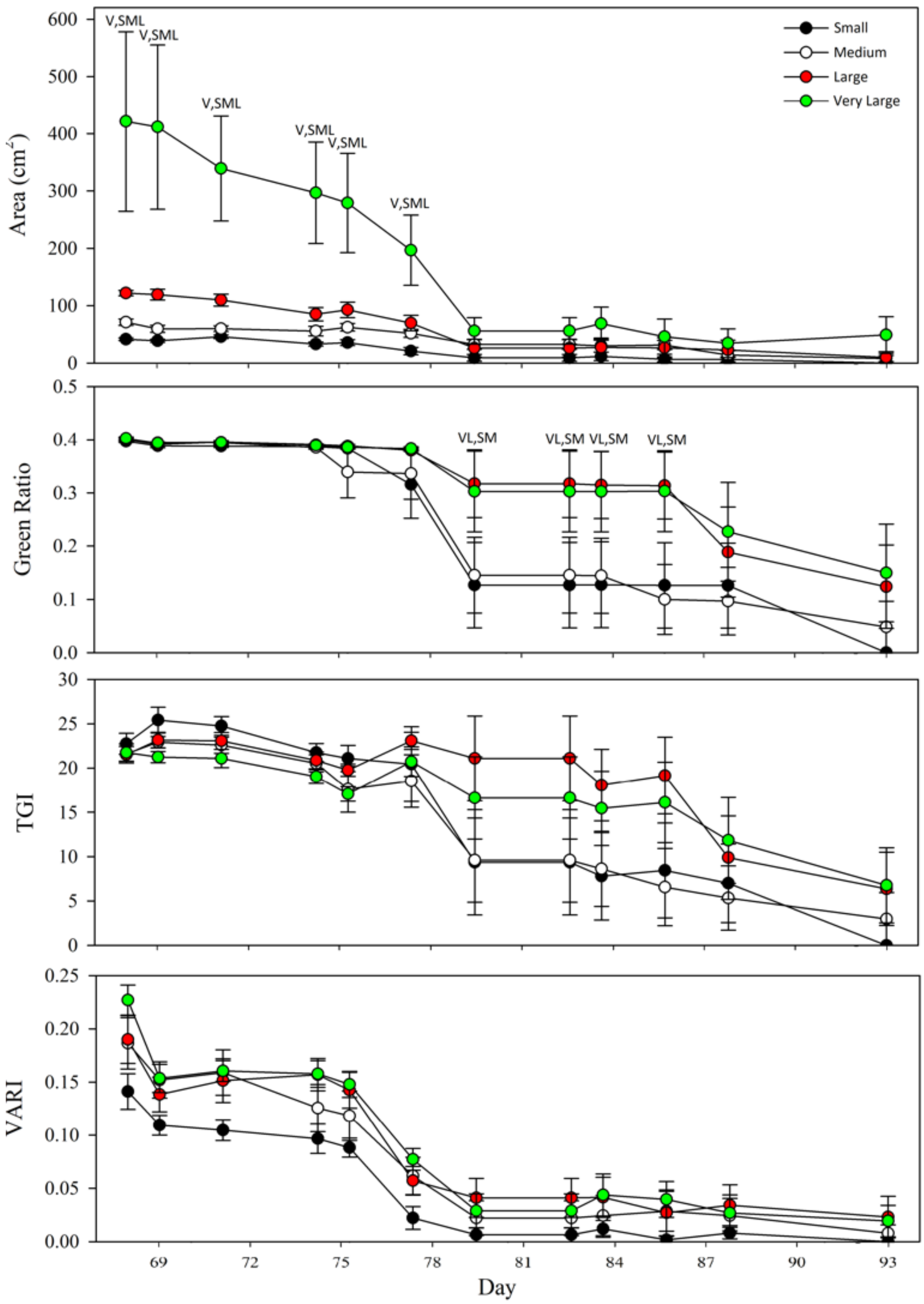

Figure 4. Daily values of object area and three visible spectrum indices for 25 target seedling objects placed into four size classes over a 24-day drought period. Small (S): < 50 cm² at day 68. Medium (M): 50–100 cm² at day 68. Large (L): 100–150 cm² at day 68. Very large (V): > 150 cm² at day 68. Error bars indicate 1 s.e. of the mean. Annotated numbering indicates statistically significant pairwise groups among size classes at each time point ($P < 0.05$).

Individuals from Very large and Large size classes generally exhibited higher mean values for all metrics over the experimental period, although classes were statistically similar in all metrics by day 92 (Figure 4). There were statistically significant interactions between the area of classified groups and time ($F[3.592, 25.144] = 5.877$, $P = 0.002$, partial $\eta^2 = 0.456$, $\varepsilon =$

0.120) and the Green Ratio of classified items and time (F[7.381, 51.666] = 0.1.164, P = 0.339, partial η^2 = 0.143, ε = 0.246), while neither TGI (F[6.966, 48.786] = 1.399, P = 0.227, partial η^2 = 0.167, ε = 0.232) nor VARI (F[9.814, 52.516] = 0.795, P = 0.602, partial η^2 = 0.102, ε = 0.250) interacted significantly with time among groups.

4. Discussion

4.1. The Effect of Daily Climatic Conditions on Spectral Indices

The strong influence of daily weather conditions on spectral indices indicates that these metrics provided a useful measure for plant response to weather conditions. Images were calibrated with the aid of a reflectance target to control for the effect of daily climatic variance on reflectance values, as the ratio of reflectance of various wavelengths of light determine the values of vegetation indices utilised [57]. Weather-dependent variation in indices was therefore reflective of changes in the spectral signature of seedlings in response to factors such as moisture, wind and temperature [59]. Object area and green-dependent visible spectrum indices (TGI, VARI and green ratio), which are considered indicators of leaf chlorophyll content [55,56], increased in periods of higher soil moisture following rainfall and decreased with higher daily temperatures and solar irradiance. Previous studies suggest that higher leaf chlorophyll content during such favourable periods for growth would be expected [60–62]. NDVI and SAVI were positively associated with all climatic variables but declined towards the end of the experiment for *L. angustifolia* when drought stress was likely greatest, reflecting their utility as general indicators of plant health [57]. However, field study of plant physiological responses to stress factors such as water limitation and temperature should ideally be conducted concomitantly to validate the collection of spectral data [14,63]. Future studies should continue translational research to test the utility of spectral indices currently applied in agricultural settings to assessment of the health of non-agricultural plant species and communities and complement remote sensing data with ground truthing examination of plant ecophysiological performance.

4.2. Classification and Tracking of Seedling Communities

Target and non-target seedlings exhibited distinctly different visible-spectrum and multispectral signatures, differed statistically significantly in every spectral index examined and their spectral signatures behaved differently over the course of the drying phase of the experiment (Figure 3). These differences allowed for the discrete and accurate tracking of object groups both spatially and temporally and for the measurement of spectral index values as indicators of plant performance independently for each group. This study also provides the first evidence that accurate and reliable UAV-based monitoring of plant health is achievable not only at fine scales for establishing seedling communities but also at the scale of individual seedlings. Although our data were collected from low-altitude flights and from a constrained spatial area, they demonstrate the significant capability that UAVs will likely bring to the monitoring of ecological restoration and rehabilitation, as well as other industries such as silviculture and viticulture [64,65], with further research and development.

Application of this capability to ecological recovery monitoring could dramatically improve the accuracy and rapidity of surveys for particular species of interest in large-scale restoration plantings (e.g., spectral discrimination of a particular rare or restoration-significant species from among a biodiverse reinstated plant community) and improve the capacity to monitor plant performance at individual-scale to enable early warning of declining plant health (e.g., to identify declines in high-value or indicator species enabling early responsive restoration management). Although UAVs are likely to represent an effective monitoring tool for many restoration and rehabilitation projects, there are two major constraints to be overcome. The first is legislative restrictions, which cannot be discussed in depth due to differing laws from jurisdiction to jurisdiction and the second is cost, which remains a primary limitation to UAV application and the specific technological requirements of UAV-based remote sensing in ecological recovery are likely to differ among projects dependent upon their ecological recovery goals and monitoring outcomes. With adequate investment and demand-driven research UAVs almost certainly represent the future of monitoring in ecological recovery [14].

Studies have suggested that satellite-based remote sensing may facilitate accurate and real-time monitoring of plant health at community and landscape scale [66]. Given the more rapid turnaround times offered by UAVs compared with satellite imagery and the current application of UAVs to plant performance monitoring in agricultural contexts [43,67], we suggest that accurate real-time monitoring of plant health in ecological restoration using UAVs may already be possible with current technology and infrastructure. As the availability of only low-resolution sensors constrained our experimentation to low-altitude flights, future studies should also test the outcomes of multi-sensor UAV-based plant performance monitoring at greater flight altitudes and with higher resolution sensors. Additionally, studies should focus on testing a broader array of multispectral (and, indeed, hyperspectral) indices and robustly testing the relevance of these indices to the physiological performance of monitored plants.

4.3. Classification and Tracking of Individual Seedlings

The use of naturally occurring reference points yielded acceptable accuracy in orthomosaic alignment and undertaking OBIA on aligned orthomosaics allowed for precise tracking of specific individual seedlings over the 24-day drying period. Locational errors were low, with object centroid displacement for tracked objects averaging < 15 mm across all objects over the course of the experiment. This precision allowed individual seedlings to be tracked as they developed, while simultaneously capturing a range of ecological data relating to their growth and performance, until mortality was evident (confirmed by ground-truthing). To our knowledge this is the first time that UAV-based imagery has been empirically demonstrated to offer such fine-scale spatial and temporal resolution in monitoring plant growth and development. While we acknowledge that our study was conducted over a small area and using imagery captured at very low altitudes, our data offer compelling evidence that UAV-based remote sensing can generate meaningful, reliable and affordable empirical data about the growth and development of vegetation communities and even individual plants early in their life cycle. This capacity can only be improved by the application of more technologically advanced UAV systems (e.g., multi-sensor platforms and sensors offering greater resolution or greater spectral discrimination) and may revolutionize the manner in which the monitoring of environmental recovery activities such as rehabilitation and ecological restoration are undertaken. UAV-based aerial monitoring of rehabilitated or restored areas offers an accurate method of tracking seedling communities and even high-value individuals (e.g., rare, threatened or commercially-important species), providing practitioners with a robust tool for predicting the trajectory of these communities by remotely measuring growth and development and providing early warning signs of environmental stressors such as drought.

4.4. Sensor Misalignment

One factor reducing the utility of multispectral imagery captured in this study was poor overlap for target seedlings among captured RGB and multispectral imagery. This misalignment likely reflected a lack of synchronisation between the drone-mounted RGB camera and the multispectral sensor; although both sensors were capturing an image every two seconds, images were not captured simultaneously. Images for each sensor were thus captured at slightly different points in space, causing overlap error at the scale of several millimetres. This reduced the accuracy of individual object locations within resultant orthomosaics, yielding internal target object misalignment within even perfectly aligned orthomosaics. This error could be resolved through development of a combined single-unit RGB and multispectral sensor able to be triggered simultaneously and geotagged by the same GPS unit. Although the multispectral sensor we utilised does house an integrated RGB camera, it offered reduced resolution (16 megapixel) and possessed a rolling shutter (rather than the global shutter used on the Phantom 4 Pro). Rolling shutters can reduce orthomosaic accuracy and resolution by introducing significant image distortion from imagery taken from a moving platform such as a UAV [68]. We echo previous calls for urgent emphasis to be placed upon the development of affordable and reliable multi-sensor pods capable of triggering all sensors simultaneously and being tagged by the same GPS unit to eliminate or reduce error [14]. Further consistency and accuracy in image capture locations could be achieved through the use of real-time kinematic (RTK) GPS units, which are able to hold planned flightpaths to within centimetre-level tolerances [69]. Accuracy and precision in object location and target overlap among imagery can also be improved through the use of numerous ground control points (GCPs) [42]. GCPs allow for more accurate alignment than reliance upon fixed surface features (e.g., rocks, infrastructure) such as those employed in this study. However, in scenarios such as the monitoring of ecological restoration on post-mining landforms the placement and use of traditional GCPs may be impractical due to limited site accessibility, hazardous features (e.g., unconsolidated tailings storage facilities) or rugged terrain (e.g., angle of repose slopes on waste rock landforms). While sensor output alignment will likely be improved in the future, our data suggest that even small naturally occurring reference points can be an effective tool for relative orthomosaic alignment.

4.5. Avian Interactions with the UAV

Studies should report significant fauna interactions during UAV use to better understand their impact as environmental monitoring tools. Significant interactions during our experiment were observed with multiple individuals of Australian Black-Shouldered Kites, *Elanus axillaris*. These raptors conducted multiple shallow dives towards the UAV while it was in flight, beginning in early December and becoming increasingly frequent over the latter days of the trial. Visual surveys were conducted prior to all flights and flights did not proceed if a Black-Shouldered Kite was sighted. The UAV was immediately landed following any approach by a bird, with flights terminated for the rest of the day and no physical contact was made at any point between the UAV and the birds. One other interaction of note was of Australian Magpies, *Cracticus tibicen*, which often flew or landed underneath the UAV while it was in flight. These birds appeared unperturbed by the presence of the UAV and were regularly present in imagery captured from flights undertaken at 5 and 15 metre altitude (see supplementary materials).

5. Conclusions

This study provides evidence for the significant utility of UAV-based sensing of both visible and non-visible vegetation indices in monitoring individual target seedlings within seedling communities at fine spatial scales. OBIA classification effectively and accurately discriminated among target and non-target seedling objects and these groups exhibited distinct spectral signatures (six different visible-spectrum and multispectral indices) that responded differently over a 24-day drying period. OBIA classification from captured imagery also allowed for the accurate tracking of individual target seedling objects through time, clearly illustrating the capacity of UAV-based monitoring to undertake plant performance monitoring of individual plants at very fine spatial scales. The strongest effect sizes of regression models assessing among-group differences in spectral signature were obtained for multispectral indices (NDVI and SAVI) and green-dependent visible spectrum indices (TGI, VARI and green ratio) and these metrics were also informative for tracking individual seedlings through time. We propose that these indices represent a useful tool for plant performance assessment and discretionary classification in the context of rehabilitation and ecological restoration but that they will likely require significant further development to be as informative for native plants as they currently are in agricultural settings. With further research and investment UAV-based remote sensing will allow industry and restoration practitioners to undertake plant performance monitoring earlier in the community recovery process, with greater accuracy, precision and cost-efficiency and at much finer resolution over increasingly large spatial scales, particularly when compared to other means of monitoring such as foot surveys and manned aircraft.

Supplementary Materials: The following are available online at www.mdpi.com/xxx/s1, S1: Full eCognition Rule Sets for Automated Seedling Identification from Buters *et al.* 2019., Table S1: Measured spectral variables for 25 tracked target seedling objects over a 24-day simulated drought period.

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The use of Unmanned Aerial Vehicles (UAVs) for ecological monitoring has taken flight

~~**Introduction**~~

~~Despite the recent rapid increase in the availability of small, commercially available drones for purposes such as photography, the public perception of unmanned aerial vehicles (UAVs), or 'drones', as they are colloquially referred to, is that they are military tools – or, more recently, children's toys. Historically speaking, that perception is entirely accurate. The first UAVs ever constructed were unmanned hot air balloons that were used to bomb Venice in 1849[1], and as time has passed the application of UAVs to military operations has become deadlier; the word 'drone' is still synonymous with the idea of Predators carrying out targeted missile strikes in the Middle East. However, UAVs are increasingly becoming recognised as useful tools for peaceful purposes, and the truth is that UAVs have been in use for non-military applications for decades. For example, unmanned helicopters have been used for crop pesticide spraying in Japan since 1990[1]. UAVs are increasingly being used for niche and novel work, such as the identification of mosquito breeding sites in urban areas[2], the prevention of rhinoceros poaching in Africa[3], and the identification of areas of high radiation after nuclear accidents[4,5].~~

~~The field in which UAVs stand out most significantly is in the area of remote sensing an earth observation. UAVs have many characteristics that make them a unique choice in this field: their ability to fly low to the ground means they offer a much higher spatial resolution than manned aircraft or satellites[6], while being able to pass under clouds that might otherwise obscure a detailed view of the land[7]. The relatively low cost of their operation, rapid turnaround times and greater operational flexibility compared with manned aircraft and satellites[8] make UAVs extremely easy to integrate into a variety of projects with different user needs provided at small to medium scales (studies have recommended UAVs for areas of up to 500 hectares[9]). However, the effective area in which UAVs can operate will only increase with time as technology improves[10]. As the variety of sensors both lightweight and cheap enough to be mounted on UAVs improves[1], it is likely that UAVs will become increasingly ubiquitous tools in a wide variety of monitoring applications.~~

~~Researchers already have a wide range of sensors to choose from to fit the particulars of any job at hand. Digital Red-Green-Blue (RGB) cameras are the most common sensor employed in the scientific literature[11], and have been used for a wide variety of applications such as the detection of target species of both plants and animals[12,13], and to map out areas of geographic interest in high detail[14]. RGB cameras can also be modified by removal of the internal hot filter to allow for the detection of Near Infrared (NIR) light, which greatly improves their ability to identify key vegetation[15,16], due to the variance in spectral signatures between plant and non-plant objects. Multispectral sensors also detect NIR light, and are much more precise with the wavelengths of light they record, and record a greater variety as well, such as red edge. Analysis of multispectral sensor data allows for the calculation of a variety of multispectral vegetation indices[17], which have been commonly used in agricultural contexts to detect a variety of plant ailments such as rice sheath blight[18] and verticillium wilt in olive orchards[19]. Additionally, some multispectral cameras can be adjusted with the use of filters in order to attune them to the most relevant spectral bands for the use at hand[20].~~

~~Hyperspectral sensors further improve upon the detail of data captured offered by multispectral sensors, by detecting a far greater range wavelengths, sampled at narrow bands of light – hundreds at a time[21]. Although multispectral sensors have been employed to~~

~~provide early warning that something is wrong with a plant by identifying general stress[22], they lack the spectral resolution to pinpoint exactly what the problem factor represents, due to the specific bands needed to identify each particular problem[22]. Hyperspectral sensors predominantly solve this issue, and they have been used, for example, to accurately discriminate between different fungal diseases in sugar beets[22] and to estimate detailed plant characteristics such as leaf carotenoid content[23]. While UAV mounted hyperspectral sensors are currently limited in spectral range and spatial resolution due to the size and weight limits intrinsic to UAV mounted sensors, this is expected to improve in the future. However, while prices of most sensors are generally expected to fall as technology advances, hyperspectral sensors will likely always be significantly more expensive than their multispectral counterparts[24].~~

~~Two highly complex sensors are also available, though at significantly greater cost, in the form of thermal cameras and Lidar (Light Detection and Ranging). Thermal cameras have been utilised in the monitoring of both fauna and flora: in addition to being used to detect large warm-blooded animals such as white-tailed deer[25], they have also been used to detect water stress in plants[26,27]. Although these sensors have been utilised far less commonly in UAV based applications than other sensors, Lidar has been used to generate highly detailed Digital Elevation Models (DEMs) of areas, which provide information about the elevation of each point within the model. Although DEMs generated from UAV-captured RGB imagery now have accuracy approaching lidar[28], with RGB imagery starting to be used increasingly to model areas of bare earth without excessive vegetation, lidar remains the superior choice in areas where scanning is required to penetrate a barrier such as a forest canopy or water surface and return a model of what lies beneath[29-31], due to the fact that it is an active sensor – that is to say, it is the source of the pulse it reads the return of. This allows for selection of wavelengths that have different absorption properties, and thus can penetrate different substances[32].~~

~~Although the sensors utilised in UAV-based monitoring are clearly of significant importance as they determine the kind and quality of data captured, an equally crucial aspect of monitoring is the image processing software utilised in the classification of these data. First, and most crucial, is the image stitching software based off the Structure from Motion (SfM) technique proposed by Turner, Lucieer, and Watson in 2012[33]. While the low altitude of UAV monitoring returns imagery with a very high spatial resolution[6], the lower altitude also means that each individual image covers a smaller area on the ground[33] (the image footprint). To overcome this issue, SfM matches captured features within multiple images in order to align the inputted images and create large geo-referenced ortho-rectified mosaics (orthomosiacs); single images that cover the entire UAV flight area, but maintain the fine spatial resolution of the smaller individual images. Orthomosaics are generated from a corresponding DEMs . Although there is generally a small margin of error, this can be reduced by using ground control points or higher accuracy GPS (such as real time kinematic, post processed kinematic, or precise point processed GNSS, RTK, PPK, and PPP respectively).~~

~~One drawback of creating a single image to cover large study areas is the amount of computational power required to create it, and the time required to analyse it and identify any points of interest. Numerous methods of automated image classification have been increasingly used to reduce the amount of time required for identifying features of interest in orthomosaics. The first of two main approaches commonly used is supervised machine learning, which works by selecting training data to 'teach' the program what certain classes look like; requiring examples of both positive samples and negative samples[34]. In this manner the user is considered to be 'supervising' the machine learning. Once enough training data has been selected, the program is able to classify future images based off their resemblance to the learned classes[35]. There are numerous different approaches to achieving this, however, as each approach ultimately has the same goal, they will be referred to hereby~~

~~by the blanket term of supervised machine learning. The second main approach is Object-based Image Analysis (OBIA); a technique that splits large images up into smaller, spectrally similar 'objects' that allow for classification based not only on colour, but other contextual clues such as size, shape, and relationship to nearby objects[15]. While machine learning techniques can also be used on image objects, the general trend in the ecological monitoring literature is to use OBIA to refer to an object-based analysis approach that classifies image objects based on a user-defined ruleset[11].~~

~~Examination of the general literature focussing on UAV-based monitoring of ecological restoration efforts identified three key knowledge gaps. Firstly, there had not yet been a detailed literature review analysing, synthesising and describing which sensor applications and classification approaches were consistently successful in ecological applications. Secondly, despite the extremely high spatial resolution offered by UAVs, no studies could be identified that actually utilised this capacity in the context of ecological recovery efforts such as ecological restoration. Thirdly, studies have been conducted almost entirely utilising only a single UAV-mounted sensor, almost ubiquitously a standard RGB sensor. Where multiple sensors were used, they were generally both flown and analysed separately.~~

~~This thesis provides the first empirical research addressing these three crucial knowledge gaps. My first chapter represents a global literature review of the application of UAV-based approaches to monitoring ecological recovery. My second chapter presents a novel approach to early assessments of restoration trajectory, and utilises the 1 mm/pixel resolution available to UAVs to accurately classify and monitor seeds and seedlings from aerial imagery. My third chapter utilised both RGB and multispectral sensors, flown simultaneously and analysed in concert, to assess plant performance in a heterogenous seedling community over time in response to drought stress.~~

Review of previous studies

The first chapter of my thesis, entitled "Methodological ambiguity and inconsistency constrain unmanned aerial vehicles as a silver bullet for monitoring ecological restoration" and recently published in *Remote Sensing* [1], addresses inconsistency in the use of UAVs as a remote sensing platform in ecological recovery projects. These inconsistencies relate to the choice of UAV platform, choice of UAV-mounted sensor loadout and choice of post-capture image classification technique. The review found that, despite a large and rapidly-growing body of literature relating to UAV-based monitoring to draw on, only 56 publications made reference to restoration outcomes in UAV monitoring. Of those, only 48 studies provided experimental results applying UAV-use to rehabilitation and ecological restoration objectives. This disparity in technological application between ecological restoration and other fields (e.g., 206 studies presented experimental data in agricultural contexts [1]) indicates that UAVs are being dramatically underutilised in ecological recovery monitoring. The review provides a timely and important analysis to guide future research efforts, as highlighted by two anonymous reviewers who stated "*This is a very important and very timely topic for study and it is critical to have review papers such as this to provide a summary of the current state of the field*" and "*I enjoyed reading the MS, which is well written and touches upon an interesting topic*". The importance of a well-established knowledge base and a strong methodological framework built upon rigorous critical analysis is crucial in the field of UAV monitoring; over two thirds of the studies reviewed relating to ecological recovery monitoring had been published in the last three years, despite the literature spanning a period of nearly 25 years [1], suggesting the field is experiencing an explosion of research development.

The literature review highlighted three particularly significant gaps and inconsistencies in the literature [1]:

Single sensors, mostly simple ones

Correlation in complexity

Lack of plant performance evidence

Each of these gaps and inconsistencies represent a barrier to the use of UAVs as a single-pass method of monitoring mine site restoration, and as such further studies will need to be conducted to fill in these gaps.

One gap in studies that the literature review revealed was that studies that utilised more than one sensor were limited (13 of 48 studies), and those studies that did use multiple sensors generally flew them separately. Furthermore, the type of sensor used was generally basic, with 40 of the studies utilising an RGB camera. While this in itself is not concerning due to the variety of key indicators that can be detected with RGB imagery, 28 of these 40 studies used only RGB cameras with no additional sensors. Utilising only a single sensor, typically RGB, means that studies are limited in what can be easily detected, and are not gaining the full benefit in UAV monitoring. Flights conducted with multiple sensors take the same time to complete as flights with a single sensor (although payload weight is an issue that needs to be considered), and as such the more sensors are carried on a UAV, the more data can be gathered in the same timeframe. The review proposes a 'one-pass' approach to restoration monitoring, which involves every key indicator in a restoration project being monitored in a single UAV flight. Future studies will need to be conducted to determine the optimal combination of sensors to gain as much information as possible while keeping payload weight and cost to a minimum. Importantly, our analysis of reviewed literature found that there was a strong correlation between the complexity of sensors used, and the complexity of image classification method, suggesting that financial barriers of independent studies may be a limiting factor to fully exploring the topic. Additionally, separate studies on very similar topics commonly utilised very different approaches in terms of sensor choice and image analysis methods. While this may be due to restrictions in the UAV's flight and payload capacity, or financial restrictions, as previously stated, there seemed to be general methodological confusion, particularly regarding choice of image analysis method. Studies utilising machine learning approaches were particularly bad in this aspect – the seven studies reviewed that utilised machine learning trialled eight different approaches, and only two of these were used in more than one paper. This methodological confusion was also evident in papers utilising an OBIA approach. While each paper independently referenced previous works utilising OBIA and provided justification for why the technique was a good fit for their work, there were few examples of papers referencing previous examples of OBIA being used in restoration, suggesting a lack of knowledge of what work had previously been undertaken in the field. While we contend that UAVs represent a method of conducting a 'one pass' analysis of restoration progress, greater clarity in usable methodology is required, and higher levels of cooperation between disciplines will be needed to shine a light on the solution.

In particular, this study noted that despite the claims of many in the UAV vegetation assessment industry, we found no evidence of UAV assisted plant condition analysis being proven in a restoration context. Where vegetation monitoring was conducted, it was most often reported simply as area of vegetation cover, or classified by type of vegetation (trees, grasses, scrub, etc). Overall, despite the presence of some studies utilising more advanced and expensive sensors (such as multispectral, hyperspectral, and thermal cameras), almost all studies focussed on simple presence/absence analysis, with only one study [2] classifying vegetation according to its state of health. Even in that study however classification was merely based on whether the target plant was alive or dead, with no attempt to provide an early warning of potential mortality.

The literature review proposes the concept of a 'one-pass' solution that fills the gap in UAV studies and presents a large cost and time saving in restoration monitoring. The 'one-pass' solution proposes the use of a UAV equipped with multiple sensors to monitor every aspect of a restoration project. RGB cameras would be used to create a map of the area and for identification of plant species, multispectral and hyperspectral sensors would be used for monitoring plant health, and thermal sensors used for detection of large animals and plant water stress. Further sensors could be added as required – for example, while DEMs would typically be generated from RGB imagery, lidar could be used in scenarios where penetration of canopy or water surfaces is required. Following acquisition of all required imagery, automated classification processes such as machine learning and OBIA would be used to classify the generated orthomosaics, providing full counts of plants and large animals, plant health analysis, geomorphological information and all other pertinent information. This process would greatly reduce the amount of time restoration practitioners would need to spend on site, and improve outcomes by gaining a full understanding of the restoration area, rather than the representative segments quadrat surveys offer.

Identification and counting of seeds and seedlings

Chapter two, entitled "Seed and Seedling Detection Using Unmanned Aerial Vehicles and Automated Image Classification in the Monitoring of Ecological Recovery" and recently published in *Drones*, seeks to address a gap in UAV monitoring – utilising the extremely fine spatial resolution in order to carry out identification of target seeds and seedlings. While UAVs offer a much finer spatial resolution than manned aircraft or satellites [3], no studies to date have utilised this aspect to its full extent. In general, studies utilising UAVs have tended to use them as a cheaper, more convenient alternative to manned aircraft, without fully exploring the advantages they offer as a unique remote sensing platform. We explored the ability of UAVs to fly at extremely low altitudes over a variety of substrates, and utilise the resulting ultra-fine resolution imagery to classify seedlings at scales never before utilised in UAV monitoring.

Chapter two examined the usefulness of extremely fine spatial resolution in restoration monitoring, by assessing restoration practitioners' ability to identify seeds and seedlings of target species from UAV imagery across a variety of substrates. We found that seeds were clearly identifiable from 5 m and 10 m altitude flights, and target seedlings were clearly identifiable from 5 m (1.02 mm per pixel), 10 m (2.63 mm per pixel), and 15 m (4.04 mm per pixel) flights. We were able to create a ruleset to allow for the automated identification of seeds, and two different approaches to identify target seedlings, despite the presence of a background of commercial grasses. Accuracy in identifying target seeds was nearly 90% at 5 m with minimal false positives, and still achieved ca. 75% at 10 m, albeit with an increase in false positive rate. At both heights false positive rate was markedly higher on heterogeneous substrates. Two approaches to identifying target seedlings were identified – a single-date approach that utilised the orthomosaic and DEM generated from a single day's flight, and a layered approach that used orthomosaics from two dates layered atop each other. While both approaches achieved a high level of accuracy from 5 m flights, the single image approach achieved superior accuracy at higher level flights, particularly at 15 m. However, at all levels the layered approach had a significantly lower rate of false positive returns.

This work is important to restoration practitioners, as it provides the first ever remote sensing based method of identifying and counting seeds after sowing. There are numerous applications for this technique that would be invaluable for restoration practitioners. This technique would be immediately useful for monitoring numbers of seed in a newly sown area, and thus determining predation rates and allowing for a more accurate assessment of germination rates, both of which are bottlenecks to restoration success [4,5]. The ability to track sown seed also allows for further studies to identify microsite conditions that are the most suitable for seed germination, which is well established as being crucial to species establishment from seed [6-8]. Using the ability of UAVs to generate finely detailed DEMs, micro-topographic data could be gathered for every sown seed [9], and the ability to automatically identify seedlings as they emerge would allow restoration practitioners to identify which seeds germinated, and as such what microsite conditions are best for seed germination – a key factor in restoration successes [6,8]. This technique could be further enhanced by the incorporation of thermal cameras, to gain additional information about thermal refugia conditions and their impact on whether or not a seed germinated.

The ability to identify seedlings, with two different approaches to use depending on the situation, will be invaluable to restoration practitioners. Only one previous study has used UAVs to monitor seedlings in restoration [10], and that was operating at a much coarser scale, focussing on the identification of seedlings grown from tube stock with a minimum area of 100 cm^2, which is more than six times larger than the minimum seedling size in our study of 16 cm^2. In addition, this study focussed solely on monitoring the combined area taken up by 'woody cover', with no mention given of the species makeup, and with very little background cover. The methods detailed in chapter two lay out a method of identifying only target seedlings, despite a deliberately sown background of obscuring grasses, and thus represents a viable technique to identify and count seedlings during their most vulnerable stages, even in what may be less than ideal conditions, such as in the midst of an outbreak of invasive weeds.

However, despite the possibilities presented by this work, there is still more to be done to ensure maximum efficiency with this technique. In particular, incorporating more advanced sensors such as multispectral or hyperspectral sensors could improve the accuracy of the technique [9]. Further studies should be conducted to ensure that the seedling identification method maintains a high level of accuracy even with a wider range of background species, and ideally a number of target species should be simultaneously identifiable with a high degree of accuracy. This study focused on a single target species, and as such application of this technique to different species may require changes to the ruleset, in order to home in on the target species' most distinctive characteristics. However, with the majority of the ruleset already in place, it is very unlikely that this ruleset optimization would take more time than traditional means of surveying plant life. It should also be considered that ongoing restoration efforts require frequent monitoring[11], and once the ruleset has been modified for the target species, it can be used over and over again, representing a significant time saving that only increases as time goes on. Additionally, the approach described here is accurate for altitudes of up to 15 m, and while this is suitable for the monitoring of small areas, restoration projects are increasing in scale worldwide [12,13], and thus monitoring programs need to increase in scale along with them, which will require flights to be conducted at higher altitudes to maintain time efficiency. While technological advancements in UAV mounted sensors will improve the heights at which accurate counts can be gathered, further refinement of the technique will be required to ensure that the results it offers keep pace with the demands of restoration monitoring.

Tracking of seedlings over time

Chapter three, entitled "Monitoring plant performance of individual seedlings at fine-scale temporal and spatial resolution using Unmanned Aerial Vehicles" addresses for the first time the use of multispectral sensors to monitor individual seedling health, and presents a method to identify and track individual seedlings over time. The study focusses on the use of multispectral cameras to monitor seedling health throughout a deliberately induced droughting period, and assesses the utility of multispectral sensors to provide early warning of seedling mortality as an indicator of restoration failure. In addition, this study presents a means of individually tracking seedlings through time. This, coupled with the ability to monitor seedling health with multispectral sensors, represents the next step forward for restoration monitoring – monitoring seedling health on an individual level rather than community level.

While multispectral sensors have previously been used in agriculture to identify poor plant health due to a variety of causes such as sheath blight in rice [14], water stress in melons

[15], and bacterial spot disease in tomatoes [16], their use in restoration has been limited, and generally restricted to aiding in identification of plants rather than monitoring plant health [1]. We found that both visible and non-visible vegetation indices provided an accurate assessment of plant condition during their decline over the droughting period. This was not an unexpected result given the previous studies in agricultural fields that have yielded similar outcomes [16-19], however this study is the first to focus on such small seedlings. While most previous studies have focussed on more mature plants [10], and have used multispectral sensors more to aid in identification than to track health [2,20], this study shows that not only can multispectral sensors be used to monitor the health of plants in a restoration context, they can be used to monitor the health of much smaller seedlings than has previously been demonstrated. Additionally, the differences shown in multispectral indices between target and non-target seedlings show that the automated classification process for identifying target seedlings [9] would be improved by incorporating multispectral sensors.

While the results presented in this paper are promising, more work is needed in two key areas to make full use of multispectral sensors in seedling monitoring. Firstly, while the results presented in this chapter showed that the multispectral vegetation indices did show a decline in line with declining plant health over the droughting period, the changes were in step with the decline in the visible vegetation indices. Given that one of the key draws of multispectral sensors is the ability to provide advanced warning of poor plant health, the fact that this was not displayed in this study is disappointing. There are two possible explanations for this however. Firstly, it could be a result of the time between flights – while daily flights were intended, in general flights were only able to be conducted every second day. More frequently flights (daily, or potentially even hourly) could provide the desired early warning. Alternatively, given that the fragility of seedlings was a key factor that inspired this study[4], it could simply be that the seedlings are simply too fragile for an early warning to be detectable – by the time anything is noticeable, it could already be too late. The second area that needs work is in the capture of imagery. Lack of alignment between RGB and multispectral imagery was a limiting factor in this study, and was most likely due to images being captured at different points in space, and being geotagged with different GPS units. Ideally, multispectral and RGB imagery should be captured at the same point in space and time, and tagged by the same GPS unit. However, this would require all images to be captured from a single unit with multiple sensors, the construction of which is beyond most restoration specialists. As such, the UAV construction industry will need to make changes to their practices in order to ensure their sensor units provide optimal data capture abilities.

Drone-based remote sensing is a novel tool to assess restoration trajectory at fine-scale by identifying and monitoring seedling emergence and performance

The work presented within this thesis has presented a significant and novel technique. For the first time, a remote sensing method of identifying and counting sown seeds is presented, which allows for accurate assessments of seed numbers lost to granivores, and thus more accurate estimates of germination rate, by removing the predated seeds from calculations. This technique also represents a potential method to study and identify microsite conditions that are most suited to seed germination, seedling emergence, and seedling establishment by means of assessing the micro-topographic details of the area surrounding sown seeds from the DEMs generated from UAV imagery, and tracking germination and emergence. The ability to identify individual seeds would only be enhanced by the addition of colourful polymer coatings, which are often used in restoration projects [5], and the ability to identify, count, and track those coated seeds would open up the ability for restoration practitioners to undertake studies on coated seeds in actual restoration sites, rather than lab conditions – for example seeds could be repeatedly counted over time to determine if any seed treatments or polymer coating colours made them less vulnerable to predation by granivores, or if they make seeds more or less likely to germinate.

Additionally, this thesis presents the first published method for identifying only the desired target species, despite the presence of obscuring background grasses. As with seed counts, daily counts of seedlings would ensure restoration practitioners are able to monitor seedling predation and emergence rates, and get early warning of any potential restoration failures. Having a choice of two potential methods to count seedlings allows the restoration practitioner to choose whichever method suits their situation, and minimise incorrect classification. For example, if planting on a bare area, the higher identification accuracy of the *single-date* method would be preferred. If that area later becomes infested with invasive weeds, the previously gathered *single-date* images could become the base layer for a *layered* approach, reducing the potential number of false positives.

While both seed and seedling identification showed high levels of accuracy, it is important to note too that this is only the first study conducted on automated identification at these scales, and it is highly likely that this technique will be refined and improved across further studies. One potential means of improvement that could be implemented in the near future is the modification of the ruleset to identify more than on target species. Alternatively, given that seeds proved to be large enough to identify, flowers of many species would similarly be large enough to be reliably identified, and thus flowering seedlings could also be identified and counted.

While multispectral vegetation indices have previously been shown to be predictors of plant stress [21] and eventual mortality, this thesis is the first work proving that the theory holds true even at extremely fine spatial scales. The ability to predict mortality in seedlings would allow restoration practitioners to take early action to prevent total restoration failures. Additionally, the noticeable difference in the non-visible spectral signatures of target seedlings and non-target seedlings suggests that further studies utilising non-visible bands of light could potentially discriminate between species of target seedlings that may be too similar in the visible spectrum for the current methodology to identify them.

The ability to track seedlings individually through time provides a previously unheard of ability to monitor restoration projects during a major bottleneck period [4] in their development. Added to the ability to monitor multispectral indices at seedling level, this method of tracking individuals provides a never before seen level of detail and clarity in a restoration practitioner's understanding of a restoration project. This ability, combined with the multispectral indices, seed identification, and micro-topographical detail able to be garnered from DEMs will enable an even greater understanding of microsite characteristics. Additionally, with the low turnaround time offered by UAVs [22] monitoring could be conducted almost on demand. This ability could also be further refined with the integration of more accurate RTK GPS units, or post processed solutions to improve accuracy.

Towards a one-pass solution for monitoring ecological recovery

Despite the potential offered by the techniques presented in this thesis, there are still knowledge gaps, and there is still work to be done. One of the major issues of the studies presented within this thesis is alignment of different outputs, whether that be of different types of images, or different images over time. Aligning images manually was a difficult and painstaking process, and often produced results that were less than expected even after very careful work. While introducing additional sensors like thermal or hyperspectral cameras has the potential to improve the amount of information that can be gained from the restoration project, and thus the number of traits that could be monitored, they also introduce an additional level of complexity that would need to be overcome. Additionally, the current issue of objects appearing different from image to image due to the asynchronous capture would still remain.

One potential solution to these problems is multisensory pods. By building pods from the ground up with the intention that they be used as dedicated remote sensing platforms, sensors could be integrated with simultaneous triggering to ensure images are captured at the same point in space and time, and geotagged with the same GPS to ensure no alignment is needed post-processing. Similarly, current image processing requires that the input from different sensors be processed separately, and later aligned for analysis. It is possible that custom built software could be designed that allows for the output of multiple sensors to be processed together, with a single file created, with no alignment needed.

Ideally, these multi-sensor pods would contain everything needed to monitor every aspect of a restoration area. While some existing sensors combine multispectral and RGB cameras, such as the Sequoia, there are limitations due to the nature of the sensors used – for example, the rolling shutter employed on the Sequoia's RGB sensor makes SfM techniques less accurate [23]. Initial work leading towards the development of a one-pass solution should focus on identifying the key aspects needed in UAV mounted sensors – such as high resolution, appropriate image capturing technology, simultaneous image capture, and full integration with UAV platforms, to allow for sensors to be triggered remotely, and to allow for all sensors to be geotagged from the UAV's GPS. Further work on the one-pass solution will require the development of all in one sensor pods. While these pods could vary depending on the requirements of the job at hand, a restoration monitoring pod would need to build as complete a view of the ecosystem as possible, and as such would require many different sensors. RGB sensors would provide the recognisable base of any orthomosaics created, allowing for basic classification, and more importantly, visual confirmation of classification results [24,25]. A multispectral or hyperspectral sensor would be required to assess plant condition [14,17,26-29], however the choice of which to use will vary from project to project. While hyperspectral sensors reveal more information about the plants and can potentially be used to identify a variety of different ailments, the cost increases along with the usefulness, and they can be anywhere from four to twelve times more expensive than multispectral sensors [30]. Thermal cameras would see use in identifying large warm blooded animals [31,32], and in detecting water stress in plants [18,33], while lidar would be utilised in order to allow penetration of obscuring surfaces, whether that be plant canopies or water [34,35]. While all of these sensors can be utilised individually, the creation of sensor pods designed from the ground up would result in the most time efficient and easy to use method of monitoring ecosystems, whether they be restored or pristine – the one-pass monitoring solution. Further potential for improvement exists in the fields of deep learning and artificial intelligence. While the studies presented here are focused on the use of OBIA techniques, collaboration with computer scientists and data analysts could reveal or create new methods of classifying captured imagery. Regardless of what path to improvement is sought however, it is clear that collaboration with other specialists will be required to create the most efficient restoration techniques, and move towards a one-pass solution for UAV monitoring.

Conclusions

This thesis, "Drone-based remote sensing as a novel tool to assess restoration trajectory at fine-scale by identifying and monitoring seedling emergence and performance", has identified major gaps in the literature surrounding UAV-based restoration monitoring, and has taken steps to plug those gaps. Chapter one resulted in the creation of a literature review of every UAV-based restoration monitoring paper, and identified inconsistency and uncertainty surrounding choice of UAV platform, choice of sensor, and choice of image classification technique. Furthermore it identified deficiencies in the number of studies focussed around utilising multiple sensors simultaneously, and taking full advantage of the fine spatial resolution UAVs offer. As such, two experiments were designed to help fill that gap.

Chapter two has resulted in the creation of a novel approach to restoration monitoring, by creating an automated method of identifying seeds and seedlings in representative substrates by means of object-based image analysis. Both seeds and seedlings were able to be identified with a high accuracy (ca. 90% for seeds and ca. 80% for seedlings) with minimal false positives. This represents the first remote sensing based solution for seedling monitoring, and opens the door to future microclimate studies due to the ability to identify and track seeds.

Additionally, in chapter three a multispectral sensor was utilised to monitor the target seedlings as they underwent water stress and senescence, and showed for the first time that the vegetation indices used to monitor adult plants are still valid at seedling scale. This study provides justification for further work in the field of providing early warning of seedling mortality through the use of non-visible vegetation indices. In addition, this study showed that it is possible to track individual seedlings through time, and use vegetation indices at the individual level rather than community.

While this thesis is only a preliminary study on UAV-based restoration monitoring at seedling scale, it proves beyond doubt that current technology can remotely monitor individual seedlings, and paves the way towards future one-pass restoration monitoring.

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Appendices

Supplementary material for Buters et al. (2019), "Methodological ambiguity and inconsistency constrain unmanned aerial vehicles as a silver bullet for monitoring ecological restoration".

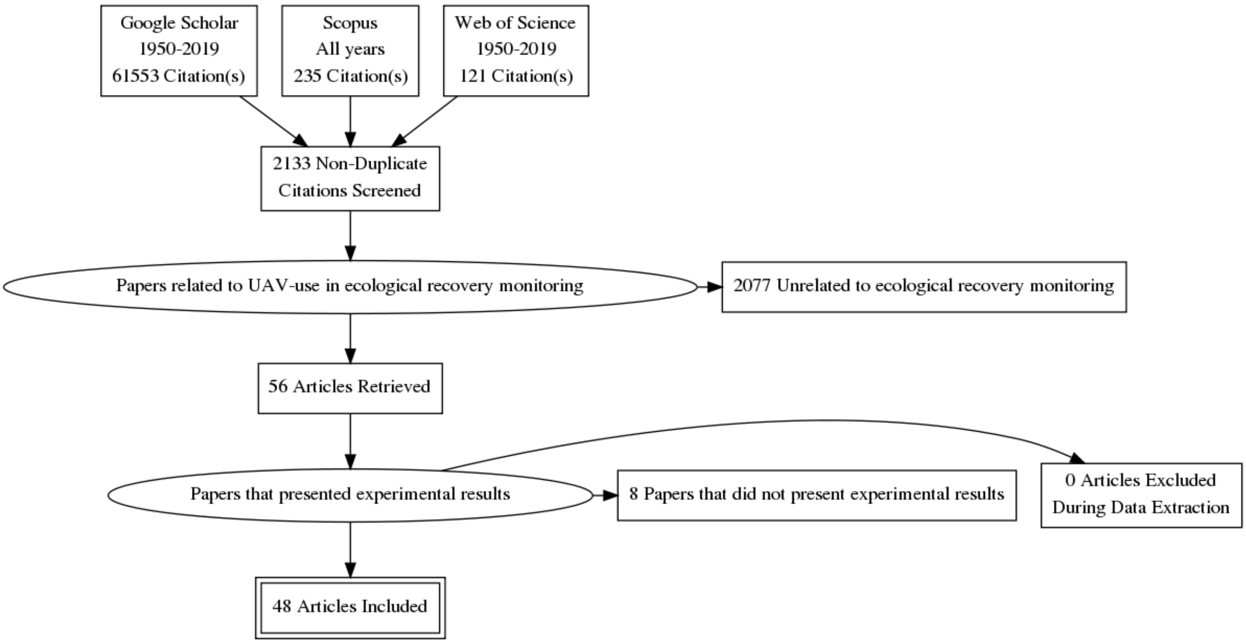

Figure S1. The PRISMA flow diagram for Buters *et al.* (2019), detailing the databases searched, the number of non-duplicate articles found, and the final number of relevant articles retrieved, after discarding papers that did not present experimental results.

Citation	Year	Region	Landscape	Recovery terminology	UAV platform	Image analysis technique	Number of sensors	Most advanced sensor
Baena *et al.*, 2017	2017	South America	Forest	Restoration	Fixed-wing	OBIA	1	Modified-RGB
Bedell, 2016	2016	North America	Riparian	Restoration	Multi-rotor	Manual	1	RGB
Cao *et al.*, 2018	2018	Asia	Mangrove	Restoration	Multi-rotor	Machine Learning	1	Hyper-spectral/Thermal/LiDAR
Cara et al., 2017	2017	Europe	Post-mining	Restoration	Multi-rotor	Manual	1	RGB
Chen *et al.*, 2017	2017	North America	Forest	Recovery	Multi-rotor	Manual	1	RGB
Dufour et al., 2013	2013	Europe	Riparian	Restoration	Fixed-wing	Manual	1	RGB
Esposito *et al.*, 2017	2017	Europe	Post-mining	Rehabilitation	Multiple	Manual	1	RGB
Fletcher & Erskine, 2013	2013	Australia	Post-mining	Rehabilitation	Multi-rotor	Manual	1	RGB
Gao et al., 2018	2018	Asia	Alpine	Restoration	Multi-rotor	Manual	1	RGB
Guillot *et al.*, 2018	2018	Europe	Coastal dunes	Recovery	Multi-rotor	Manual	1	RGB
Hird *et al.*, 2017	2017	North America	Forest	Recovery	Multi-rotor	Manual	1	RGB
Iizuka *et al.*, 2018	2018	Southeast Asia	Forest	Restoration	Multi-rotor	Machine Learning	2	Hyper-spectral/Thermal/LiDAR
Johansen *et al.*, 2019	2019	Australia	Post-mining	Rehabilitation	Fixed-wing	OBIA	3	Multi-spectral
Klein Hentz et al., 2018	2018	North America	Riparian	Restoration	Multi-rotor	Manual	1	RGB
Knoth et al., 2013	2013	Europe	Wetland	Restoration	Multi-rotor	OBIA	2	Modified-RGB
Laliberte et al., 2007	2007	North America	Rangeland	Restoration	Fixed-wing	OBIA	1	RGB
Laliberte & Rango, 2008	2008	North America	Rangeland	Restoration	Fixed-wing	OBIA	1	RGB
Lehmann et al 2017	2017	South America	Rainforest	Restoration	Fixed-wing	Machine Learning	2	Modified-RGB
Lejot et al 2007	2007	Europe	Riparian	Restoration	Paramotor	Manual	1	RGB
Levy et al., 2018	2018	North America	Coral reef	Recovery	Multi-rotor	Manual	1	RGB
Lishawa et al., 2017	2017	North America	Wetland	Restoration	Fixed-wing	Manual	2	Multi-spectral
Lobo et al., 2012	2012	Europe	Forest	Restoration	Fixed-wing	Manual	1	Multi-spectral
Marteau et al., 2016	2016	Europe	Riparian	Restoration	Multi-rotor	Manual	1	RGB
McIntosh et al., 2018	2018	Australia	Island	Recovery	Multi-rotor	Manual	1	RGB
Mitchell et al., 2012	2012	North America	Forest	Restoration	Fixed-wing	Machine Learning	1	Hyper-spectral/Thermal/LiDAR

Messinger & Silman, 2016	2016	North America	Post-mining	Recovery	Fixed-wing	Manual	1	RGB
Moudry et al., 2019	2019	Europe	Post-mining	Restoration	Fixed-wing	Manual	1	RGB
Nagai et al., 2007	2007	Asia	Riparian	Restoration	Helicopter	Manual	2	Multi-spectral
Nyquist, 1996	1996	North America	Post-mining	Restoration	Fixed-wing	Manual	1	RGB
Padro et al., 2019	2019	Europe	Post-mining	Restoration	Multi-rotor	Machine Learning	1	Multi-spectral
Rango et al., 2017	2017	North America	Grassland	Remediation	Multiple	OBIA	1	RGB
Reis et al., 2019	2019	South America	Grassland	Restoration	Not stated	Machine Learning	1	Multi-spectral
Ruessink et al., 2018	2018	Europe	Coastal dunes	Restoration	Fixed-wing	Manual	1	RGB
Ruisheng et al., 2018	2018	Asia	Grassland	Restoration	Multi-rotor	Manual	1	RGB
Sankey et al., 2017	2017	North America	Grassland	Restoration	Multiple	Machine Learning	3	Hyper-spectral/Thermal/LiDAR
Strohbach et al., 2018	2018	Africa	Post-mining	Restoration	Fixed-wing	Manual	2	Modified-RGB
Sykora-Bodie et al., 2017	2017	South America	Ocean	Recovery	Fixed-wing	Manual	1	Multi-spectral
Talavera et al., 2018	2018	Europe	Coastal dunes	Recovery	Multi-rotor	Manual	1	RGB
Tian et al., 2017	2017	Asia	Mangrove	Restoration	Not stated	Manual	2	Multi-spectral
van Iersel et al., 2016	2016	Europe	Riparian	Restoration	Not stated	Manual	2	Modified-RGB
van Iersel et al., 2018	2018	Europe	Riparian	Restoration	Fixed-wing	Manual	2	Modified-RGB
Waite et al., 2019	2019	Southeast Asia	Rainforest	Restoration	Multi-rotor	Manual	1	RGB
Whiteside & Bartolo, 2016	2016	Australia	Post-mining	Revegetation	Fixed-wing	OBIA	3	Multi-spectral
Whiteside & Bartolo, 2018	2018	Australia	Post-mining	Revegetation	Fixed-wing	OBIA	3	Multi-spectral
Woellner & Wagner, 2019	2019	Europe	Riparian	Restoration	Multi-rotor	Manual	1	RGB
Zawahi et al., 2015	2015	South America	Forest	Restoration	Multi-rotor	Manual	1	RGB
Zhao et al., 2017	2017	Asia	Riparian	Restoration	Not stated	Manual	1	RGB
Zhao et al., 2017	2017	Asia	Grassland	Recovery	Not stated	OBIA	1	RGB

Table S1. All analysed unmanned aerial vehicle (UAV) studies providing empirical data on the use of UAVs in the monitoring of ecological recovery by Buters *et al.* 2019, including year of publication, region of study interest, landscape of study interest, ecological recovery terminology utilised, UAV platform employed, captured imagery analytical technique, maximum number of sensors utilised in a single flight, and most advanced sensor type utilised during the study.

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Supplementary material for Buters *et al.* 2019. "Seed and Seedling Detection Using Unmanned Aerial Vehicles and Automated Image Classification in the Monitoring of Ecological Recovery"

Identification of seeds:

1: Multiresolution segmentation (scale parameter 10, shape: 0.4, compact: 0.7) creating 'New Level'

2: Assign class – *unclassified* with Total diff to scene ≥100 at New Level: *Seed*

3: Merge – *Seed* with Brightness ≥0 at New Level: merge region

4: Assign class – *Seed* with Length/Width >1.5 at New Level: *unclassified*

5: Assign class – *Seed* with Area <0.8 cm^2 at New Level: *unclassified*

6: Assign class – *Seed* with Roundness ≥0.8 at New Level: *unclassified*

***Identification of target seedlings* (*L. angustifolia*), *single image approach*:**

1: Multiresolution segmentation (scale parameter: 20, shape: 0.3, compact: 0.7) creating 'New level'

2: Assign class – *unclassified* with green ratio* >0.36 at New Level: *target*

3: Spectral difference segmentation – *target* at New level: spectral difference 30

4: Assign class – *target* with Mean diff. to neighbours DEM (0) ≥0.007 at New Level: *definitely target*

5: Assign class – *target* with HSI Transformation Saturation (R = Layer 3, G = Layer 2, B = Layer 1) <0.1 at New level: *unclassified*

6: Assign class – *target* with HSI Transformation Hue (R = Layer 1, G = Layer 2, B = Layer 3) ≥0.2 and HSI Transformation Saturation (R = Layer 1, G = Layer 2, B = Layer 3) ≥0.2 at New level: *maybe target*

7: Assign class – *maybe target, target* with Perimeter/Width >18 at New level: *unclassified*

8: Assign class – *maybe target* with Compactness <2.5 at New level: *definitely target*

9: Assign class – *maybe target*, *target* with TGI <21 at New level: *unclassified*

10: Assign class – *maybe target*, *target* with TGI ≥26 at New level: *definitely target*

11: Pixel based object resizing – *maybe target* at New level: shrink using *maybe target* where rel. area of object pixels in 5 x 5 ≥0.2

12: Assign class – *definitely target, maybe target*, *target* with Length/Width ≥5.5 at New level: *unclassified*

13: Assign class – *definitely target*, *maybe target*, *target* with Area ≤16 cm^2 at New level: *unclassified*

14: Assign class – *target* with Brightness ≥0 at New level: *unclassified*

15: Assign class – *maybe target* with Brightness ≥0 at New level: *unclassified*

*Green ratio= Mean Green/(Mean Red + Mean Green + Mean Blue)

***Identification of non-target grass cover* (applied following *Identification of target seedlings*):**

1: Assign class – *unclassified* with TGI >6.5 and green ratio >0.35 at New level: *non-target grass*

***Identification of target seedlings* (*L. angustifolia*)*, layered approach*:**

1: Multiresolution segmentation (Scale parameter: 10, shape: 0.3, compact: 0.7) creating 'New level'

2: Assign class – *unclassified* with First Green* ≥ 0.35 at New level: *Target*

3: Merge Region – *Target* at New level: Merge region

4: Assign class – *Target* at with Area < 2 cm^2 at New level: *unclassified*

5: Loop Assign class – *unclassified* with Second green** ≥ 0.35 and Existance of *Target*(0)= 1 at New level: *Target*

6: Merge Region – *Target* at New level: Merge region

7: Assign class – *Target* with Compactness > 2.5 at New level: *unclassified*

8: Assign class – *Target* with Area < 16 cm^2 at New level: *unclassified*

*First Green = Green ratio of earlier image layer

**Second Green = Green ratio of later image layer

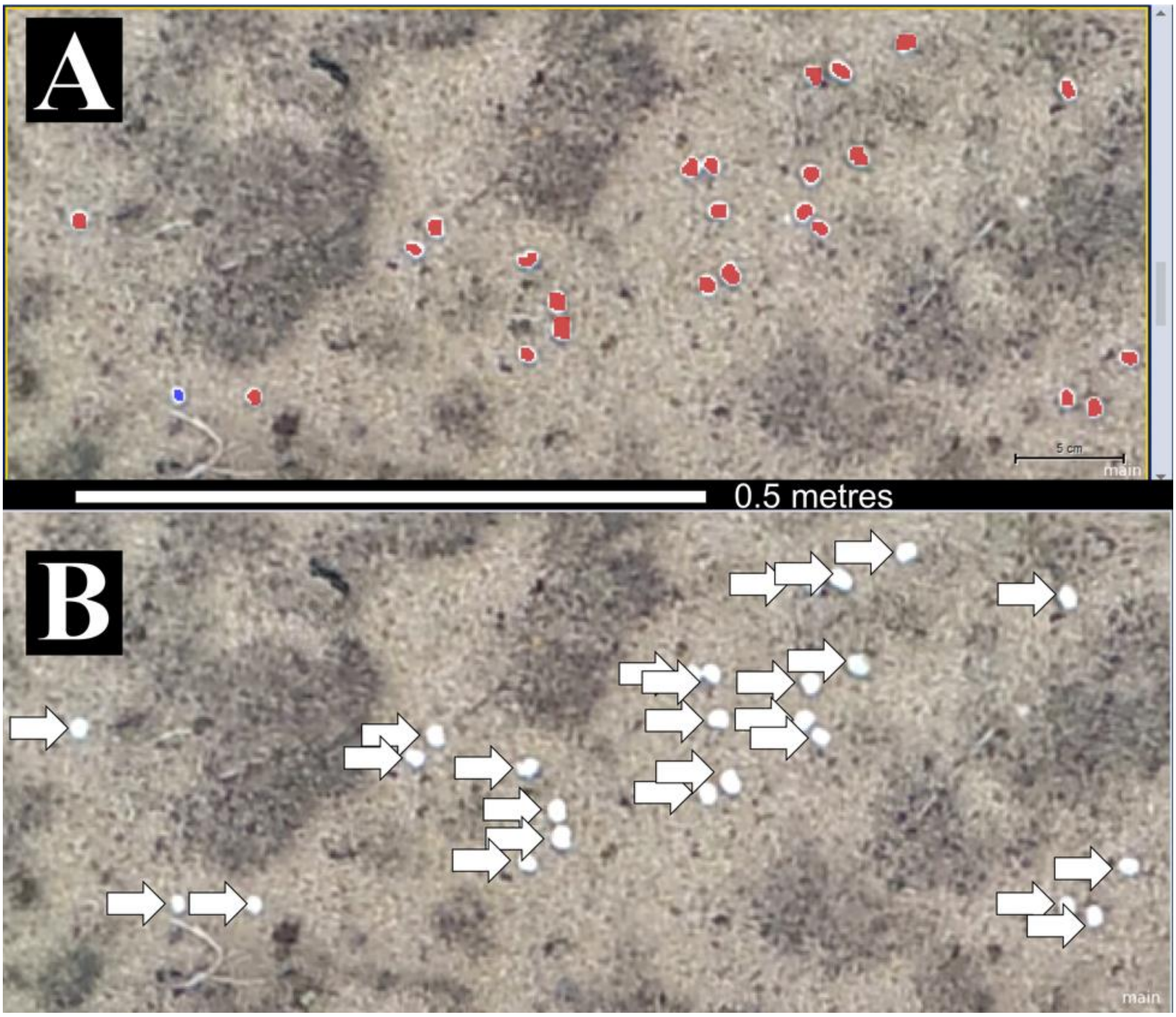

Figure S1. Example output image of a processed target area identifying seeds from eCognition rulesets, highlighting classes '*seeds*' (red), as well as 'missed target' (blue) manually identified post-processing (A), with corresponding unprocessed image target area (B) in which target seeds have been manually identified (annotated arrows). Image taken from an altitude of 5 m with a DJI Phantom 4 Pro UAV.

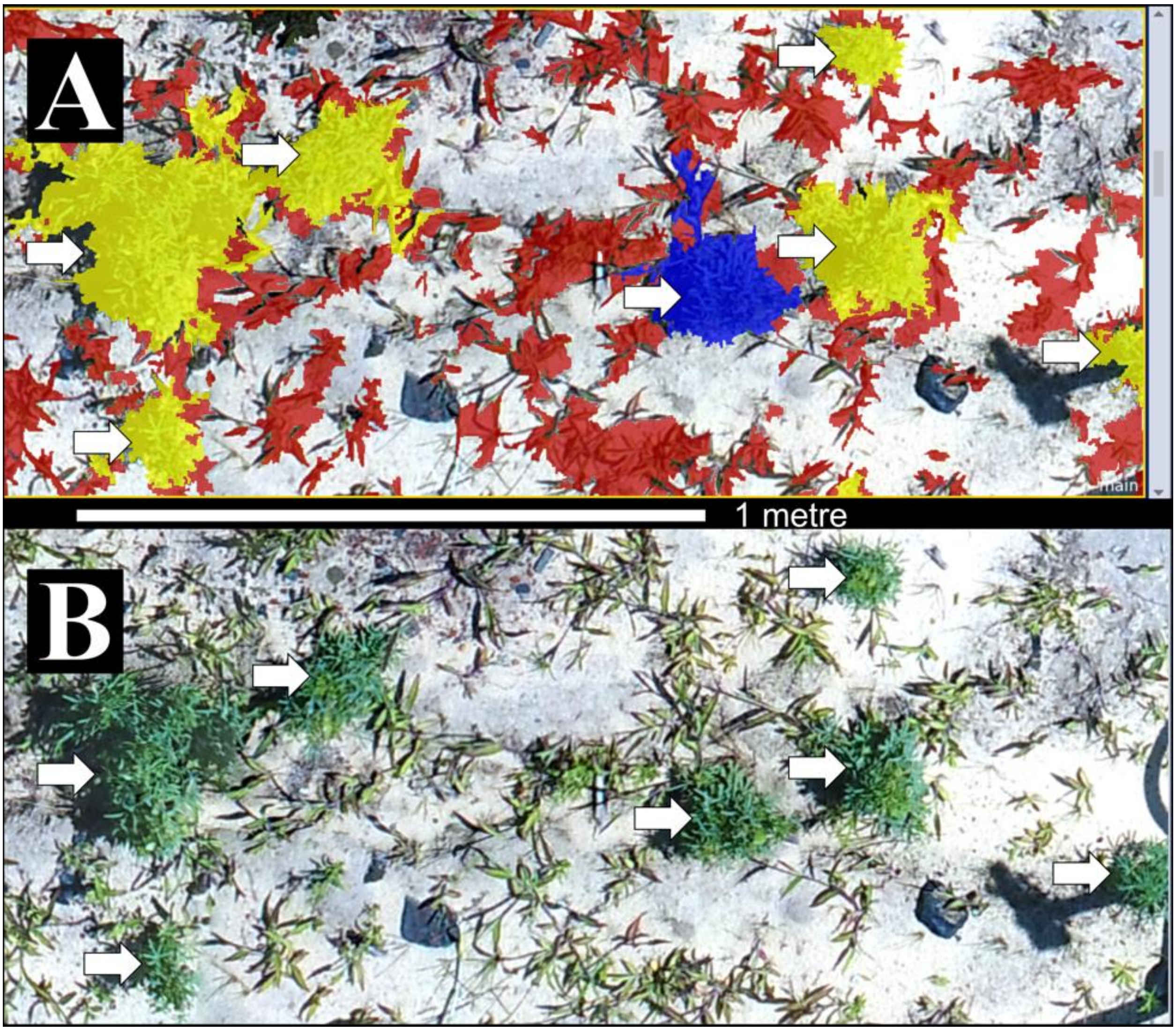

Figure S2. Example output image of a processed target area identifying seedlings from eCognition rulesets, highlighting classes ‘*definitely target*’ (yellow) and ‘*non-target grasses*’ (red), as well as ‘missed target’ (blue) manually identified post-processing (A), with corresponding unprocessed image target area (B) in which target seedlings have been manually identified (annotated arrows). Image taken from an altitude of 5 m with a DJI Phantom 4 Pro UAV.